Analysis of soil nematode community of alpine meadow in the middle of the Tibetan Plateau

Lei Hou1, 2, 3, Huiying Xue1, 4
1College of Resources & Environment, Tibet Agricultural and Animal Husbandry University, Nyingchi 860000, Tibet, China
2Key Laboratory of Forest Ecology in Tibet of Ministry of Education, Nyingchi 860000, Tibet, China
3Center for Ecological Research, Northeast Forestry University, Harbin 150040, China.
4Corresponding author’s e-mail: 472425717@qq.com

Abstract. The alpine meadow of Tibet Plateau is extensively distributed, and the soil nematode community may exhibit certain regional differences. To understand whether there were differences among soil nematode communities, soil samples were collected in the Bangjietang alpine meadow which is in the middle of the Tibet Plateau, and the composition of soil nematode communities was determined by high-throughput sequencing technology. It was found that from 0-10 cm to 10-20 cm, the composition of soil nematode community changed significantly, the relative abundance of Chromadorea at the class level decreased while that of Enoplea increased, the relative abundance of Rhabditida at the order level decreased while that of Dorylaimida increased. There were only eight genera of soil nematodes at the genus level, which was significantly different from the Northern Tibet region. The soil nematode communities were dominated by a bacterivorous trophic group. The soil chemical properties such as soil organic matter (SOM), available nitrogen (AN), total nitrogen (TN), total phosphorus (TP), Zn, Cu, Pb, and Cd affected the class and order level of soil nematodes, specifically, they both had negative effects on Chromadorea and Rhabditida, and positive effects on Enoplea and Dorylaimida. In general, our results indicate that the composition of soil nematode communities in the alpine meadow which was in the middle of the Tibet Plateau was similar but different from that in Northern Tibet Plateau, specifically, they were mainly bacterivorous trophic group, but Bangjietang alpine meadow contained few genera, and the dominant nematode communities were different at class and order levels.

1. Introduction
Soil organisms have received much attention in recent years due to their ecosystem functioning, which has central roles in global biogeochemistry, influencing the fertility of soils and the exchange of gases with the atmosphere [1]. As a dominant component of soil organisms, the nematode is the most abundant and accounts for an estimated four-fifths of all animals on land [2, 3]. They feature in all major trophic levels of the soil food web [4], play crucial parts in regulating carbon and nutrient dynamics, and are a good indicator of biological activity in soils [5-7]. However, despite our understanding of soil nematode at a global pattern [8], we still lack a basic understanding of biogeographical patterns in the Tibet Plateau, where is a crucial area but rarely studied. As we knew, the alpine meadow is one of the most important and extensively distributed ecosystems in the Tibet Plateau where is very fragile and sensitive to anthropogenic disturbance [9]. Previous studies on alpine meadow soil nematodes were
concentrated in the northern Tibet Plateau [10-12], but no studies were undertaken on other alpine meadows of the Tibet Plateau. Therefore, the information lacking the soil nematodes in other regions of the Tibet Plateau alpine meadow is impeding the understanding of biogeography in nematodes in the Tibet Plateau.

The traditional morphological identification method is used to study the diversity of nematodes, which is time-consuming and laborious [13]. The development of high-throughput sequencing technology provides a convenient tool for soil biological community research [14] and results are considered to have better consistency in nematodes [15]. Here, we used high-throughput sequencing methods to describe the soil nematode diversity and abundance in Bangjietang alpine meadow which is located in the middle of the Tibet Plateau to examine the difference of alpine meadow soil nematode communities in areas with different latitude in the Tibet Plateau. It provided a data foundation for the biogeographical pattern of the soil nematode communities in the Tibet Plateau alpine meadow.

2. Materials and Methods

2.1. Study site
In August 2019, we sampled soils in Bangjietang alpine meadow with a mean elevation of about 4450 m (92°26′19.9″E, 29°54′28.27″N), in the middle of the Tibet Plateau, belonged to Tibet Autonomous Region, China. The location has mean annual precipitation of 550 mm, a mean annual temperature of -3.8 °C, and a mean annual wind speed of 3 m/s. The main plants include *Kabresia pygmaea*, *Stipa purpurea*, *Androsace gmelinii*, *Potentilla ansrina*, *Pedicularis reaupinanta*, *Lamiophlomis rotate* and *Lancea tibetica*, etc [16].

