ABSTRACT: In concurrent with global warming, precipitation regimes are predicted to change as well around the world. In this study, two experimental sites were selected with different nutrient availability along a slope to study the effects of simulated increased precipitation on soil β-glucosidase activities in an Inner Mongolian grassland. Soil samples were adjusted to 55% of water holding capacity and incubated at 22°C in the dark for 32 days. Soil β-glucosidase activities were measured prior to and after the incubation. Results showed that soil β-glucosidase activities had differential responses to increased precipitation with a significant increase in the down-slope site, but not in the up-slope site. Correlation analysis showed that the initial soil β-glucosidase activities exerted a significantly negative relationship with soluble organic nitrogen (N). Our results indicated that both water availability and soil soluble N availability played important roles in regulating β-glucosidase activities in this semi-arid region.

KEY WORDS: increased precipitation, β-glucosidase activity, incubation, Inner Mongolia, grassland

Soil enzyme activity can serve as an indicator of soil quality and fertility (Chen 2003). They have been shown to be strongly correlated with nutrient availability (Sardans and Peñuelas 2005). Microorganisms synthesize and secrete extra-cellular enzymes such as β-glucosidase into the soil matrix. Soil β-glucosidase plays an important role in carbon (C) cycling, hydrolysing carbohydrates with β-glucoside bonds, such as maltose and cellobiose, and producing sugars, an important energy source for microorganisms (Verchot and Borelli 2005). Therefore, factors influencing soil β-glucosidase activities exert controls on C availability and soil fertility. Until now, soil β-glucosidase activities have been studied in agricultural (Verchot and Borelli 2005) and forest soils (Chen 2003, Sardans and Peñuelas 2005). However, little information is available for soil β-glucosidase activities in a grassland soil, particularly in a semi-arid temperate grassland.

Previous studies have demonstrated that water availability is the primary limiting factor in semi-arid and arid regions, strongly affecting ecosystem functions (Chen and Wang 2000, Chen et al. 2005, Liu et al. 2009). Global and regional precipitation regimes are predicted to change with the increases in heavy rainfall predicted in most global circulation models (IPCC 2007), which may result in nutrient losses in the nearest decades. Previous studies have examined the effects...
of climate changes on plant communities (Chen and Wang 2000), ecosystem respiration and soil respiration (Liu et al. 2009) as well as microbial communities (Zhou et al. 2008) in semiarid and arid regions. However, few studies are available for the effects of increased precipitation on soil β-glucosidase activity in these regions.

The objective of this study was to examine soil β-glucosidase activities in response to increased precipitation under contrasting nutrient availability in the semiarid Inner Mongolian grassland. We hypothesized that water additions would increase β-glucosidase activities, since higher water availability would enhance nutrient flow and subsequently provide more nutrient availability to microorganisms.

The experimental site (43°32'N, 116°40'E, 1200 m a.s.l) is located in a fenced Leymus chinensis community at the Inner Mongolia Grassland Ecosystem Research Station located at Xilin River Watershed of Inner Mongolia Autonomous Region. The site is located in a smooth wide plain with low hills (20–30 m high with <5° slope) on a second-level basalt platform. It is under a temperate semiarid continental climate with dry springs and moist summers. The annual temperature averages –0.4°C with a growing season length from 150 to 180 d. The mean annual precipitation is 335 mm, most of which falls from June to August. The plant community is dominated by perennial rhizome grass, Leymus chinensis, Agropyron cristatum and Stipa grandis (Zhou and Hao 2010). The site was considered as the representative of undisturbed and climax steppe community. Two plots were selected within the fenced area along the slope; one is located on the upper, the other on the low part of the slope. The soil type is 'calcic luvisols' according to the FAO classification with about 60% of sand and 20% of silt (Chen and Wang 2000).

Three subplots were selected within each plot at 30 m intervals. Soil samples were collected by taking five random cores (5-cm in diameter) to a depth of 10 cm using a soil auger in each subplot. The five soil cores were thoroughly mixed and kept in a cooler (ca 4°C). After passing through 2-mm sieve, the soil samples were stored at 4°C prior to analysis.

Soil moisture content was determined after being oven-dried at 105°C overnight. Soil pH was measured at a 1:2.5 dry soil/water ratio. For the hot water extraction, field moist soil samples (about 5 g of dry weight equivalent) were shaken and then let passed through 0.45-μm filter membrane. The concentration of inorganic N was measured on a La-chat Quickchem automated analyzer (Quick Chem method 10-107-064-D for NH4+ and 10107-04-1-H for NO3– and NO2–). Soluble inorganic N (SIN) was calculated as the sum of NO3–-N and NH4+-N. Soil SOC and total soluble organic N (TSN) in soil extracts were determined using SHIMADZU TOC-CPN analyzer (fitted with a TN unit). Soil SON concentrations were determined by subtracting SIN from TSN for each samples (Zhou et al. 2012).

