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RESEARCH ARTICLE

Assessment of soil nematode diversity based on different taxonomic levels and functional groups

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ABSTRACT

Although soil nematode diversity has been used as an indicator of habitat characteristics and environmental change, the diversity of entire soil nematode communities has not been comprehensively evaluated at different taxonomic levels, or for different functional groups, or at a fine taxonomic level within functional groups. In this study, two taxonomic diversity indices, the Shannon-Wiener index (H′) and Simpson index (λ), were used to evaluate the following: 1) nematode diversity at different taxonomic levels for the whole community, 2) nematode diversity of different functional groups, and 3) nematode generic diversity of functional groups in the following four land-use types: forage land, cropland, secondary forest, and grass-shrubland. The results showed that significant differences in nematode diversity among land-use types were detected by assessment at the order level but not at the family or genus level. The results also showed that significant differences in nematode diversity were better revealed by assessment of trophic groups rather than *cp* groups. The generic diversities (H′) of omnivorous nematodes and *cp*3 nematodes also significantly differed among land-use types. Our results indicate that diversity at a high taxonomic level (i.e., order) may be a more useful indicator than diversity at a low taxonomic level (i.e., family or genus) of differences among land-use types. In addition, the functional group diversity (i.e., trophic group, *cp* group, and the combination of these two groups) for the whole community and the taxonomic diversity within functional groups were useful indicators of differences among land-use types.

1 Introduction

Nematodes are abundant soil animals that affect many important ecosystems processes (Freckman and Caswell, 1985; Bongers and Ferris, 1999; Ferris et al., 2001). The diversity of soil nematodes can differ greatly among ecosystems (Boag and Yeates, 1998; Nielsen et al., 2014; Song et al., 2017), and assessment of nematode diversity has become an important research area in soil ecology. Nematode diversity can reflect soil biodiversity, soil resource diversity, and resource utilization diversity, and can serve as a useful indicator of the nutrient status of the ecosystem and the structure of the soil food web (Neher and Barbercheck, 1998; Ferris et al., 2001; Ferris et al., 2012; Ferris and Tuomisto, 2015). A useful indicator should have the function of reflecting and predicting ecosystems processes accurately. However,

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the relationship between ecosystems processes and diversity is unclear, most nematode communities have a lack of understanding, and a single community diversity index cannot clearly describe the ecosystems processes (Neher and Darby, 2009). Most recent assessments of nematode communities have not been based on taxon diversity but instead have focused on the use of nematode faunal indices, such as the maturity index, structure index, enrichment index, channel index, and metabolic footprint index (Bhusal et al., 2014).

Nematodes can be classified at different taxonomic levels (order, family, genus, and species) and can also be assigned to different functional groups (Bongers and Bongers, 1998; Ferris et al., 2001; Yeates, 2003). Because most previous studies have identified nematodes to the genus level, nematodes taxomic diversity generally refers to generic diversity. However, nematode genera vary in their responses to ecological disturbances, which could cause nematode faunal indices to incorrectly indicate responses to disturbances (Fiscus and Neher, 2002; Zhao and Neher, 2013). In addition, whether species-level designations are necessary in ecological studies has been debated. Some researchers have argued that species-level designations are too costly and time consumming (Boag and Yeates, 1998; Pik et al., 1999; Terlizzi et al., 2003). Consistent with this view, Bhusal et al. (2014) reported that taxonomic identification at both a low level (genus) and a high level (order or family) can reliably indicate nematode diversity in different habitats. However, assessments of nematode diversity based on nematode order, family, genus, feeding group and/or functional guild have not been comprehensively compared, nor has the generic diversity for different feeding groups and functional guilds been comprehensively analyzed (Bhusal et al., 2014). In addition, it is unclear whether the nematode faunal indices or the diveristy indices are better predictors for distinguishing significnat diffences in nematode communities of different ecosystems or under different disturbance types.