2.2. Soil samples and determination of soil chemical properties
The soil samples were collected with a cylindrical collector at the depth of 0-10 cm and 10-20 cm from the Bangjietang alpine meadow which covered an area of 15 km². Soil was randomly selected from 3 different locations and mixed into 3 samples for each soil layer. They were packed in polyethylene bags labeled with the sample information and were kept on ice during transportation to the laboratory. The samples were air-dried and gravel, roots, and various residues removed. We crushed part of each soil sample and passed it through a 1-mm square-aperture sieve. Soil fertility determination was undertaken as follows: total nitrogen (TN) was assayed by use of the semi-trace Kelvin method, total phosphorus (TP) was assayed by use of the sodium carbonate melting method, available phosphorus (AP) was assayed by use of the hydrochloric acid-ammonium fluoride method, available nitrogen (AN) was assayed by use of the alkali hydrolysis diffusion method, and soil organic matter (SOM) was determined using the high-temperature external hot dichromate oxidation-capacity method. Soil heavy metal determination was described as: the total amounts of Cu, Zn, Pb, and Cd were determined by flame atomic absorption spectrometry (F-AAS) with a nitric acid-hydrofluoric acid-perchloric acid digestion system, pH was measured using the potential method [17].

2.3. High-throughput sequencing

2.3.1. DNA extraction and PCR amplification. Soil DNA was extracted according to the instructions of the E.Z.N.A. SOIL kit (Omega Bio-Tek, Norcross, GA, USA). The DNA concentration and purity were determined by NanoDrop2000, and DNA extract was checked by 1% agarose gel. PCR amplification was performed using NF15′ -GGTGGTGCATGGCCTTCTTAGTTATGT-3' and 18Sr2bR 5′ -TACAAAGGGCCAGGGACGTAAT-3′ primers [14, 18].

2.3.2. Illumina Miseq sequencing and analysis. The data were analyzed on the free online platform of Majorbio I-Sanger Cloud Platform (www.i-sanger.com). Specifically, the NCBI (NT) was used as a sequencing data comparison database. The vegan package in R was used for redundancy analysis (RDA)
to ascertain the correlation between soil nematode communities and soil chemical properties. The trophic group composition map of soil nematodes was prepared by GraphPad Prism 5.

3. Results

3.1. Analysis of sequencing data and nematode community diversity

Random sampling of optimized sequences and construction of rarefaction curves by the number of sampled sequences and the number of OTUs they can represent. OTUs (Operational Taxonomic Units) are the same markers artificially assigned to a taxonomic unit (strain, species, genus, group, etc.) in phylogenetic or population genetics studies to facilitate analysis. In rarefaction curves, both the 6 soil samples attended saturation plateau (Figure 1a). The results of rarefaction curves showed that OTUs were similar in different soil layer samples. The different soil layers shared 13 OTUs of the total 30 OTUs (Figure 1b).

![Figure 1a](image1.png)

![Figure 1b](image2.png)

**Figure 1.** Diversity analysis of Bangjietang alpine meadow soil nematode sequence reads. (a) Rarefaction curves for nematode OTUs in the different soil layers, (b) OTU Venn diagram of the soil nematode communities.

3.2. Nematode community composition analysis at the class and order levels

The classification of sequences demonstrated nematode community differences in different soil layers at the class level and order levels. In this study, the sequences were clustered into 3 nematode classes (2 identified classes) and the abundance of each class varied in two soil layers. It was obvious that Chromadorea was the most dominant class in the depths of 0-10 cm, accounting for 68.31%, followed by unclassified_p_Nematoda (31.32%) and Enpolea (0.37%). In the depths of 10-20 cm, the above 3 classes all accounted for more than 25% of the total population in descending order of unclassified_p_Nematoda, Chromadorea and Enpolea, accounted for 39.65%, 32.26%, and 28.09%, respectively (Figure 2a). These results demonstrated that Chromadorea which was identified as a dominant class and was decreasing by 36.05%, whereas Enpolea was increasing by 27.72% from the surface to deep soil.
Figure 2. The relative abundances of soil nematode communities at (a) the class level and (b) the order level in the different soil layers.

