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Experimental nitrogen deposition alters the quantity and quality of soil dissolved organic carbon in an alpine meadow on the Qinghai-Tibetan Plateau

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ABSTRACT

Dissolved organic matter (DOM) plays a central role in driving biogeochemical processes in soils, but little information is available on the relation of soil DOM dynamics to microbial activity. The effects of NO_3^- and NH_4^+ deposition in grasslands on the amount and composition of soil DOM also remain largely unclear. In this study, a multi-form, low-dose N addition experiment was conducted in an alpine meadow on the Qinghai–Tibetan Plateau in 2007. Three N fertilizers, NH_4Cl, $(NH_4)_2SO_4$ and KNO_3, were applied at four rates: 0, 10, 20 and 40 kg N ha^{-1} yr^{-1}. Soil samples from surface (0–10 cm) and subsurface layers (10–20 cm) were collected in 2011. Excitation/emission matrix fluorescence spectroscopy (EEM) was used to assess the composition and stability of soil DOM. Community-level physiological profile (CLPP, basing on the BIOLOG Ecoplate technique) was measured to evaluate the relationship between soil DOC dynamics and microbial utilization of C resources. Nitrogen (N) dose rather than N form significantly increased soil DOC contents in surface layer by 23.5%–35.1%, whereas it significantly decreased soil DOC contents in subsurface layer by 10.4%–23.8%. Continuous five-year N addition significantly increased the labile components and decreased recalcitrant components of soil DOM in surface layer, while an opposite pattern was observed in subsurface layer; however, the humification indices (HIX) of soil DOM was unaltered by various N treatments. Furthermore, N addition changed the amount and biodegradability of soil DOM through stimulating microbial metabolic activity and preferentially utilizing organic acids. These results suggest that microbial metabolic processes dominate the dynamics of soil DOC, and increasing atmospheric N deposition could be adverse to the accumulation of soil organic carbon pool in the alpine meadow on the Qinghai-Tibetan Plateau.

1. Introduction

Dissolved organic matter (DOM) mediates important processes in the cycling of soil organic carbon (C) and the transport of nutrients such as nitrogen (N), phosphorus and sulfur (Kalbitz et al., 2003). Soil DOM mainly derives from rhizo-deposition and degradation of litter and humus, and it is consumed through biodegradation and physical leaching, related to many factors such as ecosystem types, surrounding conditions and anthropogenic disturbances, etc. (Kalbitz et al., 2003; Waldrop and Zak, 2006; Tu et al., 2011; Hagedorn et al., 2012). Soil DOM is a complex mixture consisting of various labile and recalcitrant organic substances (Michel et al., 2006), and soil microbial community preferentially uses easily degradable components of them (DeForest et al., 2004a,b). Therefore, changes in soil microbial composition and metabolic activity are expected to alter the quantity and quality of soil DOM.

As an important component of DOM, dissolved organic C (DOC) in soils represents a highly sensitive indicator for how environmental change affects SOC storage (Hagedorn et al., 2012). Nitrogen is a limiting element for microbial activity, and microbial biomass is considered as a major source of DOC (Qualls and Haines, 1992). Therefore, increasing anthropogenic N deposition can alter soil

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DOC dynamics by changing microbial decomposition and humification processes. Evidences from various terrestrial ecosystems show that simulated N deposition increases (Yano et al., 2000; Campbell et al., 2000; Sinsabaugh et al., 2004; Gallo et al., 2005; Smemo et al., 2006; Manninen et al., 2011; Currey et al., 2011), decreases (Park et al., 2002; Aitkenhead-Peterson and Kalbitz, 2005; Scheuner and Makeschin, 2005) or does not change (Emmett et al., 1998; Gundersen et al., 1998; Sjöberg et al., 2003; Cory et al., 2004; McDowell et al., 2004) soil DOC content. Instead of altering DOC production and consumption rates, exogenous N inputs to terrestrial ecosystems may lead to changes in the composition and stability of soil DOM by influencing the humification process (Aitkenhead-Peterson and Kalbitz, 2005; Michel et al., 2006; Whittinghill et al., 2012). Two mechanisms are proposed for these widely divergent results: The repression of lignin-degrading white-rot fungi by mineral N leading to an increased release of water soluble soft-rot products (Sinsabaugh et al., 2004; DeForest et al., 2005; Gallo et al., 2005), or a stimulation of C demand for N immobilization by microorganisms leading to a decrease in soil DOC (Aber, 1992; Bragazza et al., 2006; Hagedorn et al., 2012). Unfortunately, the majority of these studies are from forest ecosystems, and little information is available on the relation of soil DOC dynamics to microbial activity in the alpine meadow soils. Moreover, a single nitrogenous fertilizer is commonly applied in most N addition experiments; less is known about the different effects of NO_3^- and NH_4^+ inputs.

In general, the responses of terrestrial ecosystems to N addition depend on stages of ecosystem N saturation (Aber et al., 1998). Evidences suggest that short-term or moderate increases in N inputs promote microbial decomposition, increase plant growth and soil CO_2 emission (Knorr et al., 2005; Schlesinger, 2009; Fang et al., 2012). However, long-term or excessive addition of N may lead to a reduction in rates of nutrient cycling with possible adverse effects on plant health through soil acidification and nutrient imbalances (Mo et al., 2008; Fang et al., 2011). For the alpine meadow ecosystems on the Qinghai-Tibetan Plateau, our preliminary studies revealed that three-year, low doses of N addition treatments (<40 kg N ha^{-1} yr^{-1}) significantly increased aboveground biomass, litter decomposition, and soil CO_2 fluxes (Fang et al., 2012); however, N storage (Fang et al., 2014), CH_4 uptake and N_2O release were unaltered by N additions (Jiang et al., 2010). Moreover, the promotion to soil CO_2 emission was more significant from NH_4^+–N than from NO_3^-–N inputs, which was attributed to their opposite ion charges and the impacts on soil acidity and substrate utilization (Fang et al., 2012). These results indicate that low dose of N addition can stimulate the activity of microbial community in the N-poor alpine meadow soils. Consequently, our working hypotheses are that experimental N deposition promotes the utilization of organic substrates by soil microbial community, decreases soil DOC content and alters the composition of DOM. Also, the effects of NO_3^- and NH_4^+ inputs on the quantity and quality of DOC are different because of their opposite effects on microbial community and soil acidification.

The community level physiological profile (CLPP), based on the ability of microorganisms to oxidize different C substrates, can provide a set of structurally diverse and ecologically relevant compounds (Insam, 1997). Although the CLPP method can only determine a small minority of the microbes that is actually able to grow in the Biolog plates (Ros et al., 2008), it has been widely used to evaluate microbial community differences resulting from N addition (Zhang et al., 2008; Dalmonech et al., 2010; Gomez and Garland, 2012). Three-dimensional excitation/emission matrix fluorescence spectroscopy (EEM) is a powerful tool of organic matter fingerprinting in terrestrial and aqueous ecosystems, and is widely used to study the composition of soil DOM (Chen et al., 2003). In this study, our specific aims were: (1) to investigate the effects of N dose and form on the soil DOC concentration and DOM composition; (2) to explore the effects of N dose and form on substrate utilization by soil microbial community; (3) to clarify the relationships between soil DOC quantity and quality and microbial metabolic activity.