The Shannon–Wiener index (H′) and the Simpson index (λ) are commonly used to describe the taxonomic diversity of soil nematodes (Yeats and Bongers, 1999; Neher and Darby, 2009). The Shannon-Wiener index gives more weight to rare species, and the Simpson dominance index gives more weight to dominant species (Neher and Darby, 2009; Zhao et al., 2014). The current study used both of these indices to evaluate the following for the whole nematode community: 1) nematode diversity at different taxonomic levels (e.g., order, family, and genus) for the whole community, 2) nematode diversity of different functional groups (e.g., trophic groups and/or *colonizer-persister* [*cp*] groups), and 3) nematode generic diversity of functional groups in the following four land-use types: forage land, cropland, secondary forest, and grass-shrubland. The objectives were to determine whether the analysis of nematode biodiversity requires fine-level designations to detect environmental differences associated with land-use differences and whether the generic diversity for a given nematode functional group reflects environmental differences.

2 Methods

2.1 Data collection

The data used in this research were obtained from a study that assessed soil nematode abundance and generic composition in four land-use types in south-west China. Detailed information about the study and the study sites was provided by Zhao et al. (2015). In brief, the study was conducted at Huanjiang Observation and Research Station for Karst Ecosystems (107°51′–108°43′E, 24°44′–25°33′N), Chinese Academy of Sciences (CAS), Guangxi Province, China. The four land-use types were forage land, cropland, secondary forest, and grass-shrubland. Each land-use type was represented by four replicate sites. All sites had a brown calcareous soil that developed from a dolostone base.

2.2 Data analysis

To determine the effects of taxonomic level on the assessment of diversity, the nematode genera were assigned to families and orders. Although assignments to nematode trophic group and life history strategy (also known as the *colonizer-persister* [*cp*] scale) are mainly based on generic and family identifications (Yeates et al., 1993; Bongers and Bongers, 1998), diversity based on trophic group or *cp* group may be different from diversity based on genus, family, and order. In addition, the generic diversity may differ among trophic groups or *cp* groups, and the generic diversity of trophic groups or *cp* groups may differ from the generic diversity of the whole nematode community. Therefore, nematodes were assigned to five trophic groups (i.e., bacterivore, fungivore, herbivore, omnivore, and predator) according to Yeates et al. (1993) and Okada et al. (2005), to five *cp* groups (i.e., *cp*1-5) according to Bongers and Bongers (1998), and to 16 functional guilds (i.e., a combination of trophic group and *cp* group: Ba1-4, Fu2-4, He2-5, Om4-5, and Pr3-5). The Shannon-Wiener index (H′) and the Simpson index (λ) were calculated for various classification levels and different groups as follows:

$$\text{Shannon-Wiener index, } H' = -\Sigma_{i=1}^{s} Pi * \ln Pi$$

$$\text{Simpson index, } \lambda = \Sigma Pi^2$$

where '*Pi*' is the proportion of the individuals of "*i* th" taxon in a nematode assemblage, and '*s*' is the total number of nematode taxa in the given assemblage.

Before analysis, data were $\log(x+1)$ transformed when required to improve normality and homogeneity of variance. One-way ANOVAs were used to examine differences in nematode diversity among the four land-use types. When ANOVAs were significant ($p < 0.05$), LSD was used to test for difference among land-use types. Tamhane's T2 was used to test for differences among land-use types when the variances of transformed data were not equal. The one-way ANOVAs were performed using SPSS24 software (SPSS Inc., Chicago, IL).

3 Results

3.1 Diversity indices for the whole nematode communities at different taxonomic levels and of different functional groups

The patterns of nematode generic diversity and family diversity as indicated by either H′ or λ did not significantly differ among the four land-use types (Fig. 1A,B and Fig. 2A,B). At the order level, H′ was significantly lower for the cropland than for the secondary forest or grass-shrubland (Fig. 1C) but did not significantly differ among the forage land, secondary forest, and grass-shrubland (Fig. 1C). At the order level, λ was significantly higher for the cropland than for secondary forest (Fig. 2C) but did not significantly differ between cropland/secondary forest and forage land/grass-shrubland (Fig. 2C). For trophic groups, H′ did not significantly differ among the four land-use types (Fig. 1D). For trophic groups, λ was significantly higher for the grass-shrubland than for the secondary forest (Fig. 2D). For *cp* groups, neither H′ nor λ significantly differed among the four land-use types (Fig. 1E and 2E). At the functional guild level, H′ was significantly lower for the cropland than for the grass-shrubland but did not significantly differ between the forage land and the secondary forest (Fig. 1F). At the functional guild level, λ did not significantly differ among the four land-use types (Fig. 2F).

