Soil Biology & Biochemistry 98 (2016) 74–84

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Stoichiometry of soil extracellular enzyme activity along a climatic transect in temperate grasslands of northern China

Xiaoqian Peng, Wei Wang*

Department of Ecology, College of Urban and Environmental Sciences and Key Laboratory for Earth Surface Processes of the Ministry of Education, Peking University, Beijing 100871, China

ARTICLE INFO

Article history:
Received 19 November 2015
Received in revised form
1 April 2016
Accepted 5 April 2016

Keywords:
Ecological stoichiometry
Soil extracellular enzyme
Temperate grassland
Soil depth

ABSTRACT

Ratios of specific carbon (C), nitrogen (N) and phosphorus (P) acquisition activities converged on 1:1:1 at a global scale and in tropical ecosystems. It is less clear if this pattern can be applied in temperate grasslands. The questions of whether the pattern remains stable across different soil depths and what the relative contributions are from the influence of climatic, edaphic abiotic and biotic factors on soil extracellular enzyme activity (EEA) stoichiometry remain uncertain. We measured potential activities of one C-acquiring enzyme (β-1, 4-glucosidase), two N-acquiring enzymes (β-*N*-acetylglucosaminidase and leucine aminopeptidase) and one organic P-acquiring enzyme (acid phosphatase) and major influential factors along a climatic transect in temperate grasslands of northern China during the growing season of 2013. We found lower enzyme C: N (0.47) and C: P (0.18) ratios and a higher enzyme N: P activity ratio (0.40) in 0–20 cm soil depth in temperate grasslands than in tropical soils (C: N, 1.83; C:P, 0.21; N: P, 0.13). The enzyme C: N and C: P ratios decreased with soil depth except for the enzyme C: N ratio in desert steppes. However, there were no significant differences in enzyme N: P ratio with soil depth. Among all the factors, soil total C, N and P contents accounted for the most variation in soil EEA and ecoenzymatic stoichiometry in 0–10 cm surface soil, which implied that soil EEA stoichiometry was largely controlled by soil nutrient stoichiometry. Moreover, edaphic factors had less influence on soil EEA stoichiometry in subsoil than in surface soil and edaphic abiotic factors had a larger effect on soil EEA stoichiometry than climatic and biotic factors. Our results suggest that soil extracellular enzyme activity ratios were not in homeostasis but resource dependent on soil and microbial biomass stoichiometry.

1. Introduction

The polymeric carbon (C), nitrogen (N), and phosphorus (P) components of soil are structurally complex and highly diverse (Burns et al., 2013). Soil extracellular enzymes produced by plants and microorganisms are the proximate agents of soil organic matter decomposition. The expression of enzymes is a product of cellular metabolism, particularly regulated by environmental nutrient availability (Sinsabaugh et al., 2009). The relative abundance of enzymes involved in C, N and P cycling, namely extracellular enzyme stoichiometry, reflects the biogeochemical equilibrium between metabolic and nutrient requirements of microbial assemblages and nutrient availability of the environment (Sinsabaugh et al., 2009; Sinsabaugh and Follstad Shah, 2010; Hill et al., 2012; Sinsabaugh and Follstad Shah, 2012). Soil EEA stoichiometry could provide a functional assessment of the threshold at which control of community metabolism shifts from nutrient to energy flow (Sinsabaugh et al., 2009).

Previous studies have shown that many abiotic factors can affect soil EEA stoichiometry. For instance, climate could impact soil EEA stoichiometry by affecting microbial growth efficiency and the relative availability of soil nutrients (Kivlin and Treseder, 2013; Waring et al., 2013). Moreover, the stoichiometry of soil EEA was also affected by soil nutrient status, soil pH (Sinsabaugh et al., 2008) and soil texture (Alvarez and Lavado, 1998). In addition to abiotic factors, biotic factors might influence soil EEA stoichiometry, such as above-ground biomass (Zhao et al., 2014), root biomass (Reboreda and Cacador, 2008; Edwards and Jefferies, 2013) and microbial biomass stoichiometry (Cleveland and Liptzin, 2007). For example, microbial biomass C: N: P stoichiometry could influence soil EEA stoichiometry, such that microbes produce enzymes to target the most limiting nutrient from complex substrates available

* Corresponding author.
E-mail address: wangw@urban.pku.edu.cn (W. Wang).

http://dx.doi.org/10.1016/j.soilbio.2016.04.008
0038-0717/

in the soil (Allison and Vitousek, 2005). Although the impact of abiotic and biotic factors on soil EEA stoichiometry have received attention, the relative contribution from the influence of climatic, edaphic abiotic and biotic factors on soil EEA stoichiometry are rarely investigated (Kivlin and Treseder, 2013).

Abiotic and biotic factors change with soil depth. The strong soil nutrient and environmental gradients observed within the soil profile might influence the abundance, composition and function of soil microbial communities (Šnajdr et al., 2008; Delgado et al., 2012; Stone et al., 2014), and consequently cause soil EEA to change with soil depth. However, most previous studies have focused exclusively on relatively C and nutrient-rich surface soils (Stone et al., 2014), despite the fact that soil microbes can influence biogeochemical processes throughout soil profiles (Buss et al., 2005).

