

*Full Length Research Paper*

# Soil water soluble organic carbon under three alpine grassland types in Northern Tibet, China

Xuyang Lu<sup>1,2\*</sup>, Jihui Fan<sup>1</sup>, Yan Yan<sup>2</sup> and Xiaodan Wang<sup>1,2</sup>

<sup>1</sup>Key Laboratory of Mountain Environment Evolvement and Regulation, IMHE, CAS, Chengdu 610041, China.

<sup>2</sup>Shenzha Alpine Steppe and Wetland Ecosystem Observation and Experiment Station, IMHE, CAS, Shenzha 853100, China.

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**Water soluble organic carbon (WSOC) is the most mobile and reactive soil carbon source available. It plays an important role in many biogeochemical processes. In this study, we assessed WSOC in the upper 0 to 15 cm soil layer, during the growing season of three representative alpine grassland types of Northern Tibet, with an average elevation of over 4500 m. We also evaluated the contributions of soil environmental factors on the three types of grassland. We found that the WSOC was typically higher at the first sampling in May and decreased with subsequent samples. Furthermore, over the short growing season, the alpine meadow steppe ecosystem had significantly higher WSOC content than the alpine meadow and alpine steppe ecosystems. Soil WSOC of alpine grasslands also negatively correlated with both soil temperature and moisture. These results indicated that soil WSOC is considerably different among the different types of grassland in the same alpine area, and we conclude that soil environmental conditions including soil temperature and moisture are important influencing factors that control soil WSOC content.**

**Key words:** Water soluble organic carbon, soil temperature, soil moisture, alpine grassland, Northern Tibet.

## INTRODUCTION

Northern Tibet is the source of many important rivers in China and Asia, such as the Yangtze, Nu (Salween River), Lancang (Mekong River), among numerous other rivers and high mountain lakes. The average elevation of the region is more than 4,500 m above sea level and it is the highest part of the Tibetan Plateau (Zhang et al., 2006; Gao et al., 2009a, b). It is also a major livestock production centre in Tibet which is one of the nation's five key livestock raising provinces. Alpine grasslands play a vital role in local environment protection and stockbreeding. These grasslands are the dominant ecosystem occupying about 94% of Northern Tibet. As a result of the extremely harsh natural environment and an average elevation of over 4500 m (Gao et al., 2009a), the alpine grassland of Northern Tibet is a fragile ecosystem (Gao et al., 2009b, c). Being a fragile ecosystem, the alpine grassland ecosystem in Northern Tibet is extremely sensitive to climate change and human activities (Gao et al., 2009c).

Water soluble organic carbon (WSOC) was defined as the entire pool of water soluble organic carbon either sorbed on soil or sediment particles or dissolved in interstitial pore water (Tao and Lin, 2000a). WSOC accounts for a small portion of the total soil organic carbon content (Tao and Lin, 2000a; Ohno et al., 2007; Barbara and Fabrizio, 2009). Nevertheless, WSOC are considered as a most mobile and reactive soil carbon source which modulates a number of physical, chemical and biological processes in both aquatic and terrestrial environments (Schnabel et al., 2002; Marschner and Kalbitz, 2003; Halvorson and Gonzalez, 2008). In most soils, the majority of organic carbon is in insoluble form except for a small fraction that is water soluble and not yet leached out. This fraction of organic carbon is called the water soluble organic carbon (WSOC). It is the most important carbon source for soil microorganisms (Sparling et al., 1998; Schnabel et al., 2002; Marschner and Kalbitz, 2003), so both the quantitative and qualitative aspects of WSOC are very important for soil ecosystem studies (Gao et al., 1999; Gregorich et al., 2003; Kalbitz et al., 2003; Shamrikova et al., 2006; Embacher et al., 2007; Barbara and Fabrizio, 2009).

\*Corresponding author. E-mail: [xylu@imde.ac.cn](mailto:xylu@imde.ac.cn).

**Table 1.** Chemical and physical properties (mean  $\pm$  SD) of soils under alpine meadow (AM), alpine meadow steppe (AMS) and alpine steppe (AS).

