

## RESEARCH ARTICLE

# Changes in Soil Hot-Water Extractable C, N and P Fractions During Vegetative Restoration in Zhifanggou Watershed on the Loess Plateau

XUE Sha<sup>1,2</sup>, LI Peng<sup>3</sup>, LIU Guo-bin<sup>1,2</sup>, LI Zhan-bin<sup>1,2</sup> and ZHANG Chao<sup>1,2</sup><sup>1</sup> State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau of Northwest A&F University, Yangling 712100, P.R.China<sup>2</sup> Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry Water Resources, Yangling 712100, P.R.China<sup>3</sup> Key Laboratory of Northwest Water Resources and Environment Ecology, Ministry of Education/Xi'an University of Technology, Xi'an 710048, P.R.China

## Abstract

The study was conducted in Zhifanggou Watershed, Shaanxi Province, China, to evaluate the effect of different vegetation types on hot-water extractable C, N and P fractions, with the aim to determine whether hot-water extractable fractions could be used as indicators of soil quality change in Loess Plateau. The six vegetation types established in 1975 were (i) *Robinia pseudoacacia* L., (ii) *Caragana korshinkii* Kom., (iii) *Pinus tabulaeformis* Carr., (iv) *P. tabulaeformis*-*Amorpha fruticosa* L., (v) *R. pseudoacacia*-*A. fruticosa*, and (vi) grassland. A cropped hillslope plot and a *Platycladus orientalis* L. native forest plot were used as references. The results indicated that the conversion of native forest to cropland resulted in a significant decline in the hot-water extractable C, N and P fractions. Hot-water extractable C, N, and P increased when cultivated land was revegetated, but after 30 years the amount of hot-water extractable C, N, and P in revegetated fields was still much lower compared to native forest. Hot-water extractable fractions increased more under mixed-forest than under pure-forest stands. Furthermore, there was a significant correlation between the hot-water extractable fractions and soil chemical and microbiological properties. The results showed that hot-water extractable fractions could be used as indicators of soil quality change on the Loess Plateau.

**Key words:** soil hot-water extractable fraction, vegetative restoration, Loess Plateau

## INTRODUCTION

Summer rain storms combined with steep slopes, intensive cultivation, overgrazing and improper management have made the Loess Plateau become one of the most severely eroded areas in the world (Jiang 1997). The Chinese government attempted to control soil erosion and restore the damaged environment since the 1950s (Fu *et al.* 2002). As part of this ongoing effort, the government initiated the "Grain-for-Green" project in 1999. One of the primary

objectives of this project was to reduce soil erosion and improve soil quality by converting cropped hill slopes to permanent grass or forest cover. But scientists did not reach a unanimous conclusion on indicator to reflect the effectiveness of the project. It is necessary to find a standardized and sensitive indicator of soil quality which would provide important information in future policy decision making.

Soil organic matter (SOM) affects many soil physical, chemical, and biological properties. Researchers have emphasized the value of SOM as an indicator of soil quality (Craswell and Lefroy

Received 25 October, 2012 Accepted 15 May, 2013

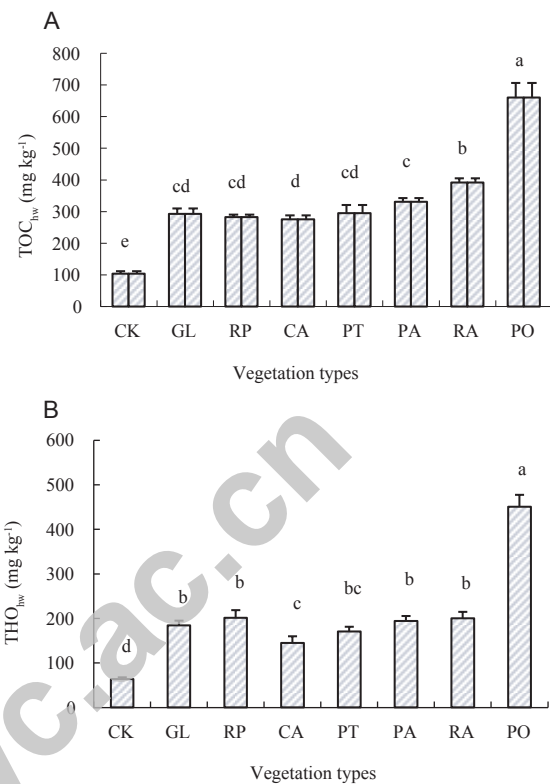
XUE Sha, Mobile: 15209242415, E-mail: xuesha100@163.com; Correspondence LI Peng, Tel: +86-29-82312658, E-mail: lipeng74@163.com

2001; Jinenez *et al.* 2002), however the short medium term in SOM are difficult to detect because of spatial variability and relatively high background concentrations of SOM (McGill *et al.* 1986; Ghani *et al.* 1996; Bolinder *et al.* 1999). Labile soil organic matters such as particulate organic matter, light fraction, water-extractable organic matter, and microbial biomass, respond rapidly to land use changes (Gregorich and Janzen 1996; Six *et al.* 1998; Lützow *et al.* 2002; Leifeld and Kögel-Knabner 2005), and have been used as early and sensitive indicators of SOM change (Chilima *et al.* 2002; Ghani *et al.* 2003). Of these, attention has been paid to the hot-water extractable organic fractions for determining impacts of soil management in soil-plant ecosystems (Sparling *et al.* 1998; Haynes 2000; Ghani *et al.* 2003; Wang and Wang 2007; Bu *et al.* 2011; Uchida *et al.* 2012). The hot-water extractable pool of C (HWC), which includes microorganisms, soluble carbohydrates, and other simple compounds, tended to relate well with microbial biomass-C (Sparling *et al.* 1998). Puget *et al.* (1999) showed that the amounts of C extracted by hot-water procedure strongly correlated with soil micro-aggregate characteristics. Haynes (1993) showed an increase in the amount of HWC when cultivated sites were under pasture and a decline when soils were cultivated. There is little information in the literature about the potential use of hot-water extractable N and P as soil quality indicators.

The objectives of this study were: 1) to reveal the change in the soil hot-water extractable C, N, and P fractions under different vegetation types; 2) to evaluate if the soil hot-water extractable C, N and P fractions could be used for indicating soil quality in the Loess Plateau.

