Effect of litter addition on amino acid content and composition in alpine meadow soil

Feng, Y.L.*; Li, X.Y.†; Zong, W.Z.†; Wang, J.†
*†State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, Gansu Province, 730020, P.R.China.

Key words: Alpine meadow; exchangeable amino acids; litter; protease activity; soil.

Abstract
Litter plays an important role in plant-soil nutrient cycling. However, the response of soil amino acid pools to litter input is unclear. Therefore, the aim of this study was to explore the effects of litter addition on the content and composition of soil amino acids in an alpine meadow soil. Soil was amended with litter of its dominant species, *Kobresia graminifolia*, and incubated for four weeks. Our results show that litter addition significantly increased the exchangeable amino acid content and protease activity in the soil. These results are crucial for understanding the amino acid cycling in soil.

Introduction
In ecosystems such as arctic tundra, boreal forest and alpine meadows that are strongly limited by nitrogen mineralization, soil free amino acid pool was comparable to or even one order of magnitude higher than inorganic nitrogen pool (Jones et al. 2005; Näsholm et al. 2009). Soil amino acids are not only good source of carbon and nitrogen for soil microorganisms, but also can be directly absorbed by many plants (Xu et al. 2006). Amino acids in soil are mainly derived from the hydrolysis of proteins and peptides by extracellular enzymes, and the rate of protein hydrolysis depends on the substrate availability of aboveground plant litter and belowground root inputs (Lipson and Näsholm 2001). Therefore, it is essential to understand the effect of litter input on soil amino acid pools. Litter decomposition is one of the important pathways of nitrogen cycling between plants and soil. However, at present, most studies about the effects of litter input on soil nitrogen transformation mainly focus on the influence of litter quality, quantity and type on soil nitrogen mineralization (Gao et al. 2016; Meier and Bowman 2008), and there are few investigations on the response of soil amino acid pools to litter addition.

Methods
This research was conducted in a fenced grassland at the Gannan Grassland Ecosystem National Observation and Research Station. The study site was located in the northeast of the Tibetan Plateau, China (33°59′ N, 102°00′ E, altitude 3500 m) with a humid and cold climate. Litter of the dominant plant *K. graminifolia* and soil samples were collected in October 2018. Litter was

mixed with soil at a rate of 0.02g litter g^{-1} soil. During the soil incubation (0, 1, 5, 9, 17 and 28 day) , the activity of protease was determined using the method described by Geisseler and Horwath (2010). After 28 days of soil incubation, the content and composition of soil exchangeable amino acids (EAA) extracted by 2 M KCl were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) on an Agilent 1100 system (Agilent, CA, U.S.A.). Data are presented as the mean ± standard error of three replicates. Statistical analyses were performed with SPSS 20.0 software (SPSS Inc., Chicago, IL). The effects of litter addition on the pool size of soil amino acids and soil protease activity were examined using independent sample t-tests.

Results and Discussion

In this study, litter addition significantly increased the content of soil exchangeable amino acids (Fig. 1), which may be due to two possible reasons. First, the input of litter may increase the activity soil protease and accelerate the decomposition of complex organic nitrogen, producing more small soluble organic nitrogen compounds such as amino acids (Schimel and Bennett 2004). Second, litter can directly release large amounts of free amino acids during its leaching or decomposition (Näsholm and Persson 2001). The composition of exchangeable amino acids in soil amended with litter was similar to that of control soil. A total of 16 amino acids were detected, which were divided into five types according to the properties of substituent groups, namely non-polar aliphatic (such as 4-aminobutyric acid, L-methionine and L-valine), aromatic (such as L-phenylalanine and L-phenylalanine), polar uncharged (such as L-asparagine, L-threonine, L-glutamine and L-serine), positively charged (such as L-citrulline, L-ornithine, L-arginine, L-histidine and L-lysine) and negatively charged (such as L-citrulline, L-aspartic acid and L-glutamic acid) amino acids (Fig. 2). The content of the five types of amino acids in the soil was significantly increased after the addition of litter, and the content of the positively charged amino acids increased the most (Fig. 2). The finding also reveals that litter addition significantly increased protease activity in soil (Fig. 3). The addition of litter provides sufficient substrate for enzymatic reactions, which may lead to enhanced soil enzyme activity. In addition, soil microorganisms become active due to the stimulation of carbon sources in litter. The increased nitrogen requirements by the microorganisms promotes soil protease activity and produce more small nitrogen molecules for microbial use (Schaeffer and Evans 2005; Zong et al. 2018).

Conclusions

Our results show that litter addition significantly increased the content of soil amino acids, which is related to soil protease activity. The information obtained in this study may help in understanding the influence of plant-soil feedback on soil amino acid pools.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (31870434) and the National Key Research and Development Program of China (2021YFD1300504, 2018YFC1706301).

References

Gao, J.Q., Feng, J., Zhang, X.W., Yu, F.H., Xu, X.L. and Kuzyakov, Y. 2016. Drying-rewetting cycles alter carbon and nitrogen mineralization in litter-amended alpine wetland soil. *Catena*, 145:285-290.