Parameter	AM	AMS	AS
BD(g·cm <sup>-3</sup> )	1.15b (0.18)	1.72a (0.05)	1.76a (0.04)
pH	8.75a (0.0)	8.81a (0.1)	8.78a (0.1)
SOC(g·kg <sup>-1</sup> )	13.11a (0.8)	13.68a (2.3)	11.12a (2.1)
Total N(g·kg <sup>-1</sup> )	1.09b (0.1)	1.63a (0.2)	1.03b (0.2)
Total P(g·kg <sup>-1</sup> )	0.46a (0.02)	0.52a (0.04)	0.52a (0.03)
Total K(g·kg <sup>-1</sup> )	27.38b (0.2)	28.51b (0.4)	31.22a (0.4)
Particle-size fraction (%)			
2 - 1 mm	7.27 (2.9)	3.75 (1.7)	2.02 (0.9)
1 - 0.5 mm	21.3 (4.9)	15.72 (3.8)	13.15 (1.5)
0.5 - 0.25 mm	23.18 (2.8)	25.7 (2.7)	24.79 (0.7)
0.25 - 0.05 mm	26.53 (8.3)	40.18 (2.9)	45.41 (3.2)
0.05 - 0.02 mm	5.73 (1.1)	5.18 (1.4)	5.86 (1.1)
0.02 - 0.002 mm	12.13 (3.8)	7.31 (2.0)	7.24 (1.2)
<0.002 mm	3.86 (1.5)	2.15 (0.7)	1.53 (1.5)

Different letters (a and b) across different types of grasslands are different from each other at  $P \leq 0.05$  level.

Many environmental factors affect WSOC in soils, such as ecosystem acidification (Shamrikova et al., 2006; Karavanova et al., 2007), litter quality as influenced by forest composition (Shamrikova et al., 2006; Ohno et al., 2007), pig slurry addition (Hernández et al., 2007), steam soil disinfestations (Roux-Michollet et al., 2010) and soil temperature (Tao et al., 2000b). Northern Tibet is located in a cold and semi-arid plateau monsoon climate region. Soil temperature and moisture are key factors that control plant growth, distribution, and soil ecology in this cold and semi-arid ecosystem (Baumann et al., 2009; Gao et al., 2009b). It is well established that soil temperature and moisture are key factors influencing soil microbial activity and soil organic matter decomposition (Chen et al., 2007; Dijkstra and Cheng, 2007; Gao et al., 2009b). However, currently, little is known about WSOC and the factors affecting it in alpine grassland soil of the Northern Tibet. The aim of the present study is to analyze variations in soil WSOC, under three types of alpine grasslands (alpine meadow, alpine meadow steppe and alpine steppe) during the growing season, and study their relationships with soil environmental factors.

## MATERIALS AND METHODS

### Study site

To identify WSOC and the possible effects of soil environmental factors on it, we compared three different types of grasslands (alpine meadow, AM; alpine meadow steppe, AMS; alpine steppe, AS) at Shenzha Alpine Steppe and Wetland Ecosystem Observation and Experiment Station (N 30°57', E 88°42', 4675 m a.s.l.) located in Northern Tibet, which has a cold and semi-arid plateau monsoon climate. The annual mean air temperature was 0°C. The average annual precipitation was 300 mm, and most of it

occurred during May to September. There was no absolute frost-free season and the frosty period was up to 279.1 days. The annual mean time of solar radiation was 2915.5 h. Vegetation coverage of AM was about 70%, which was dominated by *Kobresia humilis* and occasionally distributed with individuals of *Oxytropis* spp., *Grindelia squarros* and *Aster tataricus* L. In AMS, the distributed species were similar to AM, but coverage was at 40%. *Stipa purpurea*, *Artemisia capillaris* Thunb and *Rhodiola rotundaia* assemblages dominated the vegetation species in AS with less than 20% coverage.