## RESULTS

Soil hot-water extractable organic C ( $\text{TOC}_{\text{hw}}$ ) and hot water extractable carbohydrate ( $\text{CHO}_{\text{hw}}$ ) contents were 128 to 278% greater in revegetated plots compared to the cropped hillslope (CK), but 41 to 68% lower than the *Platycladus orientalis* L. (native forest) (Fig. 1). Among the revegetated plots,  $\text{TOC}_{\text{hw}}$  and  $\text{CHO}_{\text{hw}}$  contents tended to be the greatest in the *Robinia pseudoacacia*-*Amorpha fruticosa* treatment



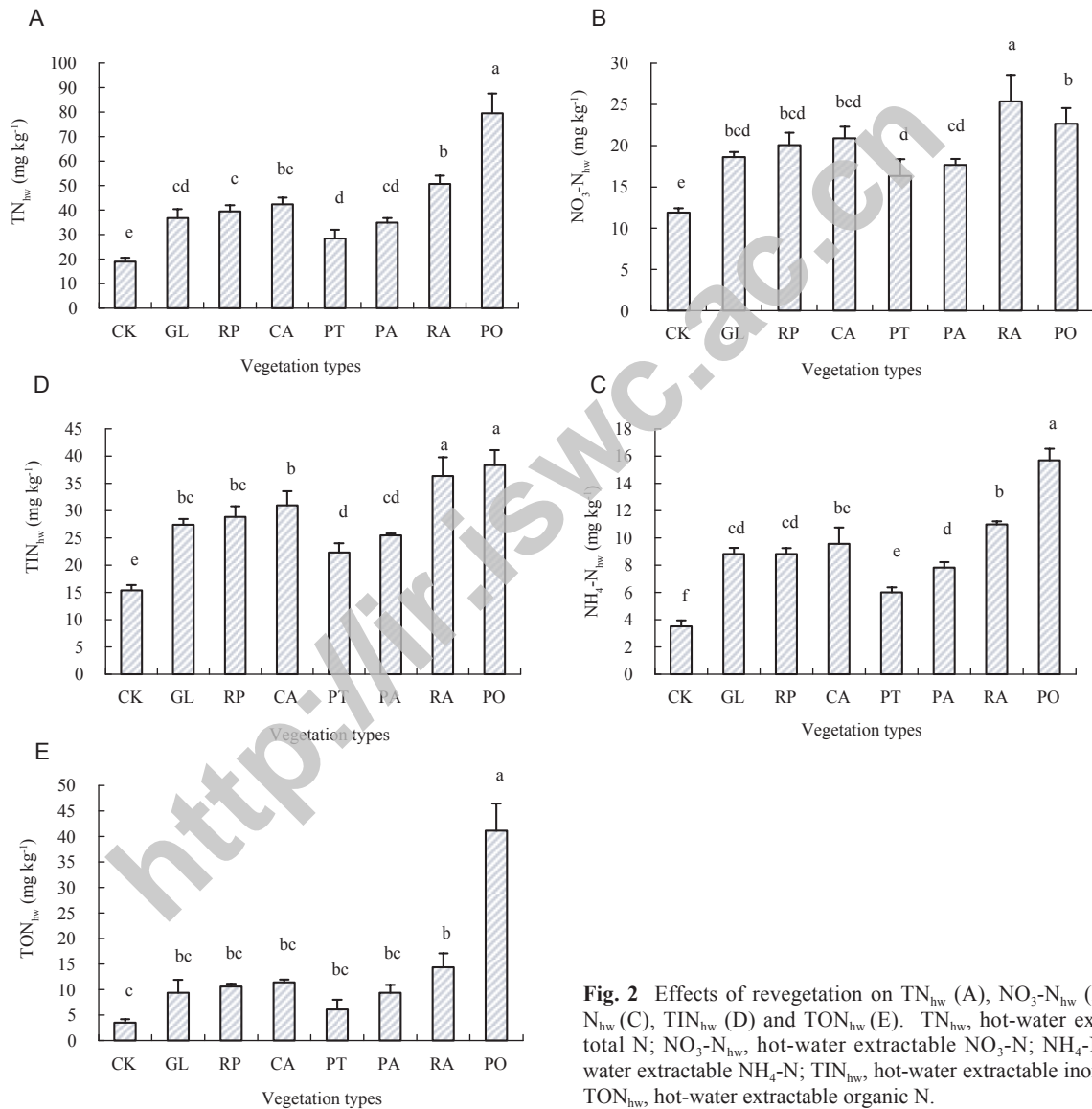
**Fig. 1** Effects of revegetation on  $\text{TOC}_{\text{hw}}$  (A) and  $\text{CHO}_{\text{hw}}$  (B). Data are given as mean $\pm$ SD.  $\text{TOC}_{\text{hw}}$ , hot-water extractable organic C;  $\text{CHO}_{\text{hw}}$ , hot-water extractable carbohydrate; RP, *Robinia pseudoacacia* L.; CA, *Caragana korshinkii* Kom.; PT, *Pinus tabulaeformis* Carr.; PA, *P. tabulaeformis*-*Amorpha fruticosa* L.; RA, *R. pseudoacacia*-*A. fruticosa*; GL, grassland; CK, cropped hillslope; PO, *Platycladus orientalis* L. Values with the same letters are not significantly different at  $P < 0.05$  level. Values with different letters are significantly different at  $P < 0.05$  level. The same as below.

and the lowest in the *Caragana korshinkii* treatment. There were no significant differences among the other vegetation types. The percentage of  $\text{TOC}_{\text{hw}}$  ranged between 3.17-4.84% whereas that of  $\text{CHO}_{\text{hw}}$  between 2.16-3.39%.  $\text{CHO}_{\text{hw}}$  accounted for 51.1-71.3% of  $\text{TOC}_{\text{hw}}$ .

Hot-water extractable total N ( $\text{TN}_{\text{hw}}$ ),  $\text{NH}_4^+$ -N ( $\text{NH}_4^+$ - $\text{N}_{\text{hw}}$ ),  $\text{NO}_3^-$ -N ( $\text{NO}_3^-$ - $\text{N}_{\text{hw}}$ ), total inorganic N ( $\text{TIN}_{\text{hw}}$ ) and organic N ( $\text{TON}_{\text{hw}}$ ) were 50-168, 71-312, 37-113, 45-136 and 73-308% greater in revegetated plots compared to the cropped hillslope, respectively, but 36-64, 38-70, 72-112, 72-95 and 15-35% less than that in *Platycladus orientalis* L. (native forest) (Fig. 2). The exception was the *R. pseudoacacia*-*A. fruticosa* treatment which had a  $\text{NH}_4^+$ - $\text{N}_{\text{hw}}$  content that

was 12% greater than in *P. orientalis* L. soil. Among the revegetated plots, hot-water extractable N fractions were the largest in the *R. pseudocacia*-*A. fruticosa* treatment and the smallest in the *P. tabulaeformis* treatment. Differences in hot-water extractable N fractions among the grassland, *R. pseudoacacia*, and