Previous studies concerning soil EEA stoichiometry were mainly focused on meta-analysis at a global scale (Sinsabaugh et al., 2008) and in tropical ecosystems (Sinsabaugh et al., 2009; Waring et al., 2013). These studies showed that ratios of specific C, N and P acquisition activities converged on 1:1:1. However, in the previous global analysis, the data were derived from 40 ecosystems, among which only two were grassland ecosystems. Grassland is one of the most widespread vegetation types in the world, occupying one-fifth of the earth's land surface (Hall et al., 1995; Scurlock and Hall, 1998). Chinese grasslands covered 41.7% of its territory and were distributed mainly in the Inner Mongolian and Tibetan plateaus (National Statistics Bureau China, 2002). The arid and semi-arid grasslands on the Mongolia plateau, representative of the Eurasian steppe region, are extremely water-limited (Bai et al., 2012). These grasslands are very sensitive to climate and environment changes since they are located in the vulnerable ecotone. It is less clear if the pattern of soil EEA stoichiometry at a global scale and in tropical ecosystems can be applied in temperate grasslands.

Our study was designed along a climatic transect of Inner Mongolian temperate grassland to explore: (1) Are soil extracellular enzyme C: N: P activity ratios in temperate grassland similar to the global pattern? (2) Does soil EEA stoichiometry vary with soil depth? and (3) What is the relative contribution of the influences from abiotic and biotic factors on soil EEA stoichiometry? We hypothesized that: (1) The soil extracellular enzyme C: N: P activity ratio in the soil surface layer in temperate grassland is similar to that in global and tropical ecosystems, which converged on 1:1:1; (2) The stoichiometry of soil EEA is variable with soil depth; and (3) Abiotic factors were more related to soil EEA stoichiometry than biotic factors and the combination of abiotic and biotic factors had less influence on soil EEA stoichiometry in subsurface soils than in surface soils.

2. Materials and methods

2.1. Site description

Seventeen study sites were selected (latitude 38.98°–50.17°N and longitude 107.97°–119.38°E) without significant human disturbance along temperature and precipitation gradients in Inner Mongolia, China (Supplemental material Fig. S1). These study sites covered three community types, including meadow steppe, typical steppe and desert steppe. The meadow steppe is located in the eastern part of Inner Mongolia. It is dominated by *Stipa baicalensis*, *S. grandis and Leymus chinensis*, with the highest species richness and above-ground productivity among the three community types. The typical steppe is located in the middle part of Inner Mongolia. It is dominated by *S. krylovii*, *L. chinensis*, *S. grandis and Artemisia frigida*. The desert steppe is located in the western part of Inner Mongolia. It is dominated by *S. breviflora*, *Cleistogenes songorica* and *Neopallasia pectinata* with the lowest species richness and productivity among the three community types. From northeast to southwest, the mean annual temperature (MAT) increases from −1.9–7.5 °C and mean annual precipitation (MAP) declines from 433 to 198 mm. Mean annual temperature and mean annual precipitation data of our study sites were obtained from the closest meteorological stations. Generally, the soils in Inner Mongolia steppe are calcareous and the development of outcrop in bedrock is poor. The soil types are defined as chernozems in meadow steppe, castanozems in typical steppe and brown calcic soils in desert steppe in China's soil classification system (Xiong and Li, 1987). And they are also categorized as chernozems, kastanozems and haplic calcisols, respectively, in the soil taxonomy system of the Food and Agriculture Organization (FAO) of the United Nations (Yang et al., 2012). The basic properties of these sites are listed in Table 1.

2.2. Soil sampling and processing

Three soil pits (1 m × 1 m × 1 m) were excavated with 10 m between each pit to 100 cm soil depth and soil samples were collected from five discrete depth intervals (0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–100 cm) for each study site in July 2013, the peak period for plant growth. For each quadrat, above-ground standing biomass was clipped to the ground and dead plant parts were removed. Soil blocks (10 cm × 50 cm × 10 cm) were collected from five discrete depth intervals in three quadrats at each site and roots were separated from soil to estimate root biomass. All plant materials were oven-dried at 65 °C for 48 h and then weighed. The soil samples were passed through a 2 mm sieve and the visible plant materials were removed from the soil samples. Half of each soil sample was divided into two subsamples, one was stored at 20 °C and the other at 4 °C for no more than 1 week, and subsequently used for measurements of soil EEA and microbial biomass C, N and P, respectively. The remaining soil samples were used to measure soil chemical and physical characteristics.

2.3. Soil chemical and physical characteristics

At each site, the following soil characteristics were examined: soil temperature, moisture, pH, texture and soil total C, N and P contents. Soil temperature and moisture were measured using a WET sensor (WET-2 sensor, Delta-T Devices Ltd: Cambridge, UK). Soil pH was determined in a 2:5 soil: distilled water slurry with a pH meter (Model PHS-2, INESA Instrument, Shanghai, China). Soil texture was measured using a laser particle size analyzer (Mastersizer 2000, Malvern, UK). Soil total C and N concentrations were measured using an elemental analyzer (Vario EL III, Elementar, Hanau, Germany). Soil total P was determined by the H_2SO_4–$HClO_4$ fusion method (Sparks et al., 1996).

2.4. Microbial biomass measurements

The chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987) was used to measure microbial biomass C (MBC) and N (MBN). Two 25 g soil subsamples were extracted with 50 ml 0.5 M K_2SO_4 for 30 min on a shaker and another two subsamples were fumigated with chloroform for 24 h in a vacuum desiccator, followed by the same extraction procedure as for the unfumigated samples. The extracts were measured for total dissolved organic C and total dissolved N using a total organic C analyzer (Multi N/C3100, Analytik Jena AG, Germany). Soil microbial biomass C and N were estimated as the difference in extractable C and N between fumigated and unfumigated soils based on extractability correction factors: $K_C = 0.45$ for C and $K_N = 0.54$ for N (Joergensen and Mueller, 1996).