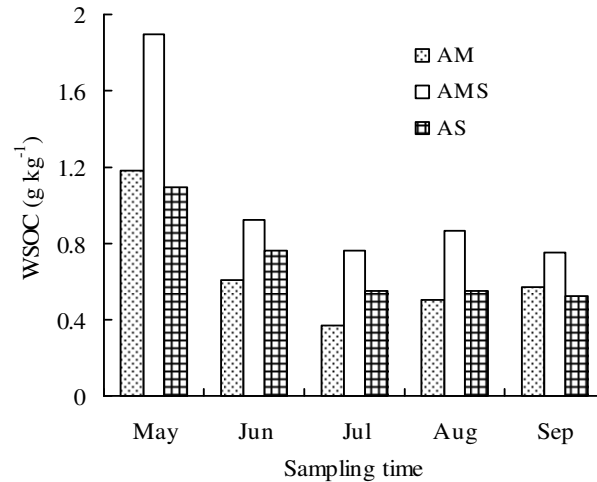
### Soil sampling and analyses

Soils were sampled from each type of grassland during May to September to capture the dynamic variabilities during the 2010 growing season. Three replicate samples with a depth of 0 to 15 cm were collected at each grassland site. Soil samples were kept at 4°C in cool boxes during transport to the laboratory. Soil samples for WSOC were stored in a refrigerator at 4°C and processed within 10 sampling days. In addition, soil samples for chemical and physical properties were determined during the first sampling. Soil moisture and temperature were automatically monitored by a weather station.

Soil properties were determined using regular analysis methods (Liu, 1996). Soil bulk density was determined as the moisture-corrected (oven-dried at 105°C) mass of each sample divided by the measured excavation volume. SOC was determined using wet oxidation by  $K_2Cr_2O_7$ . The micro-Kjeldahl digestion method was used to determine total N content. Total P and K contents were determined using the NaOH and HF-HClO<sub>4</sub> digestion methods, respectively. Soil particle-size fractions were determined by a laser diffraction instrument (Malvern Mastersizer 2000 particle size analyzer, Worcs, UK). Chemical and physical properties of soils are presented in Table 1.

### Measurement of WSOC and TSN

To determine the WSOC, fresh sample of each soil was treated with



**Figure 1.** Seasonal patterns of water soluble organic carbon (WSOC) at three contrasting alpine grassland ecosystems across the plant growing season. The generalized linear mixed model (GLMM) statistics are summarized with significant effects in *italics* for WSOC: *Month*  $F_3 = 50.41$ ,  $P < 0.001$ , *site*  $F_2 = 39.91$ ,  $P < 0.001$ , *month* × *site*  $F_8 = 3.87$ ,  $P = 0.001$ .

deionized water using soil/water ratios 1:5 (W/V) for 30 min under agitation (130 times/min) in a flask. After the extraction, samples were centrifuged at 6500 r/min for 15 min. Supernatants were filtered with a 0.22  $\mu\text{m}$  millipore membrane. Water extraction organic carbon and total soluble nitrogen (TSN) of the filtrate was determined by a Vario TOC cube total organic carbon analyzer (Elementar Analysensysteme GmbH., Germany).

### Statistical analyses

The generalized linear mixed model adapted the alpine grassland type and the sampling time as the main factors for analyzing the soil WSOC. One-way ANOVA was used to test the differences of soil chemical and physical properties among the three alpine grasslands, and a LSD test was used to distinguish differences at  $p = 0.05$ . Relationship between the WSOC and soil environmental factors was tested using linear correlation analysis. All analyses were performed using the SPSS 11.5 statistical software package (SPSS Inc., USA).