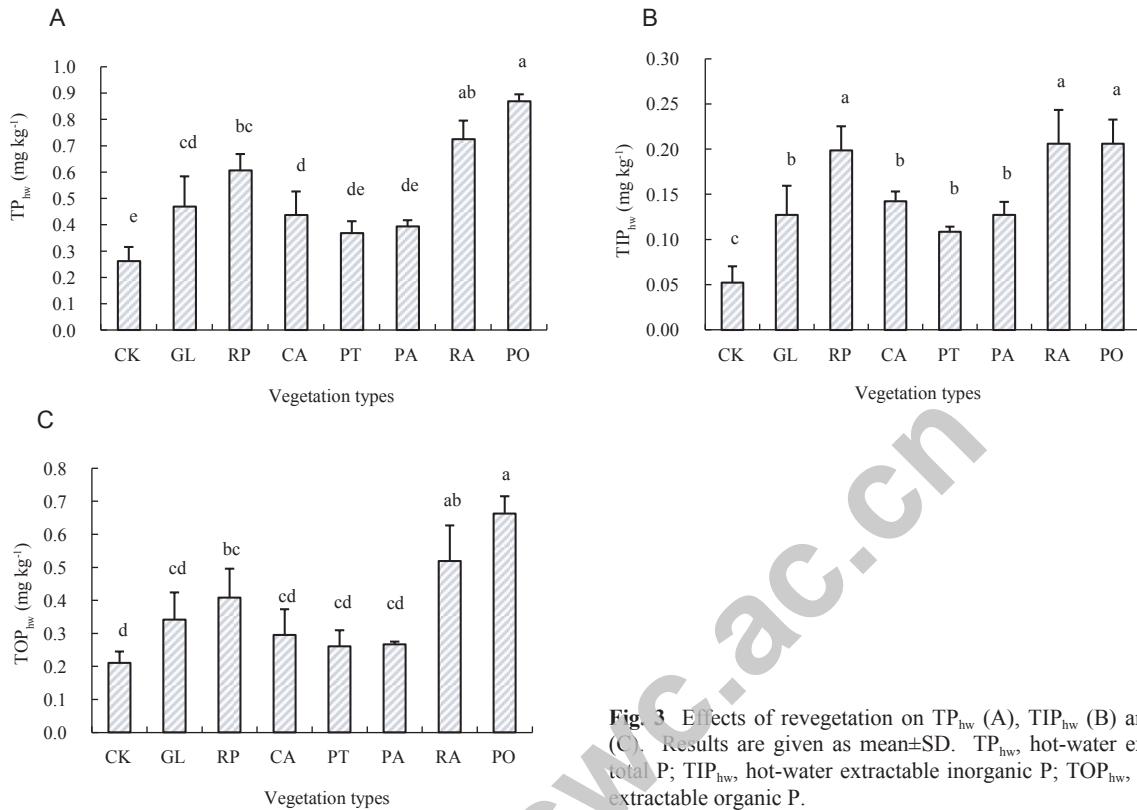
*C. korshinikii* treatments were not significant.  $TN_{hw}$  accounted for 4.20 to 5.97% of the total N in the soil.  $TIN_{hw}$  accounted for 48.2 to 81.4% of the  $TN_{hw}$  in the soil.  $TIN_{hw}$  to  $TN_{hw}$  ratio declined slightly after revegetation, but was still significantly greater compared to *Platycladus orientalis* L.



**Fig. 2** Effects of revegetation on  $TN_{hw}$  (A),  $NO_3-N_{hw}$  (B),  $NH_4-N_{hw}$  (C),  $TIN_{hw}$  (D) and  $TON_{hw}$  (E).  $TN_{hw}$ , hot-water extractable total N;  $NO_3-N_{hw}$ , hot-water extractable  $NO_3-N$ ;  $NH_4-N_{hw}$ , hot-water extractable  $NH_4-N$ ;  $TIN_{hw}$ , hot-water extractable inorganic N;  $TON_{hw}$ , hot-water extractable organic N.

Hot-water total extractable P ( $TP_{hw}$ ), inorganic P ( $TIP_{hw}$ ) and organic P ( $TOP_{hw}$ ) were 50-176%, 107-293% and 24-147% greater in revegetated plots compared to the cropped hillslope, respectively, but were only 42.4-83.5%, 52.7-99.8% and 39.2-78.3% less than that of *P. orientalis* L. (Fig. 3). Among the revegetated plots,  $TP_{hw}$ ,  $TIP_{hw}$  and  $TOP_{hw}$  were

the largest in the *R. pseudocacia*-*A. fruticosa* and *R. pseudoacacia* treatments while the smallest in the *P. tabulaeformis*-*A. fruticosa* and *P. tabulaeformis* treatments. Differences in  $TP_{hw}$ ,  $TIP_{hw}$  and  $TOP_{hw}$  between the grassland and *C. korshinikii* treatments were not significant.  $TP_{hw}$  accounted for 0.05 to 0.14% of soil total P.  $TOP_{hw}$  accounted for 67.2-80.0% of



**Fig. 3** Effects of revegetation on TP<sub>hw</sub> (A), TIP<sub>hw</sub> (B) and TOP<sub>hw</sub> (C). Results are given as mean±SD. TP<sub>hw</sub>, hot-water extractable total P; TIP<sub>hw</sub>, hot-water extractable inorganic P; TOP<sub>hw</sub>, hot-water extractable organic P.

TP<sub>hw</sub>.

The hot-water extractable fractions were respectively positively correlated ( $P < 0.01$  or  $P < 0.05$ ) with total C, total N, total P, hydrolysable N, available P, available K, and microbial biomass C, N, and P (Table 1). The hot-water extractable fractions were negatively correlated ( $P < 0.05$ ) with pH and CaCO<sub>3</sub>.

There was little or no correlation between the hot-water extractable fractions and NH<sub>4</sub><sup>+</sup>-N<sub>hw</sub> or NO<sub>3</sub><sup>-</sup>-N<sub>hw</sub>.

