Plant Soil (2017) 417:217–229
DOI 10.1007/s11104-017-3253-6

REGULAR ARTICLE

Effects of elevated nitrogen and precipitation on soil organic nitrogen fractions and nitrogen-mineralizing enzymes in semi-arid steppe and abandoned cropland

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Received: 21 October 2016 / Accepted: 5 April 2017 / Published online: 12 April 2017

Abstract
Aims Soil organic nitrogen (N) turnover is significantly influenced by elevated N deposition, precipitation and human-caused disturbances, but the underlying mechanism remains unclear. Identifying the relationships among the soil organic N fractions and N-mineralizing enzymes activities may advance our knowledge of the dynamics of soil organic N.
Methods A field experiment was conducted in a semi-arid steppe and an abandoned cropland in northern China to investigate the effects of elevated N deposition and precipitation on soil organic N fractions and their relationships with N-mineralizing enzymes, i.e., protease, amidase, urease and *N*-acetyl-β-D-glucosaminidase (NAG) activities.
Results The concentrations of N in various fractions were consistently lower in the abandoned cropland compared with the steppe. Nitrogen addition consistently decreased amino acid N content and activities of urease, protease and amidase in both sites but increased amino sugar N content and NAG activity in the steppe. Water addition decreased hydrolysable ammonium N content but increased amino sugar N content and activities of protease and NAG in both sites. Furthermore, urease and NAG activities were significantly positively correlated with the proportions of amino acid N and amino sugar N and, explained significant proportions of the variations in soil organic N fractions in the steppe. However, soil organic carbon (C), rather than N-mineralizing enzymes, explained greatest proportion of the variations in soil organic N fractions in the abandoned cropland.
Conclusions The concurrent increase of N deposition and precipitation could promote the recovery of soil N (and C) losses in the abandoned cropland resulting from previous agriculture. Furthermore, in the steppe where NH_4^+ was available at relative high concentrations, enzymatic mineralization was the dominant route involved in potential soil organic N turnover. However, the direct route may be favored over the enzymatic mineralization route with decreasing availability of C relative to N in the abandoned cropland, which is driven by the need for C. These findings confirmed that the forms of N available, and the relative availability of C and N determine N uptake pathways both through enzymatic mineralization route and direct uptake route in the semi-arid grasslands.

Responsible Editor: Klaus Butterbach.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-017-3253-6) contains supplementary material, which is available to authorized users.

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Keywords Nitrogen utilization · Land use · Nitrogen deposition · Increasing precipitation · Temperate grasslands

Introduction

Grasslands are the dominant landscape in China and cover over 40% of the nation's land surface, playing a vital role in serving environmental health and regional economy (Kang et al. 2007). As a main limiting factor for plant growth and net primary productivity, soil nitrogen (N) availability and its responses to elevated atmospheric N deposition, precipitation, and land use changes, are crucial for grassland ecosystem functions and stability (Xu et al. 2015a, b). As over 90% of soil N is present in organic forms, the mineralization rate of the soil organic N pool primarily determines soil N availability, as well as ecosystem N cycling (Stevenson 1982). There is however a lack of information on soil organic N turnover in grassland ecosystems under global climate changes.

The depolymerization and mineralization of organic N in soils is a sequence of microbial enzymatic processes (Mengel 1996). Most important enzymes involved are protease for the depolymerization of protein, amidase and urease for releasing ammonia from linear amides, *N*-acetyl-β-D-glucosaminidase (NAG) for the degradation of chitin and other β-1,4-linked glucosamine polymers (Mengel 1996; Kandeler et al. 2011). N-mineralizing enzyme activities therefore, have been widely used to predict N mineralization in response to changes in management practices or environmental conditions (e.g., cultivation, fertilization and rain events) (Kandeler et al. 2011; Landi et al. 2011). For example, it has been shown that intensive cultivation systems generally decreases protease and urease activities compared with undisturbed soils (Caravaca et al. 2002; Landi et al. 2011), which was contributed to the decreased microbial activities and soil organic matter content through tillage and soil disturbance. Other studies have reported that environmental change like N deposition or N addition can result in both increase or decrease of soil urease activities (Ajwa 1999; Saiya-Corka et al. 2002). Additionally, Bell and Henry (2011) reported that extracellular enzyme activities increase in response to water addition, which may be accounted for by immobilization of enzymes by soil organic matter. However, as organic N substrates in various fractions differ in their potentials for degradation or mineralization (Mengel 1996; Qiu et al. 2012), mechanisms in organic N mineralization will not be properly elucidated until more attention is paid to different organic N fractions involved in N turnover.

According to the classic methods (Stevenson 1982), soil organic N can be divided into total hydrolysable N and acid insoluble N, with total hydrolysable N comprising hydrolysable ammonium N, amino acid N, amino sugar N and hydrolysable unidentified N. Numerous studies have been conducted to investigate the dynamic patterns in the soil organic N pools in response to changes of environment conditions or land use, most of them are carried out in agricultural ecosystems (Xu et al. 2003; Nannipieri and Eldor 2009; Spargo et al. 2012; Lü et al. 2013). For example, Gonzalez-Prieto and Carballas (1991) and Gonzalez-Prieto et al. (1997) have reported that cultivation decrease the proportion of the soil N as amino acid N compared to adjacent virgin soils, they concluded that amino acid N could be considered as an active N pools potentially available for plants. Under intensively managed systems, N derived from fertilizer was mainly transformed into amino acid N or amino sugar N (Xu et al. 2003; Lü et al. 2013). It has been reported that water availability can significantly influenced the relative abundance of different organic N pools in protected field (Ji et al. 2007). Few studies, however, have elucidated the relative abundance of different soil organic N fractions in response to environmental and land use changes in semi-arid grasslands. Given the important interactions between organic N substrates of different composition and N-mineralizing enzymes (Sinsabaugh 1994), studies that link soil organic N fractions with N-mineralizing enzymes activities will therefore shed light on the underlying mechanisms of soil organic N mineralization turnover.