3.2 Diversity indices for genus composition of nematode functional groups

For the generic diversity of bacterivores, fungivores, herbivores, and predators, neither H′ nor λ significantly differed among the four land-use types (Fig. 3 A,B,C,E and Fig. 4 A,B, C,E). For the generic diversity of the omnivores, H′ was significantly higher for the grass-shrubland than for forage land or secondary forest (Fig. 3D) but did not significantly differ among the forage land, cropland, and secondary forest (Fig. 3D). For the generic diversity of the omnivores, λ was significantly higher for the secondary forest than for the cropland or grass-shrubland (Fig. 4D) but did not significantly differ among the forage land, cropland, and grass-shrubland (Fig. 4D). Values of λ of the *cp*1 group did not significantly differ among the four land-use types (Fig. 4F). Neither H′ nor λ of the *cp*2 group significantly differed among the four land-use types (Fig. 3F and Fig. 4G). For the *cp*3 group, H′ was significantly higher for the grass-shrubland than for the forage land (Fig. 3G) but did not significantly differ among cropland, secondary forest, and grass-shrubland (Fig. 3G). Values of λ for the *cp*3 group did not significantly differ among the four land-use types (Fig. 4H). As indicated by either H′ or λ, the generic diversity for the *cp*4 group and the *cp*5 group did not significantly differ among land-use types (Fig. 3H,I and Fig. 4 I,J).

4 Discussion

We found that the diversity at the order level was more sensitive to land-use types than diversity at the family or genus level, indicating that assessment of lower taxonomic levels is not always better than assessment at higher levels at reflecting land-use effects. Additionally, the patterns of the nematode generic diversity and family diversity were basically