### DISCUSSION

The present study confirmed the findings that soil hot-water extractable organic C (TOC<sub>hw</sub>) and hot-water

**Table 1** Correlation coefficients (*r*) among soil chemical, microbiological, and hot-water extractable fractions

	TOC <sub>hw</sub>	CHO <sub>hw</sub>	TN <sub>hw</sub>	NO <sub>3</sub> -N <sub>hw</sub>	NH <sub>4</sub> <sup>+</sup> -N <sub>hw</sub>	TIN <sub>hw</sub>	TON <sub>hw</sub>	TP <sub>hw</sub>	TIP <sub>hw</sub>	TOP <sub>hw</sub>
TOC	0.969**	0.972**	0.936**	0.548**	0.889**	0.737**	0.961**	0.822**	0.612**	0.840**
TN	0.970**	0.980**	0.944**	0.560**	0.909**	0.753**	0.964**	0.819**	0.633**	0.828**
HN	0.971**	0.936**	0.959**	0.702**	0.940**	0.845**	0.931**	0.891**	0.702**	0.897**
TP	0.675**	0.645**	0.659**	0.770**	0.730**	0.764**	0.539**	0.790**	0.820**	0.725**
AP	0.722**	0.668**	0.792**	0.707**	0.827**	0.794**	0.708**	0.722**	0.559**	0.730**
AK	0.657**	0.566**	0.753**	0.909**	0.838**	0.918**	0.569**	0.752**	0.826**	0.674**
pH	-0.891**	-0.869**	-0.914**	-0.589**	-0.868**	-0.737**	-0.937**	-0.822**	-0.555**	-0.860**
CaCO <sub>3</sub>	-0.592**	-0.673**	-0.619**	-0.061	-0.541**	-0.292	-0.760**	-0.433*	-0.105	-0.519**
NO <sub>3</sub> <sup>-</sup> -N	0.132	0.064	0.128	0.522**	0.265	0.430*	-0.079	0.319	0.483*	0.239
NH <sub>4</sub> <sup>+</sup> -N	0.003	-0.044	-0.045	0.055	-0.056	-0.033	-0.022	-0.101	-0.018	-0.124
SMBN	0.957**	0.974**	0.924**	0.510*	0.883**	0.716**	0.954**	0.775**	0.598**	0.784**
SMBN	0.887**	0.934**	0.883**	0.459*	0.836**	0.652**	0.939**	0.755**	0.579**	0.765**
SMBP	0.939**	0.943**	0.933**	0.600**	0.917**	0.789**	0.918**	0.814**	0.686**	0.804**

TOC<sub>hw</sub>, hot-water extractable organic C; CHO<sub>hw</sub>, hot-water extractable carbohydrate; NO<sub>3</sub><sup>-</sup>-N<sub>hw</sub>, hot-water extractable nitrate N; NH<sub>4</sub><sup>+</sup>-N<sub>hw</sub>, hot-water extractable ammonium N; TIN<sub>hw</sub>, hot-water extractable inorganic N; TON<sub>hw</sub>, hot-water extractable organic N; TP<sub>hw</sub>, hot-water extractable total P; TIP<sub>hw</sub>, hot-water extractable inorganic P; TOP<sub>hw</sub>, hot-water extractable organic P; TOC, total organic C; TN, total N; HN, hydrolysable N; TP, total P; AP, available P; AK, available K; SMBP, soil microbial biomass P; SMBN, soil microbial biomass N; SMBC, soil microbial biomass C. Correlation coefficient labeled by \* and \*\* indicates significant difference at  $P \leq 0.05$  and  $P \leq 0.01$  respectively (n=24).

extractable carbohydrate ( $\text{CHO}_{\text{hw}}$ ) contents are very little, but are important and highly labile components of soil organic C (Gregorich *et al.* 2003).  $\text{TOC}_{\text{hw}}$  content in this study accounted for 3.2 to 4.8% of soil organic C which was consistent with results from previous studies (Leinweber *et al.* 1995; Sparling *et al.* 1998; Chan and Heenan 1999; Chodak *et al.* 2003; Curtin *et al.* 2006), but slightly greater than the results by Wang and Wang (2007). Whereas  $\text{CHO}_{\text{hw}}$  accounted for 51-71% of  $\text{TOC}_{\text{hw}}$  and 2.2-3.4% of soil organic C, which was essentially consistent with those from the study of Ghani *et al.* (2003). Some researchers reported that  $\text{TOC}_{\text{hw}}$  primarily consisted of soil microbial biomass C, root exudates, soluble carbohydrates, and amino acids (Leinweber *et al.* 1995) and  $\text{CHO}_{\text{hw}}$  was one of the main components of  $\text{TOC}_{\text{hw}}$  and more closely related to aggregate stability than soil total carbohydrate or organic C (Angers *et al.* 1993; Haynes and Francis 1993; Ball *et al.* 1996; Degens 1997; Haynes and Beare 1997). Ghani *et al.* (2003) reported that  $\text{TOC}_{\text{hw}}$  was 3 to 7 times greater than microbial biomass C and verified the fact that microbial biomass-C was a key component of  $\text{TOC}_{\text{hw}}$  which was also found by Wang and Wang (2007). On the contrary, we found that the content of  $\text{TOC}_{\text{hw}}$  was similar with microbial biomass C supported by the result of Sparling *et al.* (1998) and Chodak *et al.* (2003), which suggested that  $\text{TOC}_{\text{hw}}$  partly derived from soil microorganisms.

Hot water was used as a mild extractant for labile organic matter which was largely composed of N-containing compounds, amino-N species and amides except carbohydrates (Leinweber *et al.* 1995). Verstraeten *et al.* (1970) and Curtin *et al.* (2006) used hot-water extractable N fractions as a measure of soil available N, but Chodak *et al.* (2003) argued that hot-water extract method did not provide any better measure than total C and N. The present studies showed that hot water was a good extractant for reflecting the change of soil nitrogen under different vegetation restoration. In this study, the content of  $\text{TN}_{\text{hw}}$  ranged from 19 to 79 mg N  $\text{kg}^{-1}$  soil, which constituted 4.20-5.97% of the TN in soils, consistent with the value by Chodak (2003) and Curtin (2006), but markedly higher than those observed by Wang and Wang (2007). There is no unified conclusion

about the potential use of extractable N fractions. Some researchers hold that dissolved N was mainly in organic forms (Gregorich *et al.* 2003; Curtin *et al.* 2006; Kranabetter *et al.* 2007), but a few studies advocated that dissolved organic N in agricultural soils represented a significant but not always dominantly fraction of dissolved total N which was regulated by land use (Christou *et al.* 2005). Nitrate N was not detected in the extracts in most researches because extracted N forms were mostly considered as the hydrolysable or distillable N (Curtin 2006). The results in the paper showed that  $\text{TON}_{\text{hw}}$  only accounted for 19-52% of  $\text{TN}_{\text{hw}}$ , and  $\text{NO}_3^- \text{-N}_{\text{hw}}$  constituted 55-77% of  $\text{TN}_{\text{hw}}$  which was a bit different from other previous researches. Curtin (2006) suggested that organic N was thermal instability and could be hydrolyzed to ammonium N, which resulted in a high content of  $\text{TN}_{\text{hw}}$ . Jia *et al.* (2011) argued that nitrate N was a main component of  $\text{TN}_{\text{hw}}$  which was consistent with our studies. Two possible reasons may explain the existence of  $\text{NO}_3^- \text{-N}_{\text{hw}}$ . Firstly, ammonium N was unstable and it was easy to nitrify to nitrate N. In addition, the soil in Loess Plateau was alkaline soil. Alkaline conditions enhanced the instability of ammonium N and accelerated nitrification rate in hot-water extracts. The highest value of  $\text{TON}_{\text{hw}}/\text{TN}_{\text{hw}}$  was found in PO, and the lowest value found in slope farmland. The result can be explained by the results of Christou *et al.* (2005) who suggested that intensive agricultural and low input systems had a low dissolved organic N to total dissolved N ratio and *vice versa*. Wang and Wang (2007) held that the content of  $\text{TN}_{\text{hw}}$  was lower than that of microbial biomass-N. But in the present studies, there was not always a lower content of  $\text{TN}_{\text{hw}}$  than microbial biomass-N, suggesting that  $\text{TN}_{\text{hw}}$  partly derived from soil microorganisms.