At the order level, the sequences were clustered into 5 nematode classes (4 identified classes), and the abundance of each order varied in the different soil layers. It was obvious that Rhabditida was the most dominant nematode order in the depths of 0-10 cm, accounting for 60.00%, followed by unclassified_p_Nematoda and Tylenchida, accounting for 31.32% and 8.31%, respectively. In the depths of 10-20 cm, unclassified_p_Nematoda predominated for 39.65%, followed by Rhabditida, Dorylaimida, Triplonchida and Tylenchida, accounting for 30.44%, 23.48%, 4.61% and 1.82%, respectively (Figure 2b). The results showed that Rhabditida which was identified as a dominant order and was decreasing by 29.56%, whereas Dorylaimida was increasing by 23.48% from the surface to deep soil.

3.3. Nematode community composition analysis at the family level and genus level

The heatmap analysis at the family level revealed that nematode communities were classified into 8 families (7 identified families). The overall nematode composition of the families differed in the different soil layers. It was obvious that Cephalobidae and unclassified_p_Nematoda were the most dominant family in the depths of 0-10 cm. In the deeper soil of 10-20 cm, the dominant families were unclassified_p_Nematoda and Cephalobidae (Figure 3a). At the genus level, the heatmap analysis revealed that nematode communities were classified into 8 different genera (7 identified genera). It was obvious that Acrobeloides and unclassified_p_Nematoda were the most dominant genus in the depths of 0-10 cm and 10-20 cm (Figure 3b). The results showed that Cephalobidae and Acrobeloide which were identified were the most dominant family and genus from the surface to deep soil.
3.4. Trophic groups composition of soil nematode communities
To display the differences in trophic group composition in the different soil layers, soil nematodes were classified into the trophic group at the genus level. The bacterivorous (Ba) group predominated among trophic groups among the soil nematodes that were identified. Since unclassified_p_Nematoda (unknown) was not classified into a specific trophic group, the soil nematode communities were dominated by bacterivorous, 0-10 cm and 10-20 cm accounted for 60.18% and 35.11%, respectively (Figure 4). It should be noted that omnivores-predator nematodes (Op) were only accounted for 0.07% from 0-10 cm and suddenly increased to 23.39% from 10-20 cm which presented that the deeper soil had more nutrients available for omnivores-predator to use. These results indicated that the bacterivorous were the dominant trophic group in both soil layers.

3.5. Relationships between nematode communities and soil chemical properties
The observed soil chemical properties, including soil fertility and heavy metals, affected soil nematode community composition. In soil fertility, the RDA diagram revealed that SOM, AN, TN, and TP were
relatively more important variables than AP in explaining variations in the nematode communities composition in different soil layers at Bangjietang alpine meadow. At the class level, the 1st axis explained 86.56% and the 2nd axis for 13.44% of the variations in soil nematode communities compositions. TP was the most important influence factor to the 1st axis with a correlation coefficient of -0.90, and AP was the most important influence factor to the 2nd axis with a correlation coefficient of -0.83. For the identified nematode class, Enoplea was positively correlated with SOM, AN, TN, and TP, and no correlation with AP while Chromadorea was negatively correlated with SOM, AN, TN, and TP, and positively correlated with AP (Figure 5a). At the order level, the 1st axis explained 83.79% and the 2nd axis for 14.54% of the variations in soil nematode compositions. TP was the most important influence factor to the 1st axis with a correlation coefficient of 0.91, and AP was the most important influence factor to the 2nd axis with a correlation coefficient of -0.80. For the identified nematode order, Dorylaimida was positively correlated with SOM, AN, TN, and TP, and no correlation with AP while Rhabditida was negatively correlated with SOM, AN, TN, and TP, and positively correlated with AP (Figure 5b).

**Figure 5.** Redundancy analysis (RDA) diagram of soil nematode communities and soil fertility properties at (a) class level and (b) order level.