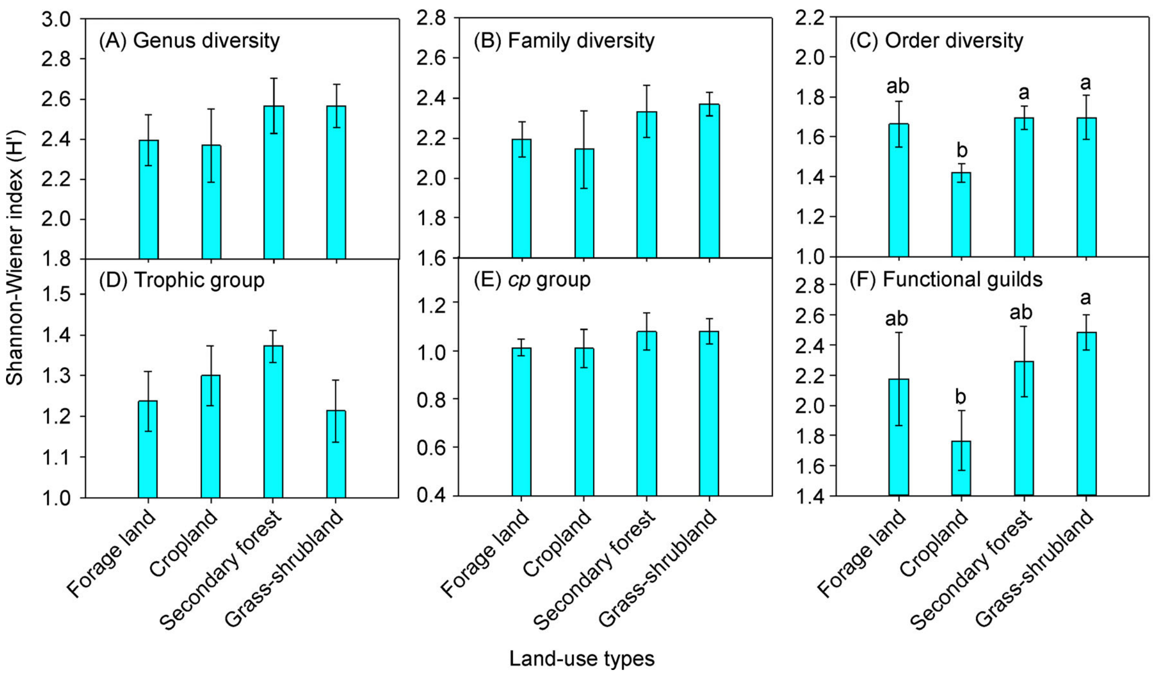

Fig. 1 The Shannon–Wiener index (H′) of the whole nematode community at the level of genus (A), family (B), order (C), trophic group (D), *cp* group (E), and functional guild (F) in the forage land, cropland, secondary forest, and grass-shrubland. Values are means±SE. Different letters indicate significant ($p < 0.05$) differences among land-use types.

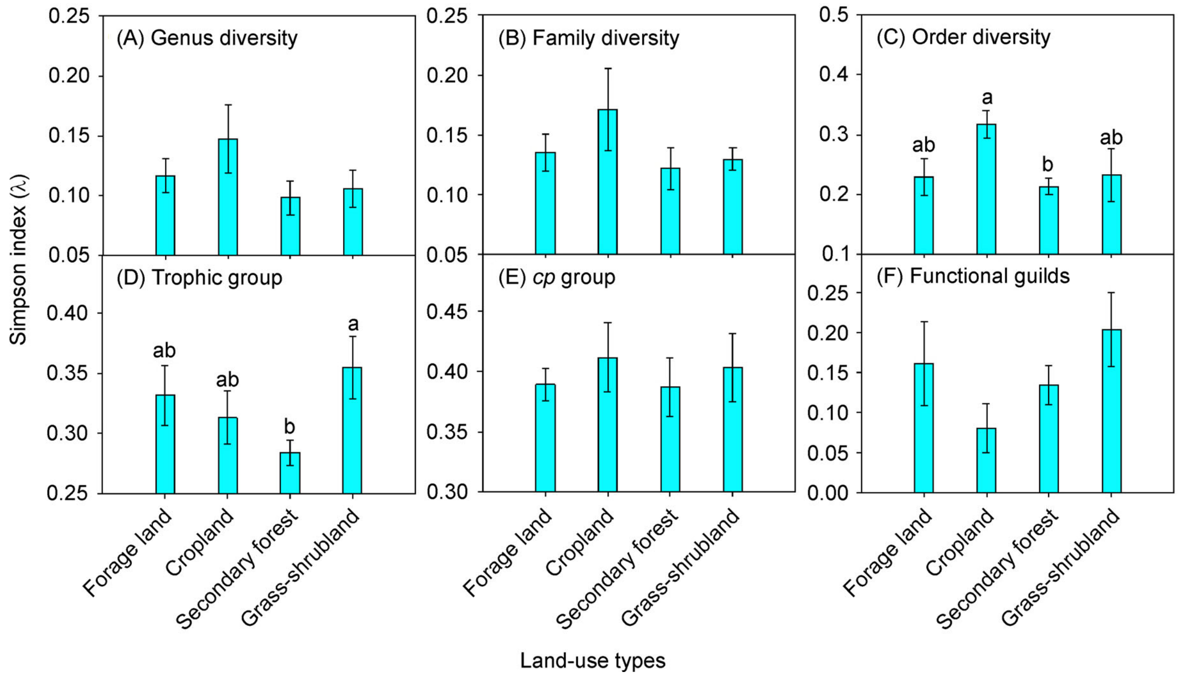

Fig. 2 The Simpson index (λ) of the whole nematode community at the level of genus (A), family (B), order (C), trophic group (D), *cp* group (E), and functional guild (F) in the forage land, cropland, secondary forest, and grass-shrubland. Values are means±SE. Different letters indicate significant ($p < 0.05$) differences among land-use types.

the same, which was consistent with previous reports (Bhusal et al., 2014). The inconsistent responses of order, family, and genus diversity to land-use types can probably be explained by taxon number. Although only 18% of the taxonomic units were reduced after assigning nematode genera to their corresponding families, 60% of the taxonomic units were reduced after assigning nematode genera to their corresponding orders. It follows that the weights of taxa (their effects on diversity assessment) did not differ between assessments of genera and families but were substantially increased for assessments of orders vs. assessments of genera and families. Whether this effect of taxon level on taxon weight is a common phenomonen warrants further study.