Dissolved P has been suggested as an indicator of ecosystem P status in terms of both the export of dissolved organic P and reactive forms of inorganic P (Neff *et al.* 2000). Dissolved organic P has an important role in maintaining the nutrient level in terrestrial ecosystems (Hedin *et al.* 1995) and is important for reflecting the element turnover rates and nutrient distribution of soils (Schoenau *et al.* 1987). Our results showed that  $\text{TP}_{\text{hw}}$  accounted for 0.05-0.14% of soil total P. Whereas  $\text{TOP}_{\text{hw}}$  accounted for

67.2-80.0% of  $TP_{hw}$ , which was consistent with the data from Chapman *et al.* (1997). Previous work has shown that dissolved inorganic P was readily available to microbial as it has been shown as a short-term source, whereas most of bio-available P and organic P represented a secondary and long-term source of bio-available P in water bodies (Sharpley *et al.* 1992). In our study, vegetative restoration resulted in an increase in the  $TIP_{hw}/TP_{hw}$  but a decrease in  $TOP_{hw}/TP_{hw}$  compared to the cultivated field, which presumed that a higher proportion of available P in the short-term might be an effective supplement to the increasing uptake phosphorus of vegetation.

A significant decrease of soil hot-water extractable C, N and P fractions accompanied by deforestation and cultivation as well as an important increase of them after vegetation restoration were shown in the study. A similar finding was observed by Shi *et al.* (2010) who held that continued cultivation of native grassland significantly reduced concentrations of organic C and total N of whole soil organic matter and some labile components. The native forest community, with a higher C stocks and a higher amount of hot-water extractable organic fractions (Ghani *et al.* 2003; Xu and Xu 2003; Wang and Wang 2007), was considered as soil-dominated climax community and the nutrient and energy cycles in the ecosystem were stable. Owing to improper tillage practices, deforestation and conversion to arable lands breaks the stability and results in an extensive deterioration of soil structure and a depletion of soil properties including dissolved organic matter (Spaccini *et al.* 2001; Zheng *et al.* 2005; An *et al.* 2008). Compared with farmland, vegetation restoration led to a higher below-ground mass and a quicker nutrients turnover in soil, which would affect the net accumulation or depletion of organic matter and hot-water extractable fractions in soil (Kuzyakov *et al.* 2001). The revegetation treatments in this study differed in litter biomass, chemical content, and decomposition rate which further affect soil hot-water extractable C, N and P fractions. Mixed forest resulted in a greater increase in the content of soil hot-water extractable C, N and P fractions than pure forest or grassland, probably due to a higher biodiversity, a higher biomass and a larger stock of organic matter. The content of them was the least in pure forest, and may be due to the fact that litter in coniferous forests

contains recalcitrant compounds such as tannin, resin, and wax.

The correlation coefficients including self-correlations between hot-water extractable C, N, and P fractions and soil chemical and microbiological properties in our study were large compared to other studies. This may have been affected by the fact that parameter values in the *P. orientalis* L. soil were much larger than in abandoned farmland soil. Therefore, a second correlation analysis was conducted in which the effect of the *P. orientalis* L. soil was excluded (data not shown). The result can be explained by the results was excluded (data not shown). The correlation coefficients were smaller but still significant ( $P < 0.01$ , or  $P < 0.05$ ) when the *P. orientalis* L. was excluded. These results are consistent with previous studies (Chodak *et al.* 2003; Ghani *et al.* 2003; Xu and Xu 2003). Compared with conventional parameters such as total P, available P, available K,  $NO_3^-$ -N, variation coefficient of hot-water extractable fractions were higher which mostly were over 40% although there was no significant difference compared with microbial biomass. Furthermore, hot-water extractable fractions can be measured more rapidly and economically than microbial biomass. The preservation of air-dried soil sample for determining hot-water extractable C, N and P fractions is also easier than that of fresh soil sample for determining biological properties. Therefore, hot-water extractable C, N, and P fractions can be more useful to reflect changes of soil quality.

## CONCLUSION

Loess Plateau ecosystems are facing serious environmental problems including land degradation due to heavy erosion. This study showed deforestation and cultivation significantly reduced the hot-water extractable C, N and P fractions, whereas revegetation significantly increased them. Hot-water extractable C, N and P fractions differ significantly under different vegetation types, and they increased more under mixed-forest than pure-forest. The significant correlation between hot-water extractable fractions and soil chemical and microbiological properties suggest that they could be used as indicators to reflect changes of soil quality in the Loess Plateau.

This study could offer help to evaluate soil quality and provide important information in future policy decisions. Further study of hot-water extractable fraction should be focused on the relationship with the active component as well as its conversion process in soil in order to quantitatively study the change of hot-water extractable fraction.

## MATERIALS AND METHODS

### Site description

The study was conducted at Zhifanggou Watershed of the Ansai Research Station of Soil and Water Conservation, located in the semi-arid region of the Loess Plateau, China (E109°13'46''-09°16'03'', N36°46'42''-36°46'28'', 1010-1431 m altitude, 8.27 km<sup>2</sup>). Zhifanggou watershed is a popular case study area for comprehensive soil and water conservation in the Loess Plateau. The landform and vegetation in the 8.27 km<sup>2</sup> watershed is typical of the Hill and Gully Region in the Loess Plateau. Average annual temperature is 8.8°C and precipitation is 549.1 mm, which have clear seasonal variation. The annual evaporation ranges from 1010 to 1400 mm and the average frost-free period is approximately 157 d according to observation and statistics in many years. The soil at the study site was loess-derived and the minimum soil depth is >10 m, which contained 640 g sand kg<sup>-1</sup>, 240 g silt kg<sup>-1</sup> and 120 g clay kg<sup>-1</sup>. Soil organic C content is (2.35±0.35) g kg<sup>-1</sup> (mean±SD); available N content is (15.73±2.52) mg kg<sup>-1</sup>; and available P content is (15.09±3.49) mg kg<sup>-1</sup>.