2. Materials and methods

2.1. Study site description

The experiment was conducted at the Haibei alpine meadow ecosystem research station, Chinese Academy of Sciences (37°37′ N, 101°19′ E). The station is located in the eastern of Qinghai-Tibetan Plateau, with an average elevation of 3280 m. The site is characterized by its plateau monsoon climate with a mean annual air temperature of −0.4 °C and mean annual precipitation of 383.3 mm. The vegetation type is a typical *Kobresia humilis* meadow. Dominant species are *Kobresia humilis*, *Saussurea superba*, *Potentilla saundersiana*, *Leontopodium nanum*, *Lancea tibetica*, *Festuca ovina*, *Festuca rubra*, *Stipa aliena*, *Elymus nutans*, *Helictotrichon tibetica*, *Koeleria cristata* and *Poa crymophila* (Fang et al., 2012). The soils developed in the Kobresia meadow are Mat-Gryic Cambisol (IUSS, 2006). The average thickness of soil profile is about 30 cm. Mean topsoil (20 cm depth) total C is 39.7 g kg^{-1}, organic C is 30.5 g kg^{-1}, total N is 5.37 g kg^{-1}, total phosphorus is 1.92 g kg^{-1}, pH is 7.5, and soil bulk density is 0.93 g cm^{-3} (Fang et al., 2012).

2.2. Experimental design

The N addition experiment is a split plot design with N doses defining the main plots and N forms as subplots. Three N fertilizers (NH_4Cl, $(NH_4)_2SO_4$ and KNO_3) were applied at 10, 20 and 40 kg N ha^{-1} yr^{-1}. A control (0 kg N ha^{-1} yr^{-1}) was set at each plot, and each N treatment had three replicates. Each subplot had an area of 9 m^2 (3 m × 3 m) and a 2-m buffer was set between subplots (Fang et al., 2012). This experiment was conducted over a span of five years beginning in May 2007 and ending in October 2011. For each subplot, N fertilizer was dissolved with 3 L of water and its solution was evenly applied using a sprayer during the first week of each month. Control subplots received equivalent applications of water only. The volume of water applied equated to ca. 4 mm of rainfall. All plots are in the winter pastures, not grazed in the growing season, and grazed in non-growing season.

2.3. Soil sampling and DOC determination

On October 15 2011, five core samples from two diagonal lines through each subplot were collected from depths of 0–10 cm and 10–20 cm using an auger (2.5 cm in diameter). Soil samples at the same layer of each subplot were immediately passed through a 2 mm sieve to remove large roots and gravel. The soil samples were then transported to the lab in chilled polystyrene boxes.

The DOM fraction of soil samples was extracted with deionized water (1:10 w/v) by shaking fresh soil samples for 24 h on a horizontal shaker at room temperature. The DOM was filtered using 0.45 μm polytetrafluoroethylene filters, and the extracts were immediately analyzed for DOC concentration using the TOC analyzer (Liqui TOCII, Elementar, Germany).

2.4. Soil DOM composition analysis

Excitation–emission matrix spectra of DOM were recorded with a fluorescence spectrophotometer (Model F-4600, Hitachi, Japan) equipped with a 150 W xenon arc lamp as the excitation source. Prior to fluorescence analysis, all samples were diluted to the uniform DOC concentration of 10 mg L^{-1} to reduce inner filter effects. Spectra were recorded using 5 nm excitation and 10 nm emission

band-pass widths, and the scan speed was 2400 nm min^{-1} (Zhang et al., 2012). The scanning wavelength range was set as: excitation wavelength (Ex) 200–400 nm, emission wavelength (Em) 300–550 nm. To avoid the scattering effects, the treatment method of the first-order Rayleigh, Raman and second-order Rayleigh scatters was applied as proposed by Bahram et al. (2006). The software MatLab 7 (MathWorks Inc., USA) was employed for handling the EEM data.

Fluorescence regional integration method was applied to compare the difference in the DOM composition elicited by N addition (Chen et al., 2003). Each EEM was divided into five zones based on its excitation and emission wavelength boundaries as follows: zone I (tyrosine-like, Ex < 250 nm, Em < 330 nm), zone II (tryptophan-like, Ex < 250 nm, Em 330-380 nm), zone III (fulvic acid-like, Ex < 250 nm, Em > 380 nm), zone IV (soluble microbial byproduct-like, Ex > 250 nm, Em < 380 nm), and zone V (humic acid-like, Ex > 250 nm, Em > 380 nm; Zhang et al., 2012). The cumulative area beneath each excitation–emission zone was calculated using MetLab 7 software. By normalizing the cumulative excitation–emission areas to relative regional areas (nm^2), the normalized excitation–emission area ($U_{i,n}$ and $U_{T,n}$, referring to the value of region i and entire region) and the percent fluorescence response ($P_{i,n} = U_{i,n}/U_{T,n}$) were calculated (Zhang et al., 2012). To express the complexity and condensation of the molecules, the humification index (HIX) deduced from emission spectra was calculated as the area in the upper quarter ($\sum$ 380–550 nm) of the usable emission spectra divided by the area in the lower usable quarter ($\sum$ 300–380 nm) (Kalbitz and Geyer, 2001).

2.5. Utilization of organic substrates

Because almost all the roots concentrate in 0–20 cm depth, and the C substrates utilized by microbial community are mainly originated from root residues in the grazing alpine meadow. We assumed that microbial metabolic activity in 0–20 cm soil layer consistently responded to N addition. On October 23 2011, soil sample in 0–20 cm depth at each subplot was collected to evaluate soil microbial metabolic activity. Community level physiological profiles were established using BIOLOG EcoPlate™ (BIOLOG Inc., CA, USA) (Garland and Mills, 1991; Insam, 1997). Briefly, 10 g of fresh soil was added to 100 ml of distilled water in a 250-ml flask and shaken for 10 min. The samples were serially diluted to 10^{-3} based on a pilot experiment, and 150 μl of each sample was inoculated into each well. The plates were incubated at 25 °C, and color development in each well was recorded as optical density (OD) at 590 nm with a plate reader (Thermo Scientific Multiskan MK3, USA) over a 7-day period (24, 48, 72, 96, 120, 144, and 168 h). The 72 h absorbance values were used to calculate average well color development (AWCD) (Bucher and Lanyon, 2005). Microbial metabolic activity in each microplate, expressed as AWCD, was determined as follows: AWCD = $\sum OD_i/31$, where OD_i is the optical density value from each well. Negative OD values were set to zero. Also, the 31 substrates in each plate can be divided into six groups: carbohydrates, polymers, miscellaneous, carboxylic acids, amino acids, and amines (Benizri and Amiaud, 2005).

2.6. Data analysis

We used a nested analysis of variance (ANOVA) to evaluate the effects of N doses and N forms on soil DOC concentration, $P_{i,n}$, HIX of DOM, AWCD, and OD values of each substrates. Differences in DOC concentration, DOM composition and microbial utilization of C substrates between nine N addition treatments and control were assessed by one-way ANOVA with a Fisher's least significant difference (LSD) test. Considering the autocorrelation of five DOM components, ridge regression was used to analyze the relationships between the net change of DOC concentration between control and N addition treatment (ΔDOC) and the net change of percentage of each DOM component between control and N addition treatment ($\Delta P_{i,n}$) in the two soil layers. Simple linear regression was used to elucidate the relationships between soil DOC concentration and microbial metabolic activity (AWCD), and between HIX of DOM and microbial metabolic activity (AWCD). Also, to clarify which C resources were highly used when HIX of DOM changed, we used principle regression to explore the relationships between HIX of DOM and microbial utilization of six C resources.