In soil heavy metal, the RDA diagram revealed that Cu, Pb, Cd, and pH were relatively more important variables than Zn in explaining variations in the nematode communities composition in different soil layers at Bangjietang alpine meadow. At the class level, the 1st axis explained 86.56% and the 2nd axis for 13.44% of the variations in soil nematode compositions. Zn was the most important influence factor to the 1st axis with a correlation coefficient of -0.89, and Cd was the most important influence factor to the 2nd axis with a correlation coefficient of -0.99. For the identified nematode class, Enoplea was positively correlated with Zn, Cu, Pb, Cd, and no correlation with pH, while Chromadorea was negatively correlated with Zn, Cu, Pb, Cd, and positively correlated with pH (Figure 6a). At the order level, the 1st axis explained 83.79% and the 2nd axis for 14.54% of the variations in soil nematode communities compositions. Zn was the most important influence factor to the 1st axis with a correlation coefficient of 0.98, and Cd was the most important influence factor to the 2nd axis with a correlation coefficient of -0.99. For the identified nematode order, Dorylaimida was positively correlated with Zn, Cu, Pb, Cd, and negatively correlated with pH, while Rhabditida was negatively correlated with Zn, Cu, and Pb, and positively correlated with pH (Figure 6b).
Figure 6. Redundancy analysis (RDA) diagram of soil nematode communities and soil heavy metal contents at (a) class level and (b) order level.

4. Discussion

Our study mainly focused on soil nematode assemblages in the Bangjietang alpine meadow. With the different soil layers, the soil properties affected nematode fauna.

4.1. Soil nematode communities composition

Soil nematode is a type of soil organism that is sensitive to environmental factors [19] and its community distribution is affected by different ecological types [13]. In the same ecological type, due to its wide range, differences may arise in soil nematode communities within the same ecological type. Among the identified soil nematode communities at class level in the Bangjietang alpine meadow in the Tibet Plateau, Chromadorea and Enpolea accounted for 68.31% and 0.37% respectively in soils at 0-10 cm depth, and Chromadorea and Enpolea accounted for more than 25% (32.26% and 28.09%, respectively) at depths of 10-20 cm. Chromadorea decreased and Enpolea increased from depths of 0-10 cm to 10-20 cm. Previous studies in the alpine meadow in northern Tibet Plateau demonstrated that Enpolea and Chromadorea were the two classes with the highest relative abundance, and Enpolea was the dominant nematode class in the 0-25 cm soil layer [20, 21], and studies on soil nematodes in alpine meadows in other parts of the world have been conducted in areas lower than ours and have mostly used traditional identification methods [22, 23]. The difference between the two regions in Tibet Plateau indicated that there were some environmental differences, and it affected the dominant community of soil nematode. For the identified soil nematode communities at order level in the Bangjietang alpine meadow of the Tibet Plateau, Rhabditida (60.00%) was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%. With increasing depth from 0-10 cm to 10-20 cm, Rhabditida decreased while Dorylaimida increased. Studies on alpine meadow soils in the northern Tibet Plateau showed that Dorylaimida was the most dominant nematode order in the 0-10 cm soil layer, and Tylenchida only accounted for 8.31%; in the 10-20 cm soil layer, Rhabditida was the most dominant nematode order, accounting for 30.44%, Dorylaimida accounted for 23.48%.
The Fourth International Workshop on Environment and Geoscience

IOP Conf. Series: Earth and Environmental Science 865 (2021) 012048
doi:10.1088/1755-1315/865/1/012048

meadow was not only different [12] but also different plant species within the similar plant community might lead to differences in dominant soil nematode communities.

Compared to the northern Tibet Plateau alpine meadow where there were 30 genera in the northern Tibet Plateau [20], there were only 8 nematode genera in the Bangjietang alpine meadow. This phenomenon may be related to the distribution of soil organic matter caused by latitude [8]. To our knowledge, the alpine meadow in northern Tibet lay at a higher latitude, so it was speculated that it had the greater soil nematode genera.

Among the identified nematodes, bacterivorous nematodes accounted for 60.18% in the 0-10 cm soil layer and 35.11% in the 10-20 cm soil layer. Therefore, the nematode trophic group in the 0-20 cm soil layer were mainly bacterivorous, and there were relatively few nematodes in other trophic groups. Our results were consistent with those reported by Xue et al in *Kobresia littledalei* community which in the northern Tibet Plateau alpine meadow [12]. Meanwhile, an increase of bacterivorous nematodes could reflect the increase of soil bacteria and soil organic matter [25], which were consistent with the previous inference.