In order to compare the effect of different vegetation types on the ecosystem, six plots were established in Zhifanggou Watershed in 1975. The vegetation types in the six plots were (i) *R. pseudoacacia* L., (ii) *C. korshinkii* Kom., (iii) *P. tabulaeformis* Carr., (iv) *P. tabulaeformis-A. fruticosa* L., (v) *R. pseudoacacia-A. fruticosa*, and (vi) grassland. The plots, which were cropped prior to the start of the experiment, were located on north-facing slopes of 20 to 32°. It was assumed that the soils in each plot were similar at the beginning of the study. A cropped hillslope (CK) and a *P. orientalis* L. (PO), which were considered

separately as start community and soil-dominated climax community in vegetation restoration were used as references. Except for cropped plots, all the vegetated plots were remained natural condition with little human disturbance and no management practices such as pruning, fertilization, cutting and forest tending. A description of each sample site is shown in Table 2.

### Soil sampling and analysis

Soil samples were collected from three sample plots (20 m×20 m) within each vegetation type. Composite (10 cores) soil samples were collected from three different locations within each vegetation plot. The three composite samples within a plot were considered true replicates as the distance between each sampling location exceeded the spatial dependence (>13.5 m) of most soil chemical and microbial properties (Mariotte *et al.* 1997). Soil samples were collected from the top 20 cm of the soil profile with a stainless steel corer (5 cm diameter). The litter horizon was removed before soil sampling. Ten soil cores were collected along an "S" type pattern from each sample plot. Sample collection points were at least 80 cm away from trees. The ten cores were mixed to form one composite sample. Roots, stones and debris were removed and each sample was divided into two parts. One part was air-dried prior to determine soil physicochemical properties. The second part was immediately sieved (2 mm) and stored at 4°C prior to determine soil microbial biomass C, N, and P.

Soil organic C was determined by wet digestion with a mixture of potassium dichromate and concentrated sulfuric acid. Soil total N was measured by the semi-micro Kjeldahl method. Soil total P was determined colorimetrically after wet digestion with H<sub>2</sub>SO<sub>4</sub>+HClO<sub>4</sub>. Hydrolysable N was determined by micro-diffusion (Conway) method after extraction with 1 mol L<sup>-1</sup> NaOH (Cornfield 1960). Available P was determined by the Olsen method. Available K was measured by flame photometry after extraction with 1 mol L<sup>-1</sup> NH<sub>4</sub>OAc. An automatic acid-base titrator was used to determine soil pH (1:5 soil/water ratio) (Metrohm 702). Soil microbial biomass C, N, and P were determined by fumigation extraction using *kc* factors of 0.38, 0.54 and 0.40, respectively (Brookes *et al.* 1985; Wu *et al.* 1990). The soil properties are presented in Table 3.

Hot-water extracts were prepared as the method descri-

**Table 2** Description of the sampling sites

Site no.	Vegetation type	Slope aspect	Slope (°)	Altitude (m)	Total coverage (%)	Main herbaceous species	Herbaceous biomass underforestry (g m <sup>-2</sup> )
CK	Sloping farmland	N	22	1175	40	<i>Setaria italic</i> L.	192.0
GL	Grassland	N	20	1206	71	<i>Artemisia sacrorum</i>	565.0
RP	<i>R. pseudoacacia</i>	NE 10°	32	1129	75	<i>Lespedeza dahurica-Stipa bungeana</i>	155.6
CA	<i>C. korshinkii</i>	N 45°W	24	1029	82	<i>Artemisia sacrorum-Stipa bungeana</i>	205.8
PT	<i>P. tabulaeformis</i>	N	27	1166	73	<i>Artemisia sacrorum-Carex lanceolat</i>	134.0
PA	<i>P. tabulaeformis-A. fruticosa</i>	N	24	1142	78	<i>Artemisia sacrorum-Stipa bungeana</i>	129.1
RA	<i>R. pseudoacacia-A. fruticosa</i>	N 56°W	27	1185	80	<i>Artemisia sacrorum</i>	341.0
PO	<i>P. orientalis</i>	N 1°W	33	1283	63	<i>Carex lanceolat</i>	204.0

**Table 3** Effect of vegetation on the chemical and microbiological properties in surface soil (0-20 cm)

Treatment <sup>1)</sup>	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Hydrolysable N (mg kg <sup>-1</sup> )	Total P (g kg <sup>-1</sup> )	Available P (mg kg <sup>-1</sup> )	Available K (mg kg <sup>-1</sup> )	pH	Microbial biomass-C (SMBC, mg kg <sup>-1</sup> )	Microbial biomass-N (SMBN, mg kg <sup>-1</sup> )	Microbial biomass-P (SMBP, mg kg <sup>-1</sup> )
CK	2.74 f	0.365 g	20.90 g	0.55 d	1.64 b	105.4 e	8.73 a	129.42 d	19.02 e	6.73 d
GL	6.59 cd	0.769 d	50.77 c	0.59 bc	2.44 ab	162.7 c	8.70 b	270.50 c	25.52 de	10.96 bc
RP	5.94 e	0.731 e	41.48 ef	0.61 a	1.97 b	174.3 c	8.74 a	285.57 c	40.69 bc	10.86 bc
CA	5.74 e	0.710 e	45.12 de	0.58 c	2.32 ab	192.3 b	8.70 b	304.04 b	41.29 b	11.33 b
PT	6.42 d	0.663 f	41.14 f	0.57 c	1.77 b	122.7 d	8.74 a	287.53 c	26.75 de	8.87 c
PA	6.83 c	0.806 c	46.12 d	0.60 ab	2.40 ab	168.3 c	8.73 a	316.07 b	34.46 bcd	12.11 b
RA	9.27 b	0.880 b	71.34 b	0.62 a	2.79 a	203.6 a	8.62 c	313.42 b	31.04 cd	11.67 b
PO	20.80 a	1.894 a	109.5 a	0.613 a	3.53 a	194.7 b	8.47 d	793.91 a	103.89 a	19.99 a

<sup>1)</sup> CK, cropped hillslope; GL, grassland; RP, *Robinia pseudoacacia* L.; CA, *Caragana korshinskii* Kom.; PT, *Pinus tabulaeformis* Carr.; PA, *P. tabulaeformis*-*Amorpha fruticosa* L.; RA, *R. pseudoacacia*-*A. fruticosa*; PO, *Platycladus orientalis* L.