3. Results

3.1. Soil DOC concentration

In the control treatment, soil DOC concentrations in 0–10 cm and 10–20 cm layers averaged 174.15 mg kg^{-1} and 204.62 mg kg^{-1}, respectively (Table 1). Nested ANOVA results showed that N dose rather than N form significantly changed soil DOC concentrations in both 0–10 cm and 10–20 cm layers (Table 2, $P = 0.017$, $P < 0.001$). Compared with the control, N addition tended to increase the concentrations of soil DOC in 0–10 cm layer by 23.5%–35.1%, but consistently decreased the concentrations of soil DOC in 10–20 cm layer by 10.4%–23.8% (Table 1).

3.2. DOM fluorescence characteristics

Three-dimensional EEM spectra were obtained by simultaneously scanning of the emission and excitation spectra. Two distinct fluorescence peaks were observed for all the DOM samples in 0–10 cm and 10–20 cm layers (Figs. 1 and 2). The emission wavelength of the peaks occurred in the visible region, ranging from 420 to 480 nm, whereas the excitation wavelength occurred in the UV region, ranging from 250 to 300 nm and 300 to 350 nm, respectively (Fig. 1 and Fig. 2). The fluorescence of five zones in soil DOM was related to tyrosine-like, tryptophan-like, fulvic acid-like, soluble microbial byproduct-like and humic acid-like materials. In the control treatment, most of soil DOM in 0–10 cm layer was soluble microbial byproduct-like ($P_{4,n}$) and humic-like ($P_{5,n}$) materials, accounting for 52.59% and 41.55% of DOM, respectively (Table 1). The percentages of these two fractions ($P_{4,n}$ and $P_{5,n}$) in 10–20 cm soil layer were 57.33% and 36.0%, respectively (Table 1). The other three fractions only accounted for 7% or less (Table 1).

The various doses of N addition significantly changed the composition of soil DOM in 0–10 cm and 10–20 cm layers, while the forms of N fertilizers had no effects on them (Table 2). Compared with the control, N addition tended to increase the intensity of fluorescence peaks (Ex/Em = 250–300 nm/420–480 nm and 300–350 nm/420–480 nm) in 0–10 cm layer, whereas an opposite response was found in 10–20 cm layer (Figs. 1 and 2). For 0–10 cm soil layer, N addition tended to decrease the percentages of tyrosine-like ($P_{1,n}$), tryptophan-like ($P_{2,n}$) and humic-like ($P_{5,n}$) materials, whereas increased those of fulvic acid-like ($P_{3,n}$) and soluble microbial byproduct-like ($P_{4,n}$) materials (Table 1). On the contrary, for 10–20 cm soil layer, N addition tended to increase the percentages of tyrosine-like ($P_{1,n}$), tryptophan-like ($P_{2,n}$) and humic acid-like ($P_{5,n}$) materials, whereas decreased those of fulvic acid-like ($P_{3,n}$)and soluble microbial byproduct-like ($P_{4,n}$) materials (Table 1). However, both N dose and N form did not change the HIX of soil DOM in 0–10 cm and 10–20 cm layers (Tables 1 and 2).

3.3. Relationships between DOC and DOM components

To evaluate the relative contribution of each DOM component to the change in soil DOC elicited by N addition, we

Table 1
The concentration of soil DOC, the percentage of each DOM component, and the HIX of DOM in different treatments in surface and subsurface layers. Data are shown as means with standard errors.

Soil layer	Treatment	Soil DOC[a] concentration (mg kg^{-1})	Percentage of fluorescence response ($P_{i,n}$, %)[a,b]					HIX of DOM[a]
			Zone I	Zone II	Zone III	Zone IV	Zone V	
0–10 cm	Control	174.15 (4.74)	0.46 (0.05)	1.07 (0.07)	4.36 (0.20)	52.59 (1.46)	41.55 (1.28)	27.27 (1.48)
	Low-NH_4Cl	220.75* (26.00)	0.35 (0.04)	0.79* (0.10)	5.40* (0.54)	59.50* (1.58)	35.14* (1.24)	31.51 (3.19)
	Low-$(NH_4)_2SO_4$	190.16 (4.73)	0.32 * (0.09)	0.76* (0.22)	4.55 (0.34)	58.73* (1.19)	35.49* (0.89)	31.60 (3.26)
	Low-KNO_3	235.25* (7.70)	0.35 (0.04)	0.81* (0.10)	5.09 (0.31)	57.69* (1.41)	35.97* (0.94)	31.73 (3.69)
	Medium-NH_4Cl	232.95* (28.01)	0.36 (0.07)	0.84 (0.13)	5.21* (0.66)	53.16 (3.57)	40.43 (2.74)	35.09 (4.40)
	Medium-$(NH_4)_2SO_4$	208.26 (26.26)	0.35 (0.04)	0.86 (0.15)	5.23* (0.66)	57.60* (3.10)	36.50* (2.16)	31.14 (3.50)
	Medium-KNO_3	230.72* (34.15)	0.32 * (0.03)	0.74 * (0.09)	5.39* (0.44)	58.38* (1.00)	36.05* (0.50)	34.99 (3.53)
	High-NH_4Cl	214.99* (20.17)	0.33* (0.02)	0.77* (0.04)	5.13 (0.28)	57.52* (0.76)	36.71* (0.71)	33.51 (1.23)
	High-$(NH_4)_2SO_4$	201.26 (9.73)	0.33* (0.01)	0.77* (0.02)	4.90 (0.21)	57.97* (1.21)	36.23* (1.17)	32.80 (0.62)
	High-KNO_3	226.31* (30.08)	0.35 (0.01)	0.81* (0.04)	4.87 (0.07)	57.95* (0.91)	35.70* (0.54)	30.75 (1.28)
10–20 cm	Control	204.62 (8.41)	0.30 (0.02)	0.75 (0.06)	5.62 (0.28)	57.33 (0.87)	36.00 (0.62)	36.48 (2.25)
	Low-NH_4Cl	175.99* (8.71)	0.31 (0.04)	0.76 (0.12)	4.22* (0.21)	55.07 (1.40)	39.73 (1.24)	38.45 (4.49)
	Low-$(NH_4)_2SO_4$	183.33* (9.20)	0.34 (0.06)	0.81 (0.15)	4.39* (0.33)	55.65 (1.97)	38.56 (1.14)	35.12 (4.43)
	Low-KNO_3	155.97* (4.30)	0. 43 * (0.06)	0.94* (0.04)	4.42* (0.40)	52.53* (0.75)	41.19* (0.85)	30.40 (2.52)
	Medium-NH_4Cl	167.44* (2.63)	0.36 (0.05)	0.94* (0.16)	4. 97 a (0.61)	52.08 * (3.13)	41.42 * (2.38)	32.89 (3.65)
	Medium-$(NH_4)_2SO_4$	167.69* (12.22)	0.36 (0.07)	0.93* (0.20)	5.23 (0.78)	53.47 (2.88)	40.00 (1.84)	33.19 (5.25)
	Medium-KNO_3	166.66* (5.46)	0.38 (0.04)	0.92* (0.10)	4. 86 (0.50)	49.78* (3.07)	43.34* (2.24)	33.73 (1.71)
	High-NH_4Cl	176.99* (7.68)	0.36 (0.08)	0.87 (0.17)	5.06 (0.60)	52.54* (2.43)	41.06* (1.76)	35.18 (4.83)
	High-$(NH_4)_2SO_4$	174.47* (4.63)	0.34 (0.06)	0.94* (0.15)	4. 45* (0.44)	51.18 * (3.17)	41.96* (2.67)	34.05 (4.33)
	High-KNO_3	162.78* (11.16)	0.35 (0.05)	0.94* (0.18)	5.42 (0.68)	50.91* (3.45)	42.19* (2.52)	34.46 (4.83)