## RESULTS AND DISCUSSION

### Seasonal variations of soil WSOC and TSN

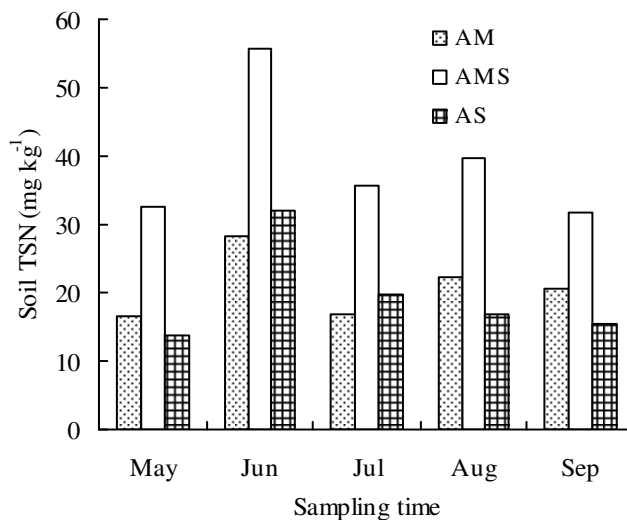
WSOC concentration changed rapidly during the early part of the growing season at all three alpine grassland sites (Figure 1). At all the three sites, the WSOC were typically high at the first sampling and decreased with subsequent samples. This seasonal variation was more obvious in the AMS site, compared to the other two alpine grassland sites. In the AM site, the WSOC decreased from May ( $1.19 \text{ g kg}^{-1}$ ) and reached minimal value in July ( $0.37 \text{ g kg}^{-1}$ ), and gradually increased after July. In contrast to the AM site, there were no significant

changes in WSOC levels at the AMS and AS sites from July to September. Our results are in agreement with Herbauts (2003), who observed a seasonal fluctuation of WSOC which appears throughout the year from fall to midsummer in three acid soils. Results from generalized linear mixed models (GLMM) demonstrated that, soil sampling times had a significant effect on WSOC concentration (Figure 1).

Early studies showed that, WSOC was significantly and positively correlated to water soluble soil nitrogen in different ecosystems (Ghani et al., 2007; Montañó et al., 2007; Halvorson and Gonzalez, 2008). Water soluble nitrogen also plays an important role in ecosystem nutrient fluxes and plant nutrition (Huang and Schoenau, 1998). In alpine grassland ecosystem, monthly soil TSN concentration showed seasonal variations ranging from  $13.63$  to  $55.61 \text{ mg kg}^{-1}$  across all alpine grassland sites and sampling dates (Figure 2). The highest TSN values of the three sites were obtained in June, and the soil TSN concentration of the AM, AMS and AS sites were  $28.40$ ,  $55.61$  and  $32.12 \text{ mg kg}^{-1}$ , respectively. Statistic analyses showed that, soil TSN concentrations were significantly different among the five sampling dates (Figure 2). However, the WSOC exhibited a weak positive correlation with soil TSN in alpine grassland ecosystems ( $r = 0.26$ ,  $P = 0.35$ ).

### Relationship between WSOC and soil environmental factors

As indicated by Tao et al. (2000b) that soil properties and soil environmental conditions are the important factors



**Figure 2.** Seasonal patterns of soil total soluble nitrogen (TSN) at three contrasting alpine grassland ecosystems across the plant growing season. The generalized linear mixed model (GLMM) statistics are summarized with significant effects in italics for WSOC: Month  $F_3 = 44.07$ ,  $P < 0.001$ , site  $F_2 = 171.35$ ,  $P < 0.001$ , month  $\times$  site  $F_8 = 3.87$ ,  $P = 0.003$ .

influencing the concentration of WSOC. WSOC concentration of alpine grassland ecosystems was grossly negative relative to the soil temperature. The mean value of the three grassland sites decreased from 1.39 to 0.76 g kg<sup>-1</sup>, with an average soil temperature of 9.57 and 11.47°C in May and June, respectively (Figure 1, Figure 3a). Decreased levels of WSOC with increasing soil temperature are attributed to its utilization as a microbial substrate. The soluble fraction of organic matter is the main energy substrate of soil and should therefore be utilized preferentially (Marschner and Bredow, 2002). As the soil temperature increases, soil microbial activity is enhanced leading to accelerated decomposition of organic matter and release of organic carbons (that is CO<sub>2</sub>) into the atmosphere. This consequently causes a decline in soluble soil organic carbon (Marschner and Bredow, 2002; Wang et al., 2008).