The patterns of the diversities differed for nematode trophic groups, *cp* groups, and functional guilds. In particular, trophic group diversity was more sensitive to land-use types than *cp* group diversity. This finding is consistent with Bhusal et al. (2014), who reported that trophic diversity could be used as a reliable indicator of the effects of environment on diversity (Bhusal et al., 2014). In addition, the functional guild diversity as indicated by the Shannon-Weiner index significantly differed among land-use types. The latter finding indicates that diversity at higher classification levels may be inferior to the diversity of functional groups in reflecting the characteristics of different ecosystems or land-use types. Soil nematodes feed on various food resources and occupy several trophic levels in the soil food web (Yeates et al., 1993; Ferris et al., 2001). The environments and food resources generally differ among ecosystems. Therefore, nematode functional group composition (i.e., the diversity-weighted abundance) may reflect the diversity and/or heterogeneity of resources and environments (Ferris and Tuomisto, 2015).

For the five trophic groups and the five *cp* groups, only the generic diversity of omnivores and of *cp*3 nematodes significantly differed among land-use types. Omnivorous nematodes feed on diverse food resources, which affect other soil organisms (including other nematodes) and may reflect the biodiversity and diversity of soil food resources (Ferris and Tuomisto, 2015; Song et al., 2017). The nematodes in the *cp*3 group are relatively sensitive to disturbances (Bongers and Bongers, 1998). Therefore, disturbance may be one of the factors affecting the distribution of nematodes in different land-use types; cropland fertilization, for example, reduced the numbers of omnivorous nematodes (Neher and Barbercheck, 1998). In the current study, the grass-shrubland had developed with little anthropogenic disturbance for more than 20 years and had substantial plant diversity that provided diverse food resources for soil organisms (Zhao et al., 2015). This suggests that diverse food resources and an undisturbed environment may be conducive to omnivorous and *cp*3 nematodes. Therefore, the taxon diversity within each functional group may also be a good indicator of soil biodiversity, soil resource diversity, and ecosystem disturbance.

As noted above, diversity assessment in different taxonomic levels and for different groups revealed significant effects of land-use types on soil nematode communities and could be served as good indicators. Surprisingly, however, the commonly used nematode faunal indices had no significant difference among the four land-use types except the plant-