[a] Asterisk (*) means significant difference between N treatments and control.
[b] Zone I to zone V refers to tyrosine-like ($P_{1,n}$), tryptophan-like ($P_{2,n}$), fulvic acid-like ($P_{3,n}$), soluble microbial byproduct-like ($P_{4,n}$) and humic acid-like ($P_{5,n}$) materials, respectively.

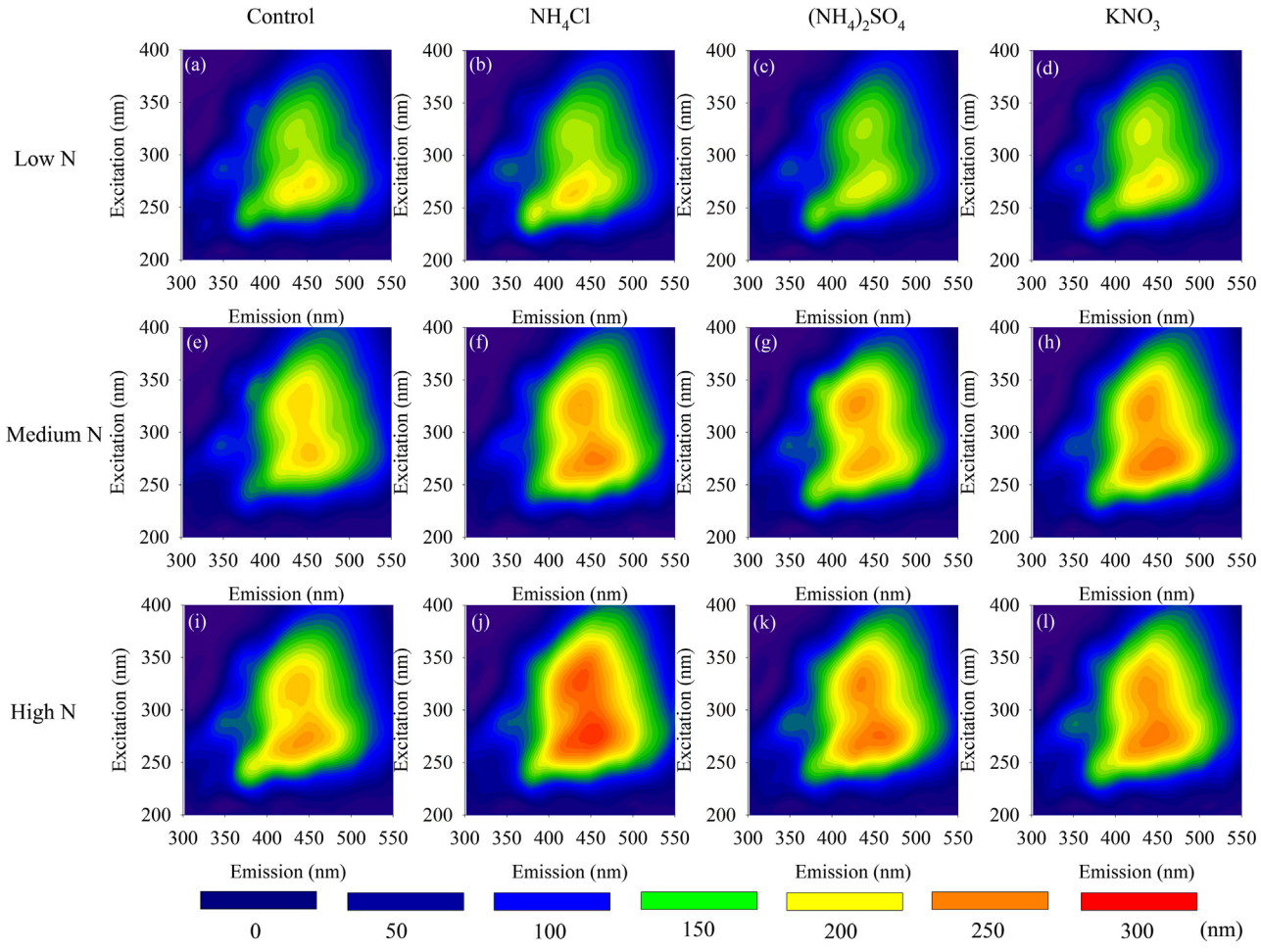

Fig. 1. Three-dimensional EEM spectra of DOM at various N dose and N form treatments in 0–10 cm soil layer.