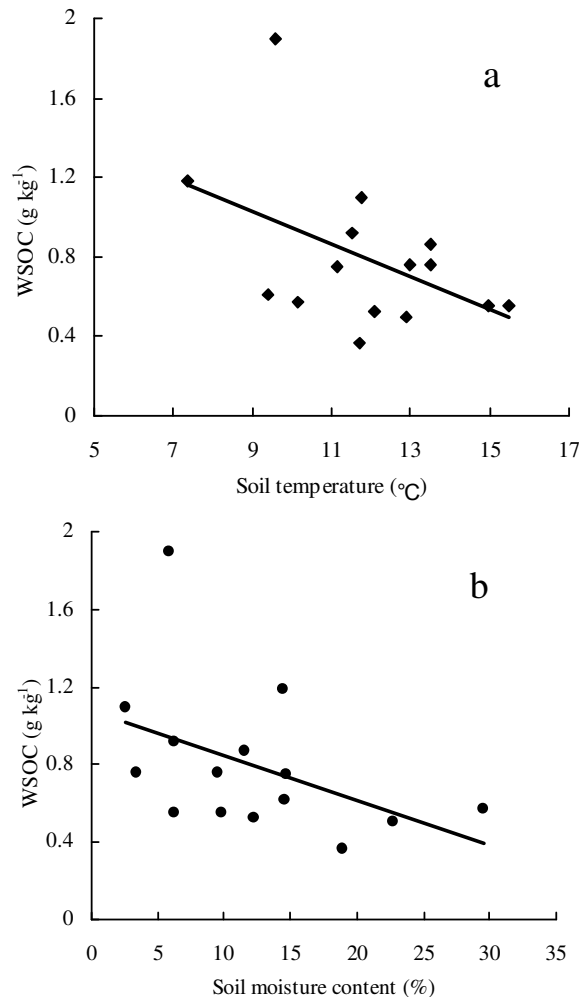
We found that WSOC concentrations were also generally negatively correlated with soil moisture content based on data collected at five different time points (Figure 3b). The highest mean value across the growing season appeared in the AMS ecosystem with medium soil moisture at an average of 9.60% from May to September. The WSOC concentration of the AMS site was 1.60 and 1.49 times higher, respectively, compared to AM and AS. Additionally, the mean soil moisture content for AM and AS sites was 20.04 and 6.88%, respectively. Previous work on alpine ecosystems of Tibet plateau mostly focused on the temperature effect on soil carbon process (Kato et al., 2004; Xu et al., 2006; Zhang et al., 2010). Soil moisture is also an important factor that regulates the process of soil ecology in alpine

semi-arid ecosystem. In this study, we explored the relationship between WSOC concentration and soil moisture content in alpine grassland ecosystem. The results show that more soluble organic carbon accumulated in the soil with moderate soil moisture content. Moreover, excess or lack of soil moisture is unfavorable for the accumulation of soil soluble organic carbon in semi-arid alpine grassland ecosystems.

### Comparison between the three alpine grassland types

The type of grassland ecosystems can profoundly impact soil soluble organic carbon through the alteration of abiotic and biotic characteristics of soils and soil physical and chemical properties (Bobby et al., 2007; Roberts et al., 2009). Among the three alpine sites, the AMS site exhibited significantly higher WSOC concentration (1.04 g kg<sup>-1</sup>) than the AM (0.65 g kg<sup>-1</sup>) and AS (0.70 g kg<sup>-1</sup>) sites from May to September. However, the total soil organic carbon contents were not statistically different among the three types of alpine grassland ecosystem (Table 1).

Therefore, the ratios of soil WSOC account for the total soil organic carbon content, were corresponding high in the AMS site. The amount of soil TSN in AMS sites was also significantly higher than the other two alpine sites, in which the mean values during the growing season of the AMS site were 1.86 and 1.99 times greater than the AM and AS sites, respectively. This is may be due to the AMS site showing significantly ( $P < 0.05$ ) greater content



**Figure 3.** Relationship between water soluble organic carbon (WSOC) and soil temperature (a), soil moisture content (b).

of soil total nitrogen than that of the AM and the AS sites (Table 1). Statistical analyses showed that, soil WSOC and TSN concentrations were significantly different among the three alpine grassland types and their interaction with the five sampling dates.

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