To assess the effects of increased soil moisture on β-glucosidase activities, soil samples at field moisture were adjusted to 55% of water holding capacity and kept in the dark at 22°C for 32 days. Soil β-glucosidase activities were determined prior to and after the incubation. The activity of β-glucosidase was assayed according to Verchot and Borelli (2005). The results were given in units of mg pNP g−1 dry soil h−1.

The statistical analysis was performed using SPSS 12.0 (SPSS Inc. Chicago, USA). One-way ANOVA were used to test differences of soil parameters and β-glucosidase activities in the both sites. The significance level was accepted at P <0.05.

Table 1. Soil properties at the two sites in the Inner Mongolian temperate grassland.

<table>
<thead>
<tr>
<th>Site</th>
<th>Moisture (%)</th>
<th>pH</th>
<th>Soluble organic C (mg kg−1)</th>
<th>Soluble organic N (mg kg−1)</th>
<th>C:N ratiob</th>
</tr>
</thead>
<tbody>
<tr>
<td>Downslope</td>
<td>0.13 ± 0.01</td>
<td>6.89 ± 0.02a</td>
<td>60.9 ± 2.5</td>
<td>10.4 ± 0.4</td>
<td>5.86 ± 0.25b</td>
</tr>
<tr>
<td>Upslope</td>
<td>0.09 ± 0.02</td>
<td>6.82 ± 0.02b</td>
<td>52.8 ± 2.1</td>
<td>3.92 ± 0.63</td>
<td>13.47 ± 1.22a</td>
</tr>
</tbody>
</table>

a data are means ± standard errors (n = 3). Different letters within columns showed significant differences at P <0.05.

b represents ratio of soil soluble organic C to soluble organic N.
The down-slope site had significantly higher soil pH, SON and the ratios of SOC to SON, as compared with the upslope site (Table 1). Soil SOC content tended to be higher at the down-slope site, although no significant differences were found between the two sites.

The upslope soil had a significantly higher \( \beta \)-glucosidase activity as compared with the downslope soil (Fig. 1). Soil \( \beta \)-glucosidase activities significantly increased after the 32-day incubation compared with the initial values in the down-slope site, but no significant differences were found in the upslope site (Fig. 1). In addition, the initial soil \( \beta \)-glucosidase activities exerted significantly negative relationships with pH \((r = -0.676, P < 0.05, n = 6)\) and soil SON \((r = -0.72, P < 0.01, n = 6)\), but had a significantly positive relationship with the ratios of SOC to SON \((r = 0.65, P < 0.01, n = 6)\). By comparison, soil \( \beta \)-glucosidase activities after the incubation had a significantly negative relationship with pH \((r = -0.66, P < 0.05, n = 6)\).

Soil \( \beta \)-glucosidase catalyzes one of the late steps of cellulose degradation, which plays an important role in C cycling. Soil \( \beta \)-glucosidase represents in different forms; a part of it is associated with living microorganisms, the other are associated with non-living particular matter of the soil matrix, and the rest is present in the soil solution (free enzyme) (Sardans and Peñuelas 2005). In general, increased precipitation can increase soil EEA via increases in enzyme and substrate diffusion (Verchot and Borelli 2005).

In this study, we found that soil \( \beta \)-glucosidase activities significantly increased after the 32-day incubation in the down-slope site (Fig. 1), which was consistent with our hypothesis. Sardans and Peñuelas (2005) found that soil drying greatly decreased soil \( \beta \)-glucosidase activities. Similarly, Geisseler and Horwarth (2009) reported that higher soil moisture significantly increased soil \( \beta \)-glucosidase activities under field studies with desert soil and with topsoil under furrow irrigated corn.

However, in contrast with our hypothesis, there were no differences in soil \( \beta \)-glucosidase activities in the upslope site. Similarly, Zornoza et al. (2006) reported no significant differences in soil \( \beta \)-glucosidase activities in response to air-drying and water additions.

Correlation showed that soil \( \beta \)-glucosidase activities were negatively affected by SON, which implied that soil SON might play an important role in regulating \( \beta \)-glucosidase activities. The higher \( \beta \)-glucosidase activities in the upslope site could be attributed to the lower soil SON as compared with those in the down-slope site. The importance of substrate availability has been addressed by Geisseler and Horwarth (2009) who found that soluble N derived from plant residue stimulated \( \beta \)-glucosidase activities in dry soils. The Inner Mongolian grassland is regarded as N deficient grassland (Chen and Wang 2000). Both water availability and soil SON could together exert roles in soil \( \beta \)-glucosidase activities, which might be the reasons for no significant differences in \( \beta \)-glucosidase activities prior to and after the incubation in the upslope site.

In conclusion, soil \( \beta \)-glucosidase activities differentially responded to increased precipitation with a significant increase in the down-slope site, but not in the upslope site. Our results showed that both water availability and soil SON played important roles in regulating \( \beta \)-glucosidase activities in this semiarid region.

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