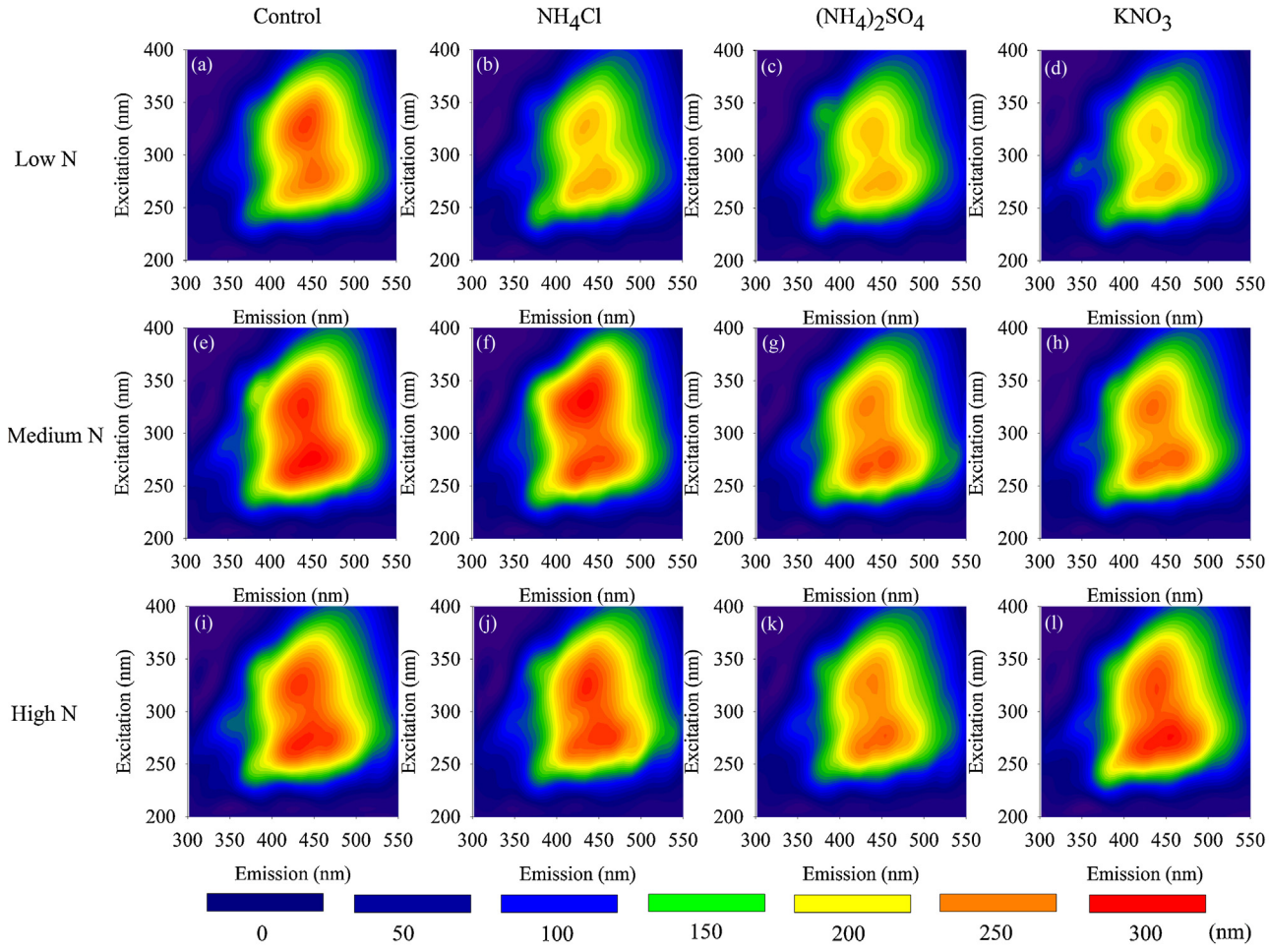

Fig. 2. Three-dimensional EEM spectra of DOM at various N dose and N form treatments in 10–20 cm soil layer.

performed a ridge regression analysis of the data. For the 0–10 cm and 10–20 cm soil samples, the change in soil DOC concentration (ΔDOC) was negatively correlated with the changes of tyrosine-like, tryptophan-like and humic acid-like materials, and positively related to those of fulvic acid-like and soluble microbial byproduct-like materials (R^2 = 0.34-0.51, P < 0.001, Fig. 3). Excluding the autocorrelation among the five components of DOM, ridge regression results demonstrated that the contribution of each component to DOC change was in decreasing order: fulvic acid-like > tryptophan-like > humic acid-like > soluble microbial byproduct-like > tyrosine-like materials (Fig. 3).

3.4. Pattern of microbial substrate utilization

Microbial metabolic activity, as reflected by AWCD, showed a significant response to the dose of N addition, and AWCD was higher in the medium N treatment than in the other treatments (Tables 3 and 4, P = 0.032). However, the effects of N forms on AWCD under the same N dose were not significant (Table 4). For the types of sole C substrates, N addition dose, rather than N form, significantly changed the utilization intensity of carbohydrates, amino acids and polymers by soil microbial communities (Tables 3 and 4, P = 0.029, P = 0.043 and P = 0.037). Moreover, the

Table 2
Results of nested ANOVA on the effects of N dose and N form on soil DOC content and DOM composition in surface and subsurface layers.

Soil layer	Source of variation	Soil DOC concentration	Percentage of fluorescence response ($P_{i,n}$)[a]					HIX of DOM
			Zone I	Zone II	Zone III	Zone IV	Zone V	
0–10 cm	Block	0.15	0.032	0.004	0.002	0.035	0.16	0.052
	Level	0.017	0.017	0.002	0.025	0.008	0.003	0.22
	Form (Level)	0.58	0.99	0.97	0.74	0.66	0.56	0.71
10-20 cm	Block	0.015	<0.001	<0.001	<0.001	<0.001	0.005	<0.001
	Level	<0.001	0.027	0.003	<0.001	0.003	0.001	0.19
	Form (Level)	0.43	0.17	0.46	0.21	0.64	0.52	0.13

[a] Zone I to zone V refers to tyrosine-like ($P_{1,n}$), tryptophan-like ($P_{2,n}$), fulvic acid-like ($P_{3,n}$), soluble microbial byproduct-like ($P_{4,n}$) and humic acid-like ($P_{5,n}$) materials, respectively.