Geisseler, D. and Horwath, W.R. 2010. Relationship between carbon and nitrogen availability and extracellular enzyme activities in soil. *Pedobiologia*, 53(1):87-98.

Jones, D.L., Shannon, D., Junvee-Fortune, T., Farrarc, J.F. 2005. Plant capture of free amino acids is maximized under high soil amino acid concentrations. *Soil Biol. Biochem.*, 37(1):179-181.

Lipson, D. and Näsholm, T. 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia*, 128(3):305-316.

Meier, C.L. and Bowman, W.D. 2008. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. *Proc. Natl. Acad. Sci. U. S. A.*, 105(50):19780-19785.

Näsholm, T. and Persson, J. 2001. Plant acquisition of organic nitrogen in boreal forests. *Physiol. Plant.*, 111(4):419-426.

Näsholm, T., Kielland, K. and Ganeteg, U. 2009. Uptake of organic nitrogen by plants. *New Phytol.*182(1):31-48.

Schaeffer, S.M. and Evans, R.D. 2005. Pulse additions of soil carbon and nitrogen affect soil nitrogen dynamics in an arid Colorado Plateau shrubland. *Oecologia*, 145(3):425-433.

Schimel, J.P. and Bennett, J. 2004. Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, 85(3):591-602.

Xu, X.L., Ouyang, H., Kuzyakov, Y., Richter, A. and Wanek, W. 2006. Significance of organic nitrogen acquisition for dominant plant species in an alpine meadow on the Tibet plateau, China. *Plant Soil*, 285(1-2):221-231.

Zong, W.Z., Wang, J., He, Y.S., Qiu, Y.F., Guo, D. and Fu, H. 2018. Net nitrogen mineralization and enzyme activities in an alpine meadow soil amended with litter tannins. *J. Plant Nutr. Soil Sci.*181(6):954-965.

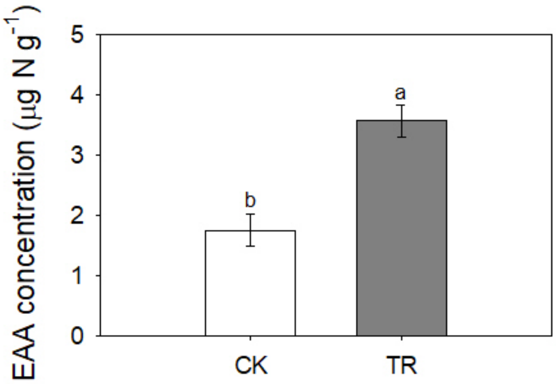

Figure 1. The effect of litter addition on the concentrations of exchangeable amino acids (EAA) in soil. Error bars indicate ± 1 SE, n = 3. CK and TR mean soil was amended without litter and with litter, respectively.

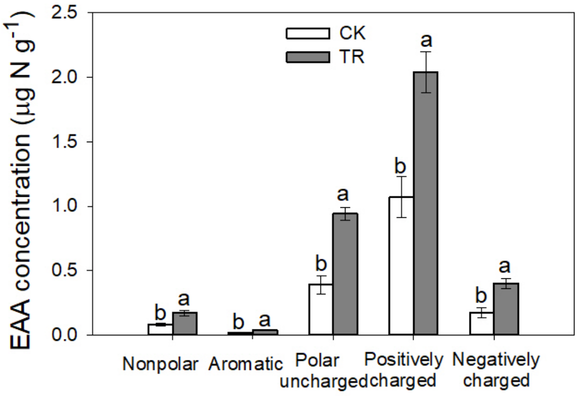

Figure 2. The effect of litter addition on different types of exchangeable amino acids in soil. Error bars indicate ± 1 SE, n = 3.

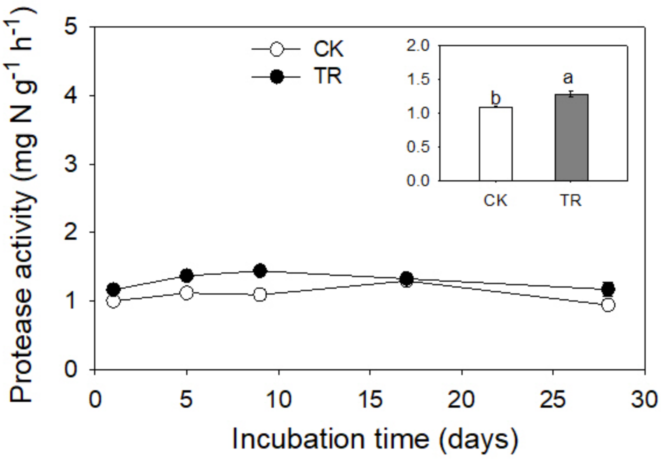

Figure 3. The effect of litter addition on soil protease activity. Error bars indicate ± 1 SE, n = 3. The embedded figure shows the weighed means of soil protease activity during the entire incubation.