4.2. Effects of soil chemical properties on soil nematode communities

Nematode communities have been found to be related to environmental variables such as rainfall and temperature [26-28], and our results showed that the soil nematode communities in Bangjietang alpine meadow are related to soil chemical properties. Previous studies have shown that phosphorus or nitrogen, and heavy metal contamination such as Cu, Cd, Pb, Zn have negative effects on nematode communities [29-32]. Combined with this study, phosphorus or nitrogen, and Zn, Cu, Pb, Cd may have negative effects on nematode communities of Chromadorea and Rhabditida, specifically. Besides, as the nematode can obtain resources from soil organic matter, the abundance of nematodes depends on the amount of soil organic matter. In our study, soil organic matter has a positive effect on Enoplea and Dorylaimida.

5. Conclusions

Our results suggest that the Chromadorea and Rhabditida are the most dominant nematode fauna in Bangjietang alpine meadow where is situated in the middle of the Tibet Plateau, and the bacterivorous nematode is the dominant trophic group. The SOM, AN, TN, TP, Cu, Zn, Pb, Cd are important factors that affect the Enopla, Chromadorea, Rhabditida, and Dorylaimida in the present study, and the SOM may be the most important factor contributing to soil nematode community species in alpine meadows of the northern and central Tibetan Plateau.

Acknowledgements

All authors have read and agreed to the published version of the manuscript. Funding: This research was funded by Key Laboratory of Forest Ecology in Tibet of Ministry of Education, grant number XZA-JYBSYS-2020-07.

References

[1] Paul E A 2015 *Soil microbiology, Ecology, and Biochemistry 4th edn* Elsevier

[2] Yeates G W 2003 Nematodes as soil indicators: functional and biodiversity aspects *Biol Fert Soils* 37 199-210

[3] Bardgett R D, Putten W H V D 2014 Belowground biodiversity and ecosystem functioning *Nature* 515 505-511

[4] Song M, Liu Y Z, Jing S S 2015 Response of soil nematodes to climate change: a review *Acta Ecologica Sinica* 35 6857-6867

[5] Freckman D W, Caswell E P 1985 The ecology of nematodes in agroecosystems *Annu Rev Phytopathol* 23 275-296