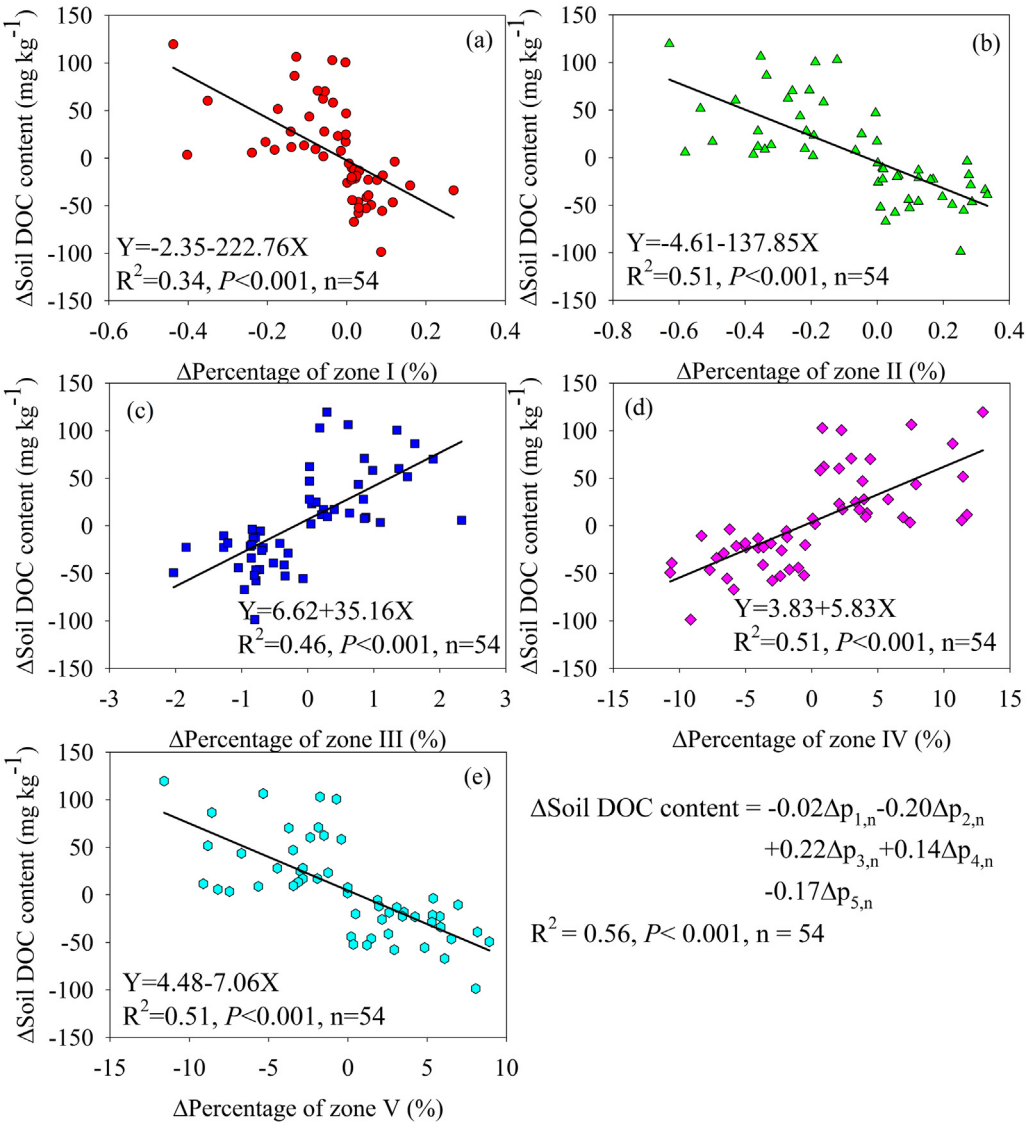

Fig. 3. Relationships between changes of soil DOC content (Δ Soil DOC content) and changes of each DOM component's percentage (Δ Percentage of zone i, $\Delta P_{i,n}$) in 0–10 cm and 10–20 cm soil layers. Zone I to zone V refers to tyrosine-like ($P_{1,n}$), tryptophan-like ($P_{2,n}$), fulvic acid-like ($P_{3,n}$), soluble microbial byproduct-like ($P_{4,n}$) and humic acid-like ($P_{5,n}$) materials, respectively.

Table 3
The microbial metabolic activity (AWCD) and microbial utilization of C substrates in different treatments in 0–20 cm soil layer. Data are shown as means with standard errors.

Treatment	AWCD[a]	Intensity of substrates utilization[a,b]					
		Group I	Group II	Group III	Group IV	Group V	Group VI
Control	0.75 (0.05)	6.01 (0.77)	3.96 (0.35)	1.45 (0.14)	5.53 (0.28)	5.06 (0.34)	1.17 (0.07)
Low-NH_4Cl	0.79 (0.11)	7.19 (1.67)	3.92 (0.43)	1.51 (0.33)	5.65 (0.59)	4.92 (0.40)	1.19 (0.16)
Low-$(NH_4)_2SO_4$	0.71 (0.05)	5.85 (0.70)	3.67 (0.29)	1.60 (0.22)	5.25 (0.34)	4.47 (0.21)	1.08 (0.04)
Low-KNO_3	0.75 (0.04)	6.39 (0.13)	3.90 (0.34)	1.17 (0.06)	5.94 (0.50)	4.82 (0.27)	1.08 (0.15)
Medium-NH_4Cl	0.84 (0.10)	7. 21 (1.44)	4.83 (0.83)	1.29 (0.26)	5.97 (1.17)	5.65 (0.89)	1.13 (0.13)
Medium-$(NH_4)_2SO_4$	0.96 * (0.04)	9.29 * (0.40)	5.15 * (0.53)	1.79 (0.09)	5.93 (0.61)	6.49 * (0.44)	1.06 (0.08)
Medium-KNO_3	0.95 * (0.02)	8.31 (0.31)	4.96 (0.48)	1.69 (0.04)	5.78 (0.20)	5.04 (0.19)	1.12 (0.11)
High-NH_4Cl	0.87 (0.13)	9.20 * (2.01)	4.67 (0.78)	1.95 * (0.16)	6.31 (0.34)	6.07 * (0.65)	1.02 (0.17)
High-$(NH_4)_2SO_4$	0.74 (0.03)	6.30 (0.56)	3.80 (0.33)	1.66 (0.10)	5.31 (0.18)	4.63 (0.37)	1.19 (0.10)
High-KNO_3	0.71 (0.10)	6.32 (0.93)	3.53 (0.52)	1.77 (0.28)	4.83 (0.80)	4.33 (0.62)	1.19 (0.11)

[a] Asterisk (*) means significant difference between N treatments and control.
[b] Group I to group VI refers to carbohydrates, polymers, miscellaneous, carboxylic acids, amino acids and amines, respectively.

Table 4
Results of nested ANOVA on the effects N dose and N form on microbial metabolic activity and utilization of C substrates in 0–20 cm soil layer.

Source of variation	AWCD value	Intensity of substrates utilization[a]					
		Group I	Group II	Group III	Group IV	Group V	Group VI
Block	0.074	0.18	0.010	0.06	0.064	0.17	0.28
Level	0.032	0.029	0.043	0.29	0.39	0.037	0.81
Form (Level)	0.27	0.32	0.59	0.24	0.23	0.10	0.87

[a] Group I to group VI refers to carbohydrates, polymers, miscellaneous, carboxylic acids, amino acids and amines, respectively.

increase from medium N was the strongest, comparing control and other N dose treatments (Table 3).

3.5. Relationships between DOC dynamics and microbial metabolic activity

The relationships between the quantity and quality of soil DOC and soil microbial metabolic activity were completely opposite in surface and subsurface soil layers. Soil AWCD in 0–20 cm layer was positively correlated with soil DOC content (Fig. 4a, $R^2 = 0.38$, $P < 0.001$) and HIX of DOM in 0–10 cm layer (Fig. 4b, $R^2 = 0.13$, $P = 0.036$). However, significant and negative relationships were observed between soil AWCD in 0–20 cm layer and soil DOC content in 10–20 cm layer (Fig. 4c, $R^2 = 0.24$, $P = 0.003$) and between soil AWCD in 0–20 cm layer and HIX of DOM in 10–20 cm layer (Fig. 4d, $R^2 = 0.45$, $P < 0.001$). The principle regression analysis showed that the microbial utilization of carboxylic acids, amino acids and amines had positive effects on HIX of DOM, and the importance was in decreasing order: carboxylic acids > amines > amino acids (Fig. 5).

4. Discussion

4.1. N addition effects on soil DOC quantity

Nitrogen addition significantly increased the concentration of soil DOC in surface layer, but consistently decreased the concentration of soil DOC in subsurface layer (Table 1). This result indicated that the responses of soil DOC to N addition changed with soil layer. Using ^{13}C tracking method, Hagedorn et al. (2012) also reported that leaching of DOC from the litter layer was not affected by NH_4NO_3 additions, but DOC fluxes from the mineral soils at 5 and 10 cm depths were significantly reduced by 17%. The divergent responses in surface and subsurface layers can be attributed to the following two aspects. First, N addition can increase aboveground biomass and subsequent litter return (Fang et al., 2012); moreover, most of roots (> 90%) in the alpine meadow mainly concentrates in 0–10 cm depth (Wu et al., 2011). Generally, DOC mainly derives from litter degradation and rhizo-deposition, and the percentage of DOC below organic layer derived from degradation of humus may increase (Steinbeiss et al., 2008; Tu et al., 2011). Inorganic N enrichment directly suppresses the activity of lignin-degrading fungi, and thereby increases release of water soluble products (DeForest et al., 2005). Furthermore, inorganic N enrichment can stimulate the activity of some lignocellulytic actinobacteria (Giroux et al., 1988). Although these organisms cannot completely metabolize lignin to CO_2, they produce soluble polyphenolics as end products of lignocellulolytic metabolism (Berrocal et al., 1997). These would promote the release of soil DOC production and release. Second, N addition decreases the root/stem ratio (Cheng et al., 2009; Amanullah and Stewart, 2013), which decreases the return of C substrates in subsurface layer. A global synthesis analysis shows that N addition significantly decreases the root/shoot ratio by 14.5%, resulting in no significant changes in C storage of both organic horizon and mineral soil in forests and grasslands (Lu et al., 2011). Therefore, litter-rich surface soils and subsurface mineral soils should be analytically discriminated during the study on soil C budget elicited by experimental N deposition. Overall, the 5 years of experimental N deposition enhanced the loss of soil DOC (Fang et al., 2012), which could be adverse to the accumulation of soil organic carbon pool.