Values are the means of three replicates and with the same letter are not significantly different at  $P < 0.05$  level.

bed by Sparling *et al.* (1998). Briefly, 20 g (oven-dry equivalent) of air-dried soil were incubated with 80 mL distilled water in a capped test tube at 70°C for 18 h. At the end of the incubation period, the test tubes were shaken on an end-to-end shaker for 5 min and centrifuged for 10 min at 3 000 r min<sup>-1</sup>. The supernatants were filtered through Whatman 42 paper and a 0.45- $\mu$ m filter membrane. The filtrates were frozen and stored for further analysis. The C, N and P fractions of filtrates were determined using standard soil test procedures of the Chinese Ecosystem Research Network (CERN Editorial Committee 1996). Hot-water extractable organic C (TOC<sub>hw</sub>) in the filtrate was determined with a High TOCII+N analyzer (Elementar, Germany). Hot-water extractable NH<sub>4</sub><sup>+</sup>-N (NH<sub>4</sub><sup>+</sup>-N<sub>hw</sub>) was measured with a continuous flow system (FIA Star 5000 analyzer, Foss Tecator, Sweden). Hot-water extractable NO<sub>3</sub><sup>-</sup>-N (NO<sub>3</sub><sup>-</sup>-N<sub>hw</sub>) was determined by ultraviolet colorimetry with a Shimadzu D2704 ultraviolet spectrophotometer (Shimadzu Ltd., Co., Japan). Total extractable N (TN<sub>hw</sub>) was determined by alkaline persulfate digestion. Hot-water inorganic N (TIN<sub>hw</sub>) was calculated as the sum of NH<sub>4</sub><sup>+</sup>-N<sub>hw</sub> and NO<sub>3</sub><sup>-</sup>-N<sub>hw</sub>. Hot-water extractable organic N (TON<sub>hw</sub>) was calculated as TN<sub>hw</sub> minus the sum of NH<sub>4</sub><sup>+</sup>-N<sub>hw</sub> and NO<sub>3</sub><sup>-</sup>-N<sub>hw</sub>. Hot-water extractable inorganic P (TIP<sub>hw</sub>) was determined by the phospho-molybdenum blue method. Hot-water total extractable P (TP<sub>hw</sub>) was measured using the ammonium molybdate spectrophotometric method. Hot-water extractable organic P (TOP<sub>hw</sub>) was calculated as TP<sub>hw</sub> minus TIP<sub>hw</sub>. Hot water extractable carbohydrate (CHO<sub>hw</sub>) content was determined by the phenol-method without acid hydrolysis (Safarik and Santruckova 1992; Ghani *et al.* 2003). 1 mL of the hot-water extract was mixed with 1 mL 5% phenol solution in the 40 mL-tube and 5 mL of concentrated sulphuric acid was added immediately. The test tubes were vortexed for 10 s and allowed to stand for 1 h. Carbohydrate content (CHO<sub>hw</sub>) was determined by absorbance of the mixture at 485 nm.

### Statistical analysis

All data are expressed on an air-dry soil weight basis. Analysis of variance (ANOVA) was used to detect

significant differences among treatments. Linear correlation analysis was used to determine relationships among soil chemical, microbiological, and hot-water extractable fractions. All statistical analysis was conducted with SAS 6.12 software.

### Acknowledgements

This work was financially supported by the Strategic Technology Project of Chinese Academy of Sciences, China (XDA05060300), the Science and Technology Research and Development Program of Shaanxi Province, China (2011KJXX63), and the Fundamental Research Funds for the Central Universities, China (ZD2013021). The authors thank the Ansai Research Station of Soil and Water Conservation of the Chinese Academy of Sciences for conducting laboratory analysis of soil samples and for providing fieldwork support. Thanks are given to the two anonymous reviewers and editors of the journal for their valuable comments, suggestions, and revisions of this manuscript.

### References

- An S S, Zheng F L, Zhang F, van Pelt S, Hamer U, Makeshin F. 2008. Soil quality degradation processes along a deforestation chronosequence in the Ziuling area, China. *Catena*, **75**, 248-256.
- Angers D A, Samson N, L  g  re A. 1993. Changes in water stable aggregation induced by rotation and tillage in a soil under barley production. *Canadian Journal of Soil Science*, **73**, 51-59.
- Ball B C, Cheshire M V, Robertson E A G, Hunter E A. 1996. Carbohydrate composition in relation to structural stability, compactibility and plasticity of two soils in a long-term experiment. *Soil & Tillage Research*, **39**, 143-160.
- Bolinder M A, Angers D A, Gregorich E G, Carter M R. 1999. The response of soil quality indicators to conservation management. *Canadian Journal of Soil Science*, **79**, 37-45.
- Brookes P C, Landman A, Pruden G, Jenkinson D S. 1985. Chloroform fumigation and the release of soil N: a rapid



- direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology & Biochemistry*, **17**, 837-842.
- Bu X L, Ding J M, Wang L M, Yu X N, Huang W, Ruan H H. 2011. Biodegradation and chemical characteristics of hot-water extractable organic matter from soils under four different vegetation types in the Wuyi Mountains, southeastern China. *European Journal of Soil Biology*, **47**, 102-107.
- Chan K Y, Heenan D P. 1999. Microbial-induced soil aggregate stability under different crop rotations. *Biology and Fertility of Soils*, **30**, 29-32.
- Chapman P J, Shand C A, Edwards A C, Smith S. 1997. The phosphorus composition of soil solutions and soil leachates: influence of soil: solution ratio. *European Journal of Soil Science*, **48**, 703-710.
- CERN Editorial Committee. 1996. *Standard Methods for Observation and Analysis in Chinese Ecosystem Research Network*. Standards Press of China, Beijing. (in Chinese)
- Chilima J, Huang C Y, Wu C F. 2002. Microbial biomass carbon trends in black and red soils under single straw application: effect of straw placement, mineral N addition and tillage. *Pedosphere*, **12**, 59-72.
- Chodak M, Khanna P, Beese F. 2003. Hot water extractable C and N in relation to microbiological properties of soils under beech forests. *Biology and Fertility of Soils*, **39**, 123-130.
- Christou M, Avramides E J, Roberts J P, Jones D L. 2005. Dissolved organic nitrogen in contrasting agricultural ecosystems. *Soil Biology & Biochemistry*, **37**, 1560-1563.
- Cornfield A H. 1960. Ammonia released on treating soils with N sodium hydroxide as a possible means of predicting the nitrogen-supplying power of soils. *Nature*, **187**, 260-261.
- Craswell E T, Lefroy R D B. 2001. The role and function of organic matter in tropical soils. *Nutrient Cycling in Agroecosystems*, **61**, 7-18.
- Curtin D, Wright C, Beare M, McCallum F. 2006. Hot water extractable nitrogen as an indicator of soil nitrogen availability. *Soil Science Society of America Journal*, **70**, 1512-1521.
- Degens B P. 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: A review. *Australian Journal of Soil Research*, **35**, 431-460.
- Fu B J, Chen L D, Qiu Y, Wang J, Meng Q H. 2002. *Land Use Structure and Ecological Processes in the Loess Hilly Area, China*. Commercial Press, Beijing. (in Chinese)
- Ghani A, Dexter M, Perrot K W. 2003. Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilization, grazing and cultivation. *Soil Biology & Biochemistry*, **35**, 1231-1243.
- Ghani A, Sarathchandra S U, Perrott K W, Wardle D A, Singleton P, Dexter M. 1996. Spatial and temporal variability in some key biological and biochemical soil properties. *Proceedings of the New Zealand Grassland Association*, **58**, 211-218.
- Gregorich E G, Beare M H, Stoklas U, St-Georges P. 2003. Biodegradability of soluble organic matter in maize-cropped soils. *Geoderma*, **113**, 237-252.
- Gregorich E G, Janzen H H. 1996. Storage and soil carbon in the light fraction and macroorganic matter. In: Carter M R, Stewart B A, eds., *Structure and Organic Matter Storage in Agricultural Soils. Series: Advances in Soil Science*. CRC Press, Boca Raton. pp. 167-190.
- Haynes R J. 2000. Labile organic matter as an indicator of organic matter quality in a tangle and pastoral soil in New Zealand. *Soil Biology & Biochemistry*, **32**, 211-219.
- Haynes R J, Beare M H. 1997. Influence of six crop species on aggregate stability and some labile organic matter fractions. *Soil Biology & Biochemistry*, **29**, 1647-1653.
- Haynes R J, Francis G S. 1993. Changes in microbial biomass, C soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. *Journal of Soil Science*, **44**, 665-675.
- Hedin L O, Armesto J J, Johnson A H. 1995. Patterns of nutrient loss from unpolluted old growth temperate forest: Evaluation of biogeochemical theory. *Ecology*, **76**, 493-509.
- Jia S Y, Han F H, Shen Y Y. 2011. Determination of soil nitrogen availability in alfalfa pasture by chemical and bioassay test. *Prataculturae Science*, **28**, 905-909. (in Chinese)
- Jiang D. 1997. *Soil Erosion and Control Models in the Loess Plateau*. Hydroelectricity Press, Beijing, China. (in Chinese)
- Jinenez M P, Horra A M, Pruzzo L, Palma R M. 2002. Soil quality: a new index based on microbiological and biochemical parameter. *Biology and Fertility of Soils*, **35**, 302-306.
- Kranabetter J M, Dawson C R, Dunn D E. 2007. Indices of dissolved organic nitrogen, ammonium and nitrate across productivity gradients of boreal forests. *Soil Biology & Biochemistry*, **39**, 3147-3158.
- Kuzyakov Y, Ehrensburger H, Stahr K. 2001. Carbon partitioning and below-ground translocation by *Lolium perenne*. *Soil Biology & Biochemistry*, **33**, 61-74.
- Leifeld J, Kögel-Knabner I. 2005. Soil organic matter fractions as early indicators for carbon stock changes under different land-use? *Geoderma*, **124**, 143-155.
- Leinweber P, Schulten H R, Körschens M. 1995. Hot water extracted organic matter chemical composition and temporal variations in a long-term field experiment. *Biology and Fertility of Soils*, **20**, 17-23.
- Lützw M, Leifeld J, Kainz M, Kögel-Knabner I, Munch J C. 2002. Indications for soil organic matter quality in soils under different management. *Geoderma*, **105**, 243-258.
- Mariotte C A, Hudson G, Hamilton D, Neilson R, Boag B,

- Handley L L, Wishart J, Scrimgeour C M, Robinson D. 1997. Spatial variability of soil total C and N and their stable isotopes in an upland Scottish grassland. *Plant Soil*, **196**, 151-162.
- McGill W B, Canon K R, Robertson J A, Cook F D. 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. *Canadian Journal of Soil Science*, **66**, 1-19.
- Neff J C, Hobbie S E, Vitousek P M. 2000. Nutrient and mineralogical control on dissolved organic C, N, and P fluxes and stoichiometry in Hawaiian soils. *Biogeochemistry*, **51**, 283-302.
- Puget P, Angers D A, Chenu C. 1999. Nature of carbohydrates associated with water-stable aggregates of two cultivated soils. *Soil Biology & Biochemistry*, **31**, 55-63.
- Safarik I, Santruckova H. 1992. Direct determination of total soil carbohydrate content. *Plant Soil*, **143**, 109-114.
- Schoenau J J, Bettany J R. 1987. Organic matter leaching as a component of carbon, nitrogen, phosphorus and sulfur cycles in a forest, grassland and gleyed soil. *Soil Science Society of America Journal*, **51**, 646-651.
- Sharpley A N, Smith S J, Jones O R, Berg W A, Coleman G A. 1992. The transport of bioavailable phosphorus in agricultural runoff. *Journal of Environmental Quality*, **11**, 247-251.
- Shi X M, Li X G, Long R J, Singh B P, Li Z T, Li F M. 2010. Dynamics of soil organic carbon and nitrogen associated with physically separated fractions in a grassland-cultivation sequence in the Qingshai-Tibetan plateau. *Biology and Fertility of Soils*, **46**, 103-112.
- Six J, Elliott E T, Paustian K, Doran J W. 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. *Soil Science Society of America Journal*, **62**, 1367-1377.
- Spaccini R, Zena A, Igwe C A, Mbagwu J S C, Piccolo A. 2001. Carbohydrates in water-stable aggregates and particle size fractions of forested and cultivated soils in two contrasting tropical ecosystems. *Biogeochemistry*, **53**, 1-22.
- Sparling G P, Vojvodić-vuković M, Schipper L A. 1998. Hot water soluble C as simple measure of labile soil organic matter: the relationship with microbial biomass C. *Soil Biology & Biochemistry*, **30**, 1469-1472.
- Uchida Y, Nishimurab S, Akiyama H. 2012. The relationship of water-soluble carbon and hot-water-soluble carbon with soil respiration in agricultural fields. *Agriculture, Ecosystems and Environment*, **156**, 116-122.
- Verstraeten L M J, Vlassak K, Livens J. 1970. Factors affecting the determination of available soil nitrogen by chemical methods. I. Comparison of extractable with mineralized nitrogen. *Soil Science*, **110**, 299-305.
- Wang Q K, Wang S L. 2007. Soil organic matter under different forest types in Southern China. *Geoderma*, **142**, 349-356.
- Wu J, Joergensen R G, Pommerening B, Chaussod R, Brookes P C. 1990. Measurement of soil microbial biomass C by fumigation-extraction: an automated procedure. *Soil Biology & Biochemistry*, **22**, 1167-1169.
- Xu Q F, Xu J M. 2003. Changes in soil carbon pools induced by substitution of plantation for native forest. *Pedosphere*, **13**, 271-278.
- Zheng F, He X, Gao X, Zhang C E, Tang K. 2005. Effects of erosion patterns on nutrient loss following deforestation on the Loess Plateau of China. *Agriculture Ecosystems & Environment*, **108**, 85-97.

(Managing editor SUN Lu-juan)