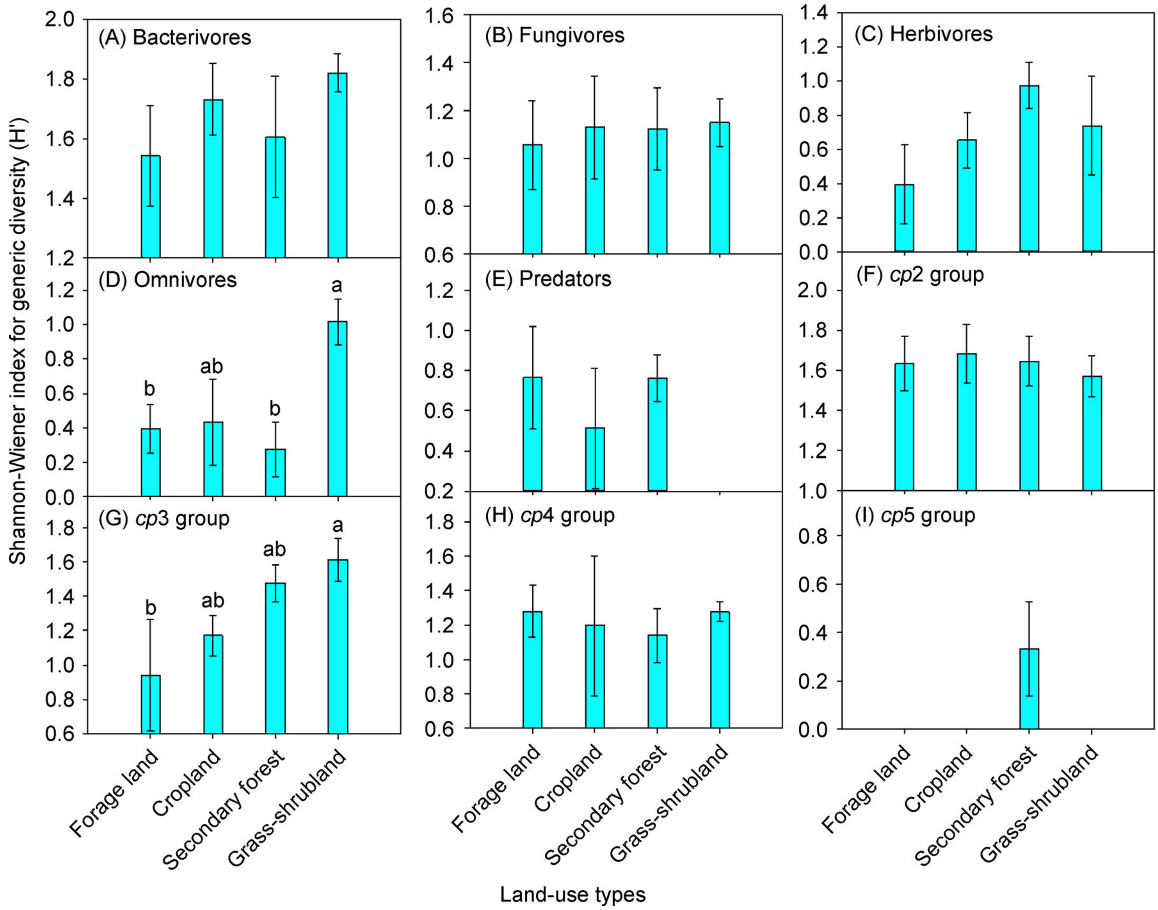

Fig. 3 The Shannon-Wiener index (H′) for the generic diversity of the bacterivores (A), fungivores (B), herbivores (C), omnivores (D), predators (E), *cp*2 group (F), *cp*3 group (G), *cp*4 group (H), and *cp*5 group (I) in the forage land, cropland, secondary forest, and grass-shrubland. Values are means±SE. Different letters indicate significant ($p < 0.05$) differences among land-use types.

parasite index (Supporting information, Table S1). Particularly, the plant-parasite index was apparent higher in the grass-shrubland than those in the other three land-use types (Supporting information, Table S1). Therefore, comprehensive assessments of the nematode diversity in different taxonomic levels and for different groups may provide important information that could not revealed by the nematode faunal assessments. The diversity indices is mainly based on analysis of relative abundance taxa and/or the number of taxa (Yeates, 1984; Yeats and Bongers, 1999), while the faunal indices is mainly based on analysis of relative abundance taxa and life history strategies (i.e. feeding types and *cp* values for nematodes) (Bongers and Ferris, 1999; Ferris et al., 2001). Since nematode life history strategies quantified, we think the basis of the diversity indices and faunal indices may be similar and both of them could reflect the nematode community compositions. Indeed, a single community diversity index cannot clearly describe the ecosystem properties (Neher and Darby, 2009). But a series of diversity indices calculated for different groups and/or in different taxonomic levels may be better in revealing the ecosystem properties. Future studies could further help determine whether the diversity indices are better indicators than the faunal indices.

In addition, data variance, as indicted by the error bar, was high for each variable in each land-use type in this study. Especially, the variances of generic diversity in predators were larger than those in bacterivores, fungivores, herbivores and omnivores, and in *cp*4 and *cp*5 groups were larger than those in *cp*2 and *cp*3 groups. The most likely reason for the high variances of generic diversity in predators, *cp*4 and *cp*5 groups is that the abundances of nematode genera in these groups are very low and may be undetectable. Number of replications is an important factor determined the data variance (Chalcraft, 2019). The larger the number of replications, the smaller the data variance. Furthermore, the statistical rule cannot be revealed if the replication number is too small (Li et al., 2000; Shao et al., 2009). We believe that more replications are conducive to reduce the variances of data and may help to reveal more information. However, increasing replications will increase the time and resource investment in nematode identification. Therefore, a reasonable replication number should be considered for given studies based on a tradeoff between potential performance improvement of statistical analysis and time and resource