In the semiarid alpine meadow, DOC leaching is not obvious and can be negligible (Fang et al., 2012). Consequently, the dynamics of soil DOC depend on the budget between biodegradation and oxidation as well as soil physical and chemical adsorption and release. In soil biology, our results showed that N addition tended to stimulate microbial metabolic activity and to increase the intensity of carbohydrates utilization (Tables 3 and 4), which partly confirmed the first hypothesis. The reason for the initial stimulation might be that N addition satisfies the N demand of soil microorganisms to decompose the relatively small pool of available labile C substrates with low N contents such as carbohydrates and holocellulose (Berg and Matzner, 1997; Carreiro et al., 2000). Furthermore, the observation of preferentially using carbohydrates in our study suggested that soil microbial communities tended to use the rhizo-deposited C. Many studies indicate that carbohydrates are the most abundant components in root exudates (Smith, 1976; Uselman et al., 2000; Melnitchouck et al., 2005; Aitkenhead-Peterson and Kalbitz, 2005). The organic compounds released by plants (via exudation) were rapidly respired by root associated or soil heterotrophic microbes before they were able to contribute to the DOC pool (Ström et al., 2003; Lu et al., 2004). For the litter-rich surface soils, the increase in microbial metabolic activity would promote the release in soil DOC; however, it simultaneously increased the consumption of low molecular weight amino acids such as tyrosine and tryptophan (Table 1). The offset between the DOC production via microbial degradation and DOC consumption via microbial respiration can explain the contrast responses of soil DOC to N addition in surface and subsurface layers.

The significant and negative relationship between soil DOC content and microbial metabolic activity in subsurface layer indicated that microbial utilization dominated the loss of soil DOC (Fig. 4c). Amino acids such as tyrosine and tryptophan are the important components of DOM, and most derive from the oxidative degradation of plant-derived organic matter and by production of microbial metabolites (Guggenberger et al., 1994; Schimel and Weintraub, 2003). Our results showed that the change of soil DOC content was closely related to the changes of tyrosine, tryptophan and other organic acids, which provided some hints for microbial origin and consumption (Fig. 3). In our previous studies, N addition increased the microbial component of soil respiration (Fang et al., 2012), suggesting increased uptake of the DOC needed to fuel microbial respiration and a net decrease in DOC concentration in root-free subsurface soils. The potential microbial mechanisms could be attributed to two aspects: (1) N addition increased the activity of glucose oxidase that links cellulose degradation, but reduced soil phenol oxidase and peroxidase activities that links lignin/humus oxidation (Sinsabaugh et al., 2004; Waldrop and Zak, 2006); (2) N addition has decreased the importance of fungi in the decomposition of microbial communities, which has probably decreased DOC

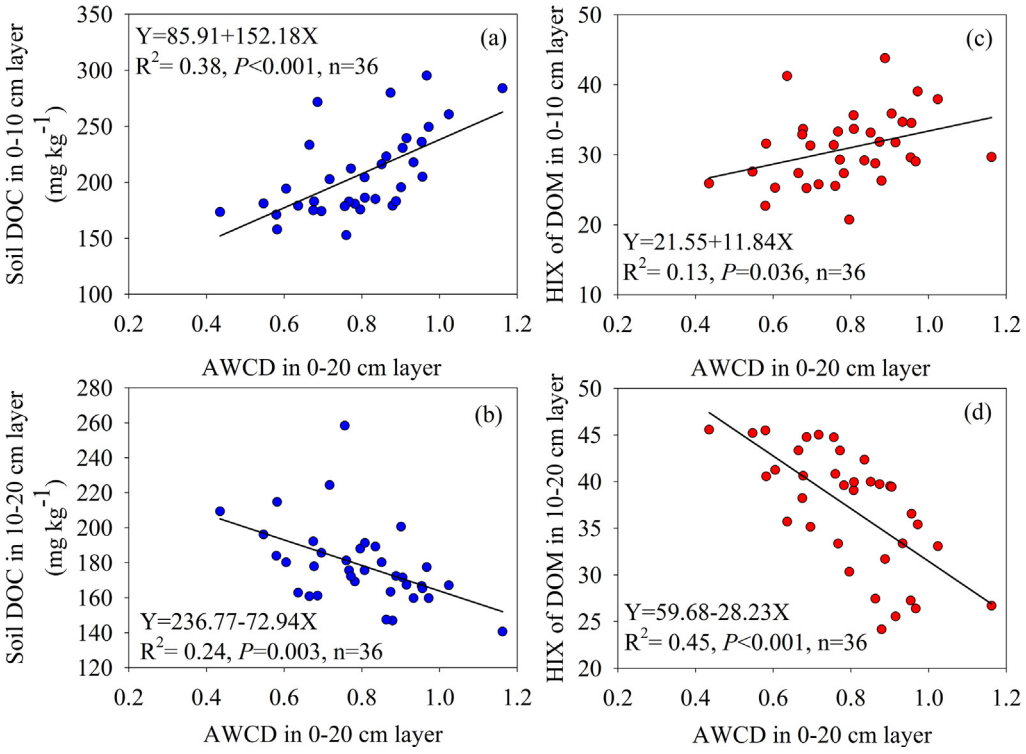

Fig. 4. Relationships between the content of DOC and stability of DOM (HIX) in 0–10 cm and 10–20 cm layers and microbial metabolic activity (AWCD) in 0–20 cm layer.

production in subsurface layer (Møller et al., 1999; Frey et al., 2004). Except for these microbial mechanisms, Hagedorn et al. (2012) proposed that the suppressed DOC contents from the mineral soil at N addition treatments could be attributed to reduced mobilization of non-litter derived 'older' DOC. They thought that experimental N deposition altered the physical and chemical adsorption capacity of mineral soil and soil organic matter due to increasing soil N availability, increasing ionic strength and decreasing soil pH (Hagedorn et al., 2012). However, in the semiarid alpine meadow, it would not be the main mechanism for DOC decline elicited by N addition due to no significant accumulation of inorganic N and soil acidification (Fang et al., 2012). Future research should be focused on the functional shifts and compositional changes in soil microbial communities in order to reveal the molecular mechanisms of soil C sequestration or loss.

There was no significant difference between NH_4^+–N and NO_3^-–N additions, which was inconsistent with the second hypothesis. It is possible that the absence of any effect was due to low dose of N addition, the short duration of the study, and livestock feeding. Our site was only fertilized for five years with small quantities of total N, and only samples collected on October, 2011 were analyzed. In our previous studies, we did find that NH_4^+ and NO_3^- fertilizers had contrary effects on aboveground biomass, soil CO_2 emission and soil CH_4 uptake (Fang et al., 2012, 2014). In the early stage of N addition, most of the applied N is being sequestered within the aboveground biomass, as alpine plants were shown to have taken up the mineral N in several ^{15}N tracing experiments (Xu et al., 2004, 2011); however, most of aboveground biomass were removed by livestock in winter, and thereby aboveground litters have limiting effects on soil microbial community.