In Inner Mongolia, grasslands are the dominant landscape and accounts for 78% of total grassland area in China (Kang et al. 2007). However, grasslands in this area have experienced serious land degeneration and desertification since 1950s because of over grazing and intensive farming, more than 2 million ha of steppe were converted to farmland until 1990s (Wei and Shuang 2001). Policies of returning cultivated land to grasslands since the end of last century have been imposed to prevent grasslands from further degradation. As a result, the natural steppe fenced after overgrazing and abandoned cropland succeeding after farmland abandonment are most widely distributed grassland types in northern China (Xu et al. 2015a). This has provided an opportunity to study the potential organic N turnover mechanisms of the two grassland types following disturbances. Given the atmospheric N deposition (Liu et al. 2013) and summer precipitation (Sun and Ding 2010) in this area are predicted to increase in the coming decades, studying the relationships between soil organic N fractions and N-mineralizing enzymes will greatly improve our knowledge of soil organic N turnover in response to changing environmental conditions, and help to improve practices in management and restoration of degraded grasslands.

In this study, a field experiment was conducted in a semi-arid steppe and an adjacent abandoned cropland under experimentally-elevated N deposition and precipitation to investigate (a) the effects of N and water additions on soil organic N fractions in semi-arid grasslands and (b) the relationships between soil organic N fractions and N-mineralizing enzymes activities in the steppe and abandoned cropland.

Materials and methods

Study sites and experimental design

The study sites were located in the typical temperate grassland in Duolun county (116° 17′ E, 42° 02′ N, elevation 1324 m a.s.l.), Inner Mongolia in northern China. This area is located in an agro-pastoral ecotone with a semi-arid continental monsoon climate. Mean annual precipitation is 385.5 mm and mean annual temperature is 2.1 °C, with mean monthly temperatures ranging from −17.5 °C in January to 18.9 °C in July (Xu et al. 2015b). The soil type in this area is classified as Haplic Calcisol according to the FAO classification (FAO/ISRIC/ISSS 1998).

A steppe and an adjacent abandoned cropland were selected for the present study. Both grassland systems were used for livestock (mainly including sheep, cattles, horses and donkeys) grazing freely before the abandoned cropland was converted to farmland in early 1980s (Xu et al. 2016). During the period of farming, *Sesamum indicum L., Avena chinensis, Triticum aestivum L.,* and *Fagopyrum sagittatum Gilib* were common crops in the abandoned cropland, the land was plowed and about 90 kg ha^{-1} diammonium phosphate fertilizers were applied every year. The steppe was free grazed until it was fenced in 2000, and the abandoned cropland was abandoned and fenced in the same year. Both grasslands have not been used in any form since 2000. At the beginning of the present experiment, the dominant plant species were *Artemisia frigida*, *Agropyron cristatum*, and *Stipa krylovii* in the steppe, and *Agropyron cristatum* and *Artemisia scoparia* in the abandoned cropland (Xu et al. 2010).

In April 2005, seven blocks (each 107 m × 8 m) were set up within each of the two grassland systems using a split-plot experimental design. Each block was divided into two main plots with water management (ambient precipitation and water addition) as treatments. The ambient precipitation plot without fertilizer inputs served as control. The water addition plots received a simulated precipitation of 15 mm weekly by sprinkling irrigation from June to August. A total of 180 mm water was added during each growing season from 2005 to 2013. Increased precipitation of similar magnitude has occurred during some of the previous years (e.g., 1979) and is expected to occur more frequently in the future under scenarios of global change (Sun and Ding 2010). Each main plot was divided into six subplots (each 8 m × 8 m separated by 1 m wide corridors) with two N treatments (ambient N vs. N addition with 100 kg N ha^{-1} year^{-1}) randomly assigned. While the other four subplots were different levels of N and phosphorus additions treatments which were not considered in this study, more detailed information about the experimental design has been reported by Zhang and Han (2008). Nitrogen (in the form of urea) was applied twice a year, half of which was applied in early May and the other half in late June. The amount of N addition is comparable to the estimated mean atmospheric N deposition rate in northern China (about 83 kg N ha^{-1} year^{-1}) (He et al. 2007). Each treatment was replicated seven times.

Soil sampling

Soil samples were collected in August 2013. Five randomly placed soil cores (0–10 cm depth) were taken from each subplot using a soil auger (3 cm in diameter) to form one composite sample. After removing visible plant residues and stones, each sample was homogenized and passed a 2-mm sieve. A portion of fresh soil samples were stored at 4 °C before the laboratory analysis of soil microbial biomass C (C_{mic}), N (N_{mic}) and enzyme activity assays. The rest of the soil samples were air-dried and ground for soil organic N fractionation. All the analyses were performed within two weeks after soil sampling.

Soil properties analyses

Methods for the analysis of soil organic C (SOC), total soil C (C_{tot}) and N (N_{tot}) were described in detail in previous publication (Tian et al. 2016). Briefly, SOC was determined by chemical oxidation using $K_2Cr_2O_7$ solution, C_{tot} and N_{tot} were determined using a Vario MACRO cube analyzer (Elementar Analysensysteme Vario MACRO cube, German). Soil C_{mic} and N_{mic} were determined using the chloroform fumigation extraction method (Joergensen 1996). Briefly, 10 g of field-moist soil was fumigated at room temperate with ethanol-free chloroform for 24 h. The fumigated and a non-fumigated control sample were both extracted with 40 ml 0.5 *M* K_2SO_4 and shaken at 150 rpm for 1 h before filtering. The C_{mic} was calculated as the difference in extractable C concentrations between fumigated and non-fumigated samples divided by an efficiency factor of 0.45. The N_{mic} was calculated as the difference in extractable N concentrations between fumigated and non-fumigated samples divided by an efficiency factor of 0.54. Extractable C and N were determined by a C and N analyzer (VarioTOC Analyzer, Elementar, Germany). Inorganic N (sum of NO_3^- and NH_4^+) was extracted by 1 *M* KCl (Mulvaney 1996), and determined using a colorimetrically method by an AutoAnalyser III continuous Flow Analyzer (Bran & Luebbe, Norderstedt, Germany). Soil organic N was calculated as the difference between N_{tot} and inorganic N.

Soil organic N fractionation

Organic N fractions were measured by hydrolyzing the soil sample with 6 *M* HCl in an autoclave at 15 lb./in^2 for 6 h (Stevenson 1982). Total hydrolysable N was determined by steam distillation with 10 *M* NaOH after Kjeldahl digestion of the acid hydrolysate. Amino acid N was determined by steam distillation of the hydrolysate with phosphate–borate buffer and, 5 *M* NaOH, ninhydrin powder was added to convert the amino N to ammonium N. Hydrolysable ammonium N was measured by steam distillation with 3.5% (*w*/*v*) MgO. Amino sugar N was calculated as the difference between the amounts of N liberated by steam distillation of the hydrolysate with phosphate–borate at pH 11.2 and the amounts of hydrolysable ammonium N. Hydrolysable unidentified N was calculated as the difference between total hydrolysable N and the N accounted for as (ammonium + amino acid + amino sugar)-N. Acid insoluble N was calculated as the difference between total soil N and total hydrolysable N. The distribution of N (%) in various fractions was calculated as the proportion of N_{tot}.

Soil N-mineralizing enzyme assays

Soil protease, amidase and urease activities were determined using colorimetrical methods as described by Landi et al. (2011) and Kandeler et al. (2011). For protease activity measurement, field-moist soil was incubated with sodium caseinate, and 0.1 *M* Tris (hydroxymethyl) amino methane buffer (pH 8.1) for 2 h at 50 °C. The released tyrosine was spectrophotometrically determined at 700 nm, and the activity of protease was expressed as μg tyrosine g^{-1} soil h^{-1} (Landi et al. 2011). Urease activity was determined by incubating field-moist soil with buffered urea solution (0.72 *M*) for 2 h at 37 °C (Kandeler et al. 2011). Amidase activity was measured based on determination of the ammonium released by amidase after soil was incubated with 0.5 *M* formamide substrate and 0.1 *M* sodium borate buffer (pH 8.5) for 2 h at 37 °C (Kandeler et al. 2011). The released ammonium was determined using colorimetric method at 660 nm. The activity of amidase and urease were expressed as μg NH_4^+ g^{-1} soil h^{-1} (Kandeler et al. 2011).

The activity of *N*-acetyl-β-D-glucosaminidase (NAG) was measured by a fluorimetric microplate assay using 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide as substrate, enzyme activities were determined as the rate of release of 4-methylumbelliferone (MUB) from the MUB-labeled substrate (4-MUB-*N*-acetyl-β-D-glucosaminide) (Marx et al. 2001). An aqueous soil suspension (soil:water =1:100, 1 *mM* NaN_3 was included to prevent microbial activity), 50 μl, was pipetted into a 96-well microplate together with 50 μl of acetate buffer (pH 5.0) and 100 μl of substrate solution, giving a final substrate concentration of 100 μM. Microplates were incubated for 1.5 h at 30 °C and the released MUB was determined immediately on a fluorimetric plate-reader (Tecan Infinite 200 PRO, TECAN Group Ltd., Mannedorf, Switzerland), with excitation at 360 nm and emission at 450 nm. The activity of NAG was expressed as nmol MU g^{-1} soil h^{-1}.

All measured enzyme activities were also expressed on a soil C_{mic} and soil organic N basis to obtain two different specific activity indices (Fig. S1 and S2 in the supplementary materials).

Statistical analysis

ANOVAs with split-plot design were executed separately in the steppe and abandoned cropland to determine the effects of N, water addition and their interactions on soil chemical and biological characteristics. In all the split-plot ANOVAs, water was the main plot factor, N was the subplot factor, and block was treated as a random effect. Multiple comparisons with Duncan test at the $P = 0.05$ level were performed to evaluate the differences among the experimental treatments. The correlation of soil parameters was based on the Pearson correlation coefficients. All statistical analyses mentioned above were conducted with SPSS 16.0 (SPSS, Chicago, IL, USA). Redundancy analysis (RDA) was selected to study the relationships between soil organic N fractions and soil environmental parameters using CANOCO software, because the lengths of gradient was less than 3 in the detrended correspondence analysis (DCA) (ter Braak 1988). The soil organic N variables were standardized [Log transformation $Y' = \log (A \times Y + B)$, $A = 1$, $B = 1$] before RDA. A Monte Carlo permutation test (999 permutations) was used to test the significance of first and second axes.

Results

Soil properties

The steppe showed higher C_{mic}, N_{mic}, C_{tot}, N_{tot}, SOC and NH_4^+ contents, as well as $(C{:}N)_{tot}$ ratio, compared with the abandoned cropland. Nitrogen addition resulted in significant decrease of C_{mic} and N_{mic} contents in both sites, no significant effects of N addition on C_{tot}, N_{tot}, SOC, NH_4^+ contents and $(C{:}N)_{tot}$ ratio were observed in both sites. Water addition significant increased C_{mic} content in the steppe, while C_{mic} for the water addition treatment was also higher than control in the abandoned cropland, the difference between them was not significant. Combined additions of N and water significantly decreased N_{mic} content but increased the $(C{:}N)_{mic}$ ratios in both sites, and significantly increased C_{tot} and N_{tot} contents in the abandoned cropland (Table 1).

Soil organic N fractions

The concentrations of N_{tot} and N in various fractions (ammonium N, amino acid N, amino sugar N, unidentified N and insoluble N) were consistently higher in the steppe compared with the abandoned cropland (Tables 1 and 2). The proportions of total hydrolysable N to N_{tot} were consistently lower in the steppe (74–82%) compared with the abandoned cropland (82–86%) (Fig. 1). Nitrogen addition decreased the concentrations and proportions of amino acid N in both sites (28% and 14% decrease for the steppe and abandoned cropland, respectively), although differences were only significant for the steppe. Also in the steppe, N addition significantly increased the concentrations and proportions of amino sugar N and hydrolysable unidentified N (Table 2; Fig. 1). Furthermore, water addition significantly decreased the proportions of hydrolysable ammonium N but increased the concentrations and proportions of amino sugar N both in the steppe and the abandoned

Table 1 Soil properties in the steppe and abandoned cropland under N and water additions

Land use	Management	C_{mic} mg kg^{-1}	N_{mic}	NH_4^+	C_{tot} g kg^{-1}	N_{tot}	SOC	$(C{:}N)_{mic}$	$(C{:}N)_{tot}$
Steppe	Control	197 B	30.9 A	16.8 A	18.7 A [a]	1.9 A	18.3 A [a]	6.4 C	9.6 A
	N	148 C	13.0 C	17.3 A	18.2 A [a]	1.9 A	17.7 A [a]	11.4 A	9.8 A
	W	296 A	34.4 A	15.9 A	19.6 A	2.0 A	18.0 A	8.6 BC	9.6 A
	NW	199 B	22.5 B	16.1 A	20.2 A	2.1 A	19.2 A	8.9 B	9.7 A
Abandoned cropland	Control	127 a	23.3 a	12.7 a	13.3 bc [a]	1.5 bc	12.0 a [a]	5.4 b	9.1 a
	N	80 b	11.8 b	12.8 a	12.7 c [a]	1.4 c	12.3 a [a]	6.8 ab	9.4 a
	W	166 a	20.2 ab	14.3 a	15.4 ab	1.7 b	14.0 a	8.3 ab	9.3 a
	NW	146 a	13.9 b	14.9 a	16.6 a	1.8 a	14.6 a	10.5 a	9.3 a

Upper and lower case indicated significant differences ($P < 0.05$) between treatments in the steppe and the abandoned cropland, respectively

N N addition, *W* water addition, *NW* N plus water additions

[a] Data from Tian et al. (2016)

Table 2 Concentrations of N (mg kg^{-1}) in the various fractions, and proportions (%) of amino acid or amino sugar to SOC, in the steppe and abandoned cropland soils under N and water additions

Land use	Management	Hydrolysable N				Insoluble N	Amino acid / SOC	Amino sugar / SOC
		Ammonium	Amino acid	Amino sugar	Unidentified			
Steppe	Control	385 A	870 A	175 C	111 C	407 B	4.8 A	1.0 B
	N	358 AB	628 B	233 B	299 A	341 B	3.5 B	1.3 A
	W	325 C	852 A	253 B	180 B	380 B	4.7 A	1.4 A
	NW	336 BC	878 A	297 A	169 BC	582 A	4.6 A	1.4 A
Abandoned cropland	Control	343 a	638 ab	168 b	64 b	238 b	5.3 a	1.4 a
	N	324 a	550 b	166 b	123 b	188 b	4.5 a	1.4 a
	W	327 a	752 a	223 a	104 b	241 b	5.4 a	1.6 a
	NW	337 a	710 a	213 a	289 a	334 a	4.9 a	1.5 a

Upper case and lower case indicated significant differences ($P < 0.05$) between treatments in the steppe and abandoned cropland, respectively

N N addition, *W* water addition, *NW* N plus water additions

cropland (Table 2; Fig. 1). Combined N and water additions significantly increased unidentified N in steppe and acid insoluble N contents in both sites, no significant effects of solely N or water addition on acid insoluble N contents were observed (Table 2; Fig. 1).

Soil N-mineralizing enzyme activities

Nitrogen addition significantly decreased urease activities in both sites, however, increased NAG activities in the steppe. Although the protease and amidase activities for the N addition treatment were lower compared with control, the differences between them were not significant. Water addition resulted in significant increase of protease and NAG activities in both sites, and amidase activities in the steppe (Fig. 2).

Relationships between soil organic N fractions and related soil properties

The proportions of total organic N and hydrolysable ammonium N showed significant positive and negative

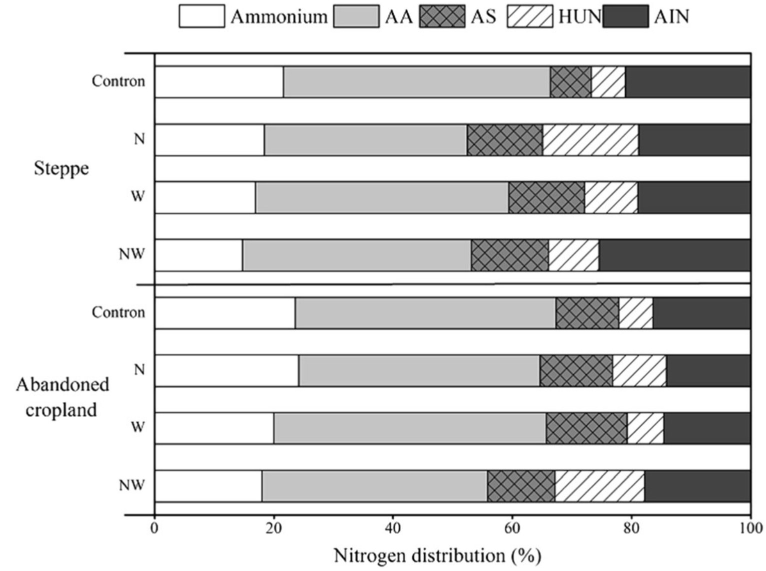

Fig. 1 Distribution (%) of soil organic N in the steppe and abandoned cropland under N and water (W) additions. *N*: N addition, *W*: water addition, *NW*: N plus water additions. *HUN*: hydrolysable unknown N, *AIN*: acid insoluble N, *AS*: amino sugar N, *AA*: amino acid N, *Ammonium*: hydrolysable ammonium N

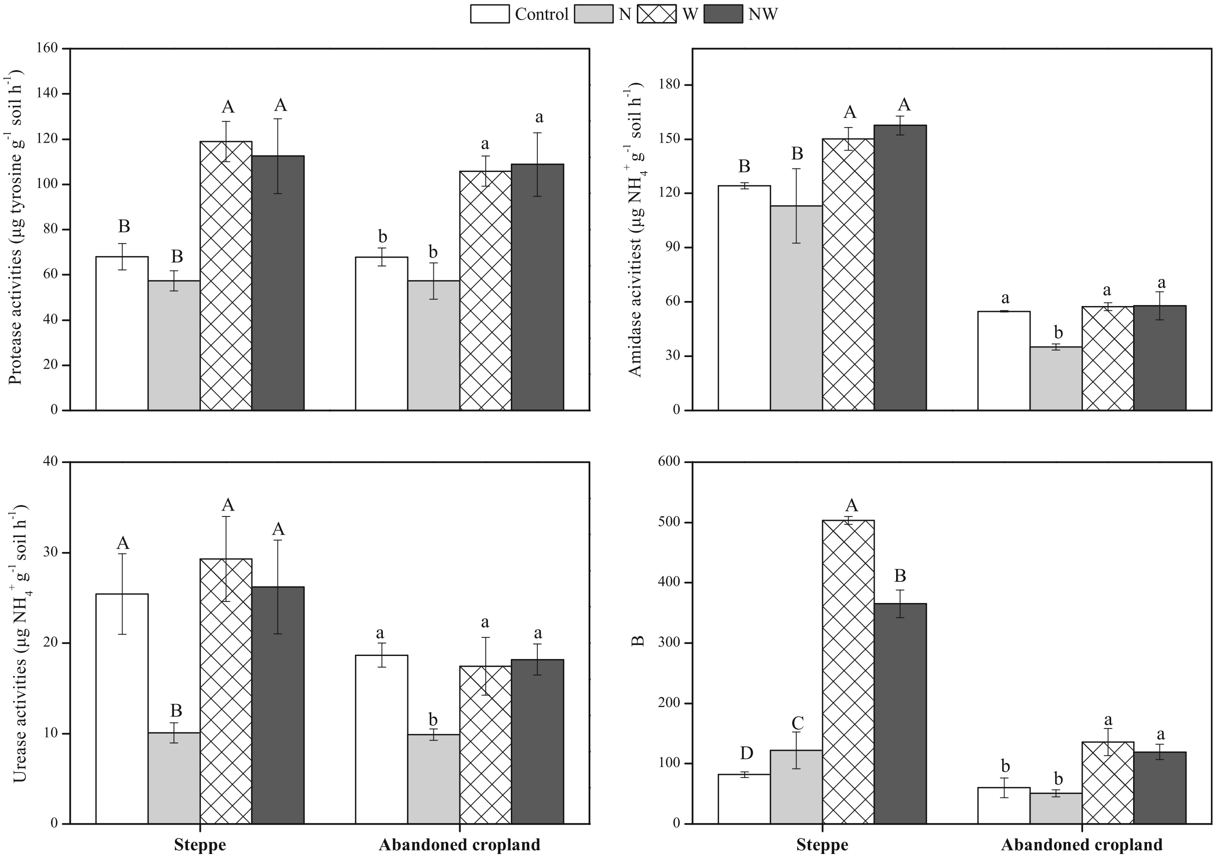

Fig. 2 Soil enzyme activities of protease, amidase, urease and *N*-acetyl glucosaminidase (NAG), in the steppe and abandoned cropland under N and water (W) additions. *N*: N addition, *W*: water addition, *NW*: N plus water additions. *Upper cases and lower cases* indicate significant difference ($P < 0.05$) of enzyme activities between treatments in the steppe and abandoned cropland soils, respectively. Error bars indicate standard deviation

correlations with all N-mineralizing enzyme (except urease and amidase in the abandoned cropland) activities, respectively. In the steppe, urease activity was significantly positively correlated with the proportions of amino acid N ($P < 0.05$) and insoluble N ($P < 0.01$), but significantly negatively correlated with the proportion of hydrolysable unidentified N ($P < 0.05$); NAG activity was significantly positively correlated with the proportion of amino sugar N ($P < 0.05$). However, no significant relationships between soil organic N fractions and N-mineralizing enzyme activities were observed in the abandoned cropland (Table 3).

Results from RDA revealed that soil NAG and urease activities explained significant proportions of the variations in organic N fractions in the steppe ($P < 0.01$) (Table 4; Fig. 3). In contrast, SOC content explained greatest proportion of the variations in organic N fractions in the abandoned cropland ($P < 0.01$), while other parameters did not (Table 4; Fig. 3). The first two axes explained 68.1 and 52.2% of the total species-environment variation in the steppe and the abandoned cropland, respectively (Fig. 3).

Discussion

Soil organic N fractions as affected by N and water additions

Previous cultivation causes markedly losses of N in various fractions ranging from 5% to 42% in the abandoned cropland. These losses can be caused by decreased plant inputs (crop removal) and accelerated decomposition of organic matter and probable erosion associated with agriculture (Standish et al. 2006). It is believed that cultivation could greatly decrease the total

Table 3 Correlations between soil organic N distribution (%) and N-mineralizing enzyme activities in the steppe and abandoned cropland

Land use		Protease	Urease	Amidase	NAG
Steppe	Ammonium N	−0.81**	−0.61*	−0.87**	−0.81**
	Amino acid N	ns	0.53*	ns	ns
	Amino sugar N	ns	ns	ns	0.54*
	Unidentified N	ns	−0.74*	ns	ns
	Insoluble N	ns	0.53**	ns	ns
	Total organic N	0.91**	0.52**	0.82**	0.91**
Abandoned cropland	Ammonium N	−0.70**	ns	ns	−0.69**
	Amino acid N	ns	ns	ns	ns
	Amino sugar N	ns	ns	ns	ns
	Unidentified N	ns	ns	ns	ns
	Insoluble N	ns	ns	ns	ns
	Total organic N	0.73**	ns	ns	0.72**

* and **: statistically significant at $P < 0.05$ and 0.01, respectively; *ns*: statistically insignificant

soil porosity due to breakup of soil aggregates, which influences the physical protection of soil organic matter and results in an increase in mineralization of soil organic matter and consequent losses of organic C and N (Balesdent et al. 1988; Evrendilek et al. 2004). However, despite the fact that the two grassland systems experienced the same situations (no land use and no removal of plant biomass) for 13 years, the abandoned cropland organic C and N pools were still not "recovered" from agricultural use. This could be explained by the fact that the residual effects of deteriorating soil structure and probable erosion can be recognized even decades after abandonment of agriculture (Standish et al. 2006). These legacies are not surprising given that the study conducted in a chronosequence of abandoned croplands suggested that recovery of soil C and N to 95% of the pre-agricultural levels is predicted to require centuries (Knops and Tilman 2000). However, it is important to note that combined N and water additions significantly increased N_{tot} and C_{tot} contents in the abandoned cropland, suggesting that concurrent increase of N deposition and precipitation in this area could promote the recovery of soil C and N pools from the residual effects of previous cultivation. This could be attributed to the stimulated root production and consequently accelerated root residue incorporation into soil organic matter (Xu et al. 2016).

The amino acid concentrations in soil were controlled by uptake and release both by microorganisms and plants (Stevenson 1982). The decrease of amino acid N content under N addition can be attributed, partly at least, to the decreased C_{mic} (Table 1). Because a large

Table 4 Statistic results of redundancy analysis (RDA) between soil organic N fractions and explanatory environmental parameters

Explanatory variables	N fractions in the steppe			N fractions in the abandoned cropland		
	F-value	*P*-value	%Explained	*F*-value	*P*-value	%Explained
Urease	**5.64**	**<0.01**	**28.7**	2.96	0.06	11.8
NAG	**9.84**	**<0.01**	**30.7**	1.01	0.32	4.1
Amidase	1.75	0.18	5.2	0.32	0.71	1.3
Protease	0.73	0.55	2.0	0.05	0.98	0.2
SOC	2.11	0.11	5.7	**7.86**	**0.01**	**36.0**

Explained variance is based on the sum of all canonical eigen values, a measurement of the explanatory power of the environment variables. *P*-values are based on a Monte-Carlo permutation test with 999 permutation, significant differences were labelled with bold

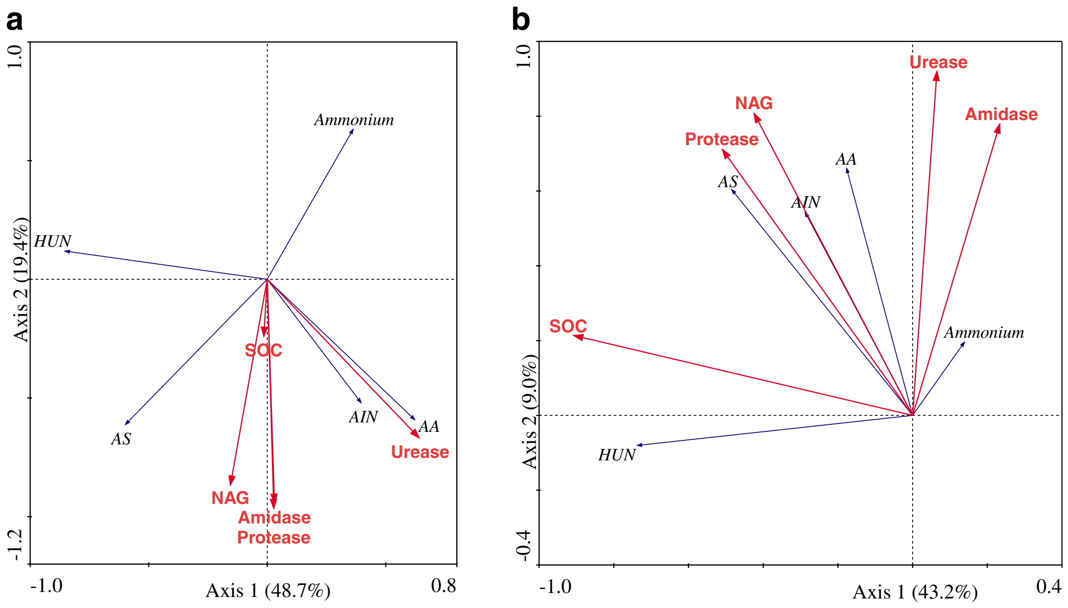

Fig. 3 Redundancy analysis (RDA) between soil N fractions and soil environmental parameters under nitrogen (N) and water (W) additions in the (**a**) steppe and (**b**) abandoned cropland. *N*: N addition, *W*: water addition, *NW*: N plus water additions. *AIN*: acid insoluble N, *HUN*: hydrolysable unknown N, *AA*: amino acid N, *AS*: amino sugar N, *NAG*: N-acetyl-β-D-glucosaminidase, *SOC*: soil organic C

proportion of amino acids in soils appear to be those present in soil microorganisms (Stevenson 1982) and, the synthesis and dynamics of amino acids in soil-plant system are closely associated with microbial metabolism (Schulten and Schnitzer 1998). In addition, the decrease of amino acid N can in part be explained by marked increases in above ground productivity by N addition (Ren et al. 2017), which may result in decrease of soil amino acid N by plant uptake. The uptake of amino acid by plants has been shown to be independent of inorganic N concentration in soil, which may significantly contribute to total N taken up by plants even in situation where fertilizer inputs are high (Jones and Darrah 1994). Furthermore, the decreased soil pH under N addition may also accelerate amino acid turnover (Table 1), as Jones and Kielland (2002) reported that amino acid turnover increased 4-fold with a drop in soil acidity of less than half a pH unit.

It is generally assumed that amino sugars in soils are derived primarily from microorganisms, and most of them come from fungi cell walls whereas little occurs in bacterial cell walls or other tissues (Olk 2008; Nannipieri and Eldor 2009). As an indicator of soil microbial community structure, the $(C{:}N)_{mic}$ ratio is often used to describe the relative contributions of bacterial and fungal cell populations to the soil microbial biomass. A high $(C{:}N)_{mic}$ ratio indicates a higher proportion of fungi in microbial biomass, whereas a low value suggests that bacteria may predominate in the microbial population (Campbell et al. 1991; Moore et al. 2000). And the (C:N)mic ratios of selected microorganisms frequently isolated from soils, and cultivated under optimal conditions, range from 7 to 12 in fungi and from 3 to 6 in bacteria (Anderson and Domsch 1980). In this study, the significantly increased amino sugar N content in the steppe by N addition may be attributed to stimulating soil fungal populations as indicated by significantly increased $(C{:}N)_{mic}$ ratio, the effect of which was non-significant in the abandoned cropland (Table 1). The stimulated fungal populations here can be explained by markedly increased above ground biomass by N addition (64% of increase in the steppe and 41% of increase in the abandoned cropland, unpublished data), as enhanced plant inputs can stimulate the fungal population and increase the fungal:bacterial ratio in soil microbial biomass (Brant et al. 2006). Similarly, the consistently increased concentrations and proportion of amino sugar N by water addition in both sites may also be attributed to the stimulating fungal populations (Table 1).

Hydrolysable ammonium N is the fraction in soil hydrolysates originated dominantly from decomposition of organic compounds as well some derived from exchangeable and clay fixed NH_4^+, which serves as a fast released and available pool of N for plant and microorganisms (Qiu et al. 2012; Lü et al. 2013). Therefore, the significantly decreased proportion of N as ammonium N under water addition (Fig. 1), could be attributed to the enhanced immobilization or uptake of mineral N by microorganisms and plants, as both soil microbial biomass and above ground biomass were significantly enhanced by water addition in both sites (Table 1) (Xu et al. 2010). Acid-insoluble N is believed to occur as a structural component of humic substances (Nannipieri and Eldor 2009), the most important sources of which are senescent materials as above and below ground detritus (Horwath 2007). The increased concentrations and proportions of acid-insoluble N by combined additions of N and water, therefore, could be explained by the consistent increase of both above ground biomass and below ground root production in both sites (Xu et al. 2015b; Xu et al. 2016). Hydrolysable unidentified N was calculated by subtracting ammonium N, amino acid N and amino sugar N from total hydrolysable N, little information is available on the nature of this fraction, the biological significance of the hydrolysable unidentified N was therefore not discussed in this study.

The potential N-mineralizing enzyme activities as affected by N and water additions

It is well established that protease, amidase, urease and NAG in soils come largely from microorganisms (Kandeler et al. 2011; Landi et al. 2011), the consistent increase of these enzyme activities (except urease) under water addition can therefore be attributed to the increased C_{mic}. In contrast, N addition significantly decreased C_{mic} in both sites, which consequently decreased protease, amidase and urease activities (Table 1, Fig. 2). However, despite the decrease of C_{mic} in the steppe, the relative abundance of fungi in soil microbial biomass may be stimulated by enhanced plant inputs under N addition (Brant et al. 2006), which is indicated by significantly increased $(C:N)_{mic}$ ratio (Table 1) (Campbell et al. 1991; Moore et al. 2000). It is widely assumed that NAG activities were mainly expressed by a diverse group of fungi (Miller et al. 1998; Muruganandam et al. 2009), the increased activities of NAG under N addition can therefore be explained by the stimulated relative abundance of fungi in soils.

Potential mechanisms involved in soil organic N turnover in the steppe and abandoned cropland

Most of the organic N in soils are in the form of polymers, which first have to be degraded into smaller units, the small organic molecules (e.g., amino acid, amino sugar etc.) can then be taken up directly (direct route), or mineralized further by extracellular enzymes and the N taken up in the mineral N (enzymatic mineralization route) (Nannipieri and Eldor 2009). In this study, the relationships between soil organic N fractions and extracellular N-mineralizing enzymes activities differed markedly between land use systems, suggesting differential potential mechanisms involved in organic N turnover. In the steppe, urease and NAG activities were significantly positively correlated with the proportions of amino acid N and amino sugar N, and explained great proportions of the variations in soil organic N fractions (Tables 3 and 4; Fig. 3). These results suggested that extracellular N-mineralizing enzymes were actively involved in potential turnover of soil organic N in the steppe. However, in the abandoned cropland, the fact that soil extracellular N-mineralizing enzymes activities were not related to organic N fractions and SOC explained greatest proportion of the variations in soil organic N fractions, suggested that the potential organic N turnover may be more C-limited compared with the steppe (Tables 3 and 4; Fig. 3).

Ammonium is considered as preferred source of N for microorganisms and plants (Merrick and Edwards 1995; Näsholm et al. 2009). Therefore, in the steppe where NH_4^+ is available at relative high concentrations (32% higher for the control compared with the abandoned cropland, Table 1), the transcription of genes encoding for enzymes systems required for the direct utilization of alternative N sources, such as organic N molecules, may be repressed (Magasanik 1993). The fact that extracellular N-mineralizing enzymes were actively involved in potential turnover of soil organic N, suggested that the enzymatic mineralization route may be favored over the direct route under these circumstances (Geisseler et al. 2009, 2010) (Tables 3 and 4; Fig. 3). In contrast, in the abandoned cropland where the NH_4^+ availability is lower, systems for the direct uptake of organic N molecules may be de-repressed and becomes relative more important. Furthermore, conversion of steppe to cultivated cropland causes long-term loss of soil organic matter, the proportionally greater loss of C than that of N, i.e., relative lower $(C:N)_{tot}$ ratios (Table 1), suggested that C may became limiting. When C becomes limiting relative to N, low molecular weight N-containing substrates, such as amino acid and amino sugar, can be taken up directly by microorganisms as C sources with intracellular deamination (Nannipieri and Eldor 2009; Geisseler et al. 2010). Additionally, when most of the available C in soils is in the form of molecules also contains N, such as amino acid and amino sugar, the direct route may even be important when inorganic N is available at high concentrations because microorganisms have to meet their C demand by taking up organic N molecules (Geisseler et al. 2010). The relative higher ratios of amino acid N and amino sugar N to SOC in the abandoned cropland (Table 2), also suggested that the direct route might be favored over the enzymatic mineralization route. Therefore, our results suggested that the relative importance of the dominant processes involved in organic N turnover is not only dependent on N availability, but also on the source of C and relative availability of C and N (Geisseler et al. 2010; Yang et al. 2016).

Conclusions

Using the long-term manipulation experiment in the semi-arid grassland, our results suggested that N addition decreased soil amino acid N, while water addition increased amino sugar N in soils. Conversion of grassland to cropland causes long-term losses of organic C and N that are not re-stablished even 13 years after abandonment. However, it is important to note that concurrent increase of N deposition and precipitation could promote the recovery of soil C and N pools from previous cultivation. Furthermore, our findings highlight that the dominant processes involved in organic N turnover differed between land use systems. In the undisturbed steppe where NH_4^+ was preferred as N source, enzymatic mineralization was the dominant route involved in potential organic N turnover. However, the direct route may be favored over the enzymatic mineralization route in the abandoned cropland with decreasing availability of C relative to N. These findings confirmed that the forms of N available, and the relative availability of C and N determine N uptake pathways both through enzymatic mineralization route and direct route in the semi-arid grasslands.

Acknowledgements This study was financially supported by the National Natural Science Foundation of China (grant numbers 41171241, 41201290, 41371251, and 31370009), the National Key Technology R&D Program of China (grant number 2012BAD14B04), and the Strategic Priority Research Program of the Chinese Academy of Sciences (grant number XDB15010403). We also thank Jiangping Cai and Shan Yang for providing soil organic matter data.

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