[6] Ferris H 2010 Contribution of Nematodes to the Structure and Function of the Soil Food Web *Journal of Nematology* 42 63-67
[7] Neher D A 2002 Role of Nematodes in Soil Health and Their Use as Indicators *Journal of nematology* **33** 161-168
[8] Hoogen J V D, Geisen S, Routh D, Ferris H, Crowther T W 2019 Soil nematode abundance and functional group composition at a global scale *Nature* **572** 194-198
[9] Xu M H, Xue X 2013 A research on summer vegetation characteristics & short-time responses to experimental warming of alpine meadow in the Qinghai-Tibetan Plateau *Acta Ecologica Sinica* **33** 2071-2083
[10] Xue H Y, Luo D Q, Wang H Y, Qu X L 2017 Effects of free grazing or enclosure on soil nematodes in alpine meadows in North Tibet, China *Acta Pedologica Sinica* **54** 480-492
[11] Xue H Y, Luo D Q, Hu F, Li H X, Wang J S, Qu X L, Wang H Y, Yu B Z, Sun Q 2016 Effect of short-term enclosure on soil nematode communities in an alpine meadow in Northern Tibet *Acta Ecologica Sinica* **36** 6139-6148
[12] Xue H Y, Hu F, Luo D Q 2013 Effects of alpine meadow plant communities on soil nematode functional structure in Northern Tibet, China *Acta Ecologica Sinica* **33** 1482-1494
[13] Geisen S, Snoek L B, Ten Hooven F C, Duyts H, Kostenko O, Bloem J, Martens H, Quist C W, Helder J A, Van der Putten W H 2018 Integrating quantitative morphological and qualitative molecular methods to analyse soil nematode community responses to plant range expansion *Methods Ecol Evol*
[14] Xia W W, Jia Z J 2014 Comparative analysis of soil microbial communities by pyrosequencing and DGGE *Acta Microbiologica Sinica* **54** 1489-1499
[15] Du X F, Li Y B, Han X, Ahmad W, Li Q 2020 Using high-throughput sequencing quantitatively to investigate soil nematode community composition in a steppe-forest ecotope *Appl Soil Ecol* **152** 103562
[16] Wang X T, Gao Y, Wei X H, Sun L, Zhao Y H, Chen D D, Yixi C M, Miao Y J 2014 Effects of different grazing intensities on the soil seed bank of alpine meadow in the bangjietang research station of Tibet *Acta Agrestia Sinica* **22** 750-756
[17] Bao S D 2000 *Soil agrochemical analysis* China Agriculture Press
[18] Zhu X Z, Li Q, Li Y P, Han H B, Ma K P 2015 Eupatorium adenophorum invasion alters soil bacterial community and diversity *Biodiversity Science* **23** 665-672
[19] Fiscus D A, Neher D A 2002 Distinguishing sensitivity of free-living soil nematode genera to physical and chemical disturbances *Ecol Appl* **12** 565-575
[20] Xue B, Hou L, H Y Xue 2019 Research on the characteristics of soil nematode communities in alpine meadow in northern Tibet by using high-throughput sequencing *Acta Ecologica Sinica* **39** 4088-4095
[21] Hou L, Xue B, Xue H Y 2019 Study on soil nematode community of northern Tibetan alpine meadow under simulated warming condition by high-throughput sequencing method *Acta Agrestia Sinica* **27** 443-451
[22] Háneš L 2017 Soil nematodes in alpine meadows of the Tatra National Park (Slovak Republic) *Helminthologia* **54**
[23] Reno M, Gmyrová E, Erevková A 2020 The Effect of Soil Type and Ecosystems on the Soil Nematode and Microbial Communities *Helminthologia* **57** 129-144
[24] Wei X H, Yang P, Xie Z K, Wang Y J 2003 Distribution and utilization of Kobresia pygmaea type pastures in Naqu prefecture of Tibet *Acta Agrestia Sinica* **67**-74
[25] Griffiths B S, Ritz K, Wheatley R E 1994 Nematodes as indicators of enhanced microbiological activity in a Scottish organic farming system *Soil Use and Management* **10** 20-24
[26] Nielsen U N, Ayres E, Wall D H, Li G, Bardgett R D, Wu T, Garey J R 2015 Global - scale patterns of assemblage structure of soil nematodes in relation to climate and ecosystem properties *Global Ecology & Biogeography* **23** 968-978
[27] Song D, Pan K, Tariq A, Sun F, Li Z, Sun X, Zhang L, Olusanya O A, Wu X 2017 Large-scale patterns of distribution and diversity of terrestrial nematodes *Applied Soil Ecology* **161**-169
[28] Thakur M P, Real I D, Cesarz S, Steinauer K, Reich P B, Hobbie S, Ciobanu M, Rich R, Worm K,
Eisenhauer N 2019 Soil microbial, nematode, and enzymatic responses to elevated CO2, N fertilization, warming, and reduced precipitation Soil Biology and Biochemistry

[29] Zhao J, Wang F, Li J, Zou B, Wang X, Li Z, Fu S 2014 Effects of experimental nitrogen and/or phosphorus additions on soil nematode communities in a secondary tropical forest Soil Biology and Biochemistry

[30] Park B Y, Lee J K, Ro H M, Kim Y H 2011 Effects of heavy metal contamination from an abandoned mine on nematode community structure as an indicator of soil ecosystem health Applied Soil Ecology 51 17-24

[31] Navas A, Flores-Romero P, Sánchez-Moreno S, Camargo J A, Megawley E C 2010 Effects of heavy metal soil pollution on nematode communities after the aznalcollar mining spill Nematropica 40 13-29

[32] Li Q, Zhong S, Li F, Lou Y 2011 Nematode community structure as bioindicator of soil heavy metal pollution along an urban?rural gradient in southern Shenyang, China International Journal of Environment & Pollution 45 297-309