4.2. N addition effects on soil DOM quality

Besides altering DOC quantity, N addition to the alpine meadow ecosystem significantly changed the composition rather than biodegradation of DOM. The increase/decrease in labile SOM fractions is often associated with increase/decrease in the proportion of recalcitrant SOM fractions under N enrichment, which further results in the changes in the composition and humification degree of DOM (Wang et al., 2011). For 0–10 cm organic layer, N addition tended to consume the labile tyrosine-like and tryptophan-like materials, activated the recalcitrant humic acid-like material, and thereby increased the fulvic acid-like and soluble microbial byproduct-like materials (Table 1). On the contrary, the reduction of plant residues return under N addition decreased soil microbial metabolic activity, and thereby resulted in the accumulation of amino acids and humic acid-like materials (Table 1). The HIX of DOM expresses the complexity and condensation of the molecules (Kalbitz and Geyer, 2001), and there is an inverse relationship between the aromaticity and complexity of DOM and its biodegradability (Kalbitz et al., 2003). Generally, a lower HIX of DOM indicates a lower aromaticity, complexity and degree of humification of DOM, which is expected to result in a higher mineralization of DOC at N addition treatments (Aitkenhead-Peterson and Kalbitz, 2005; Michel et al., 2006). Our study suggested that the utilization of organic acids and nitrogenous compounds such as carboxylic acids, amino acids and amines by microbial communities dominated the degradability of soil DOM (Fig. 5). In addition, no significant changes in the HIX of DOM between N treatments and control were observed (Table 1), which was mainly attributed to N-poor ecosystem, no enrichment of inorganic N pools and short duration of fertilization. On the contrary, in some N-rich forests, mineral N inputs increased the aromaticity of DOM and the complexity, i.e. the degree of condensation of the molecules (Park et al., 2002; Hagedorn et al., 2002; Michel et al., 2006), indicating that N addition may result in deposition of aromatic compounds in soils. The main reasons are that experimental N deposition decreases the extent of litter and soil organic matter decay (Baldock, 2007; Whittinghill et al., 2012). In the future, we should strengthen research on the relation of soil C sequestration to DOM stability

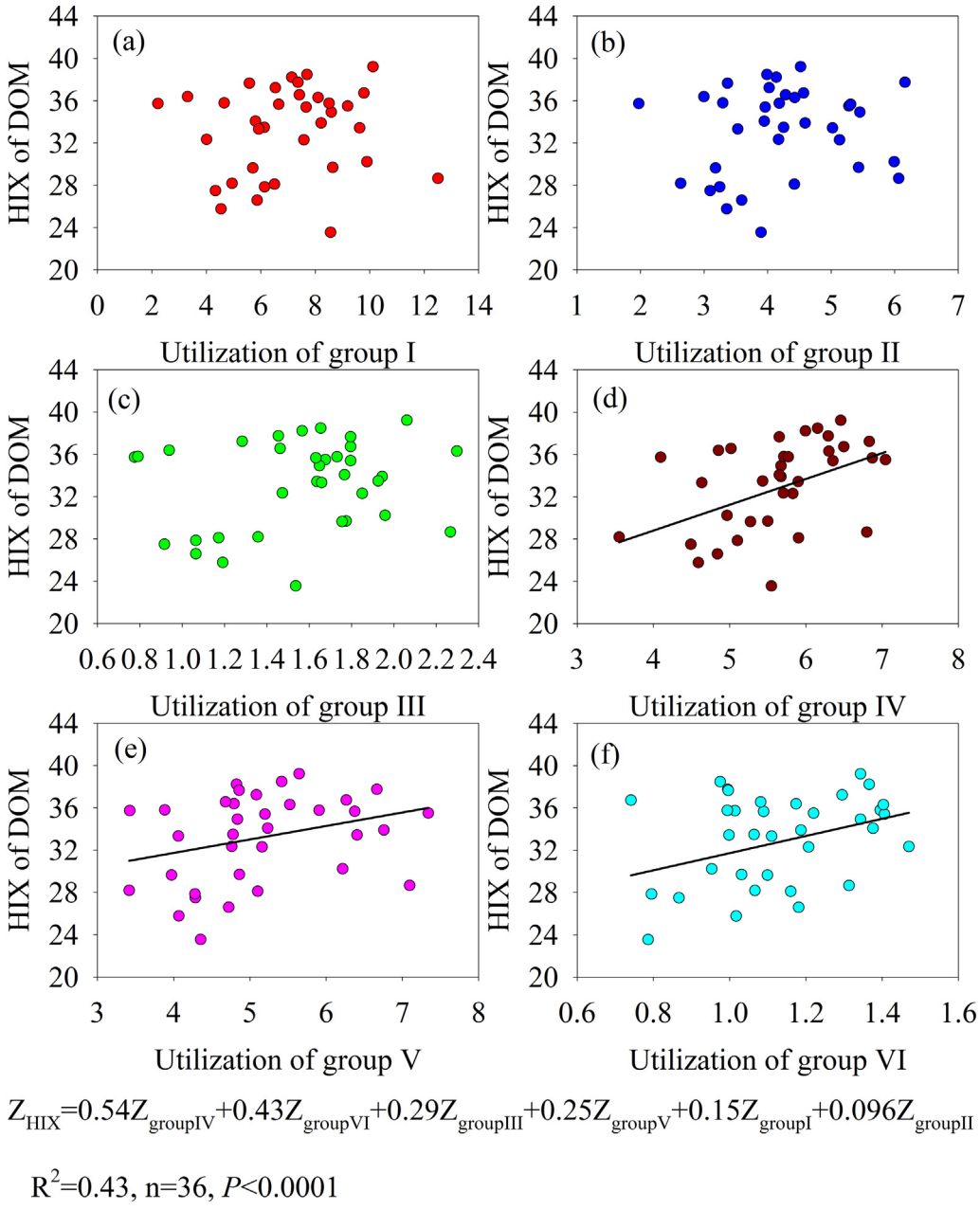

Fig. 5. Relationships between HIX of DOM and utilization of C resources in 0–20 cm soil layer. Group I to group VI refers to carbohydrates, polymers, miscellaneous, carboxylic acids, amino acids and amines, respectively.

through a combination of biochemical analyses of DOM and the molecular analyses of microbial communities.

5. Conclusions

This study characterized changes in the quantity and quality of soil DOC as well as microbial metabolic activity in the alpine meadow on the Qinghai-Tibetan Plateau. Continuous five-year N addition significantly increased DOC content in litter-rich surface soils, but consistently decreased DOC content in subsurface mineral soils. There was no significant difference between reduced NH_4^+ and oxidative NO_3^- inputs. Nitrogen addition significantly promoted microbial metabolic activity and utilization of C substrates, which was the main driver for changes in soil DOC amount and composition. However, CLPPs were used as a snapshot of the microbial activity and further studies should explore more the variations in relation to N and DOC at different soil depths. Overall, these results suggest that increasing atmospheric N deposition would stimulate soil microbial metabolic activity and could be adverse to the accumulation of soil organic carbon pool in the alpine meadow on the Qinghai-Tibetan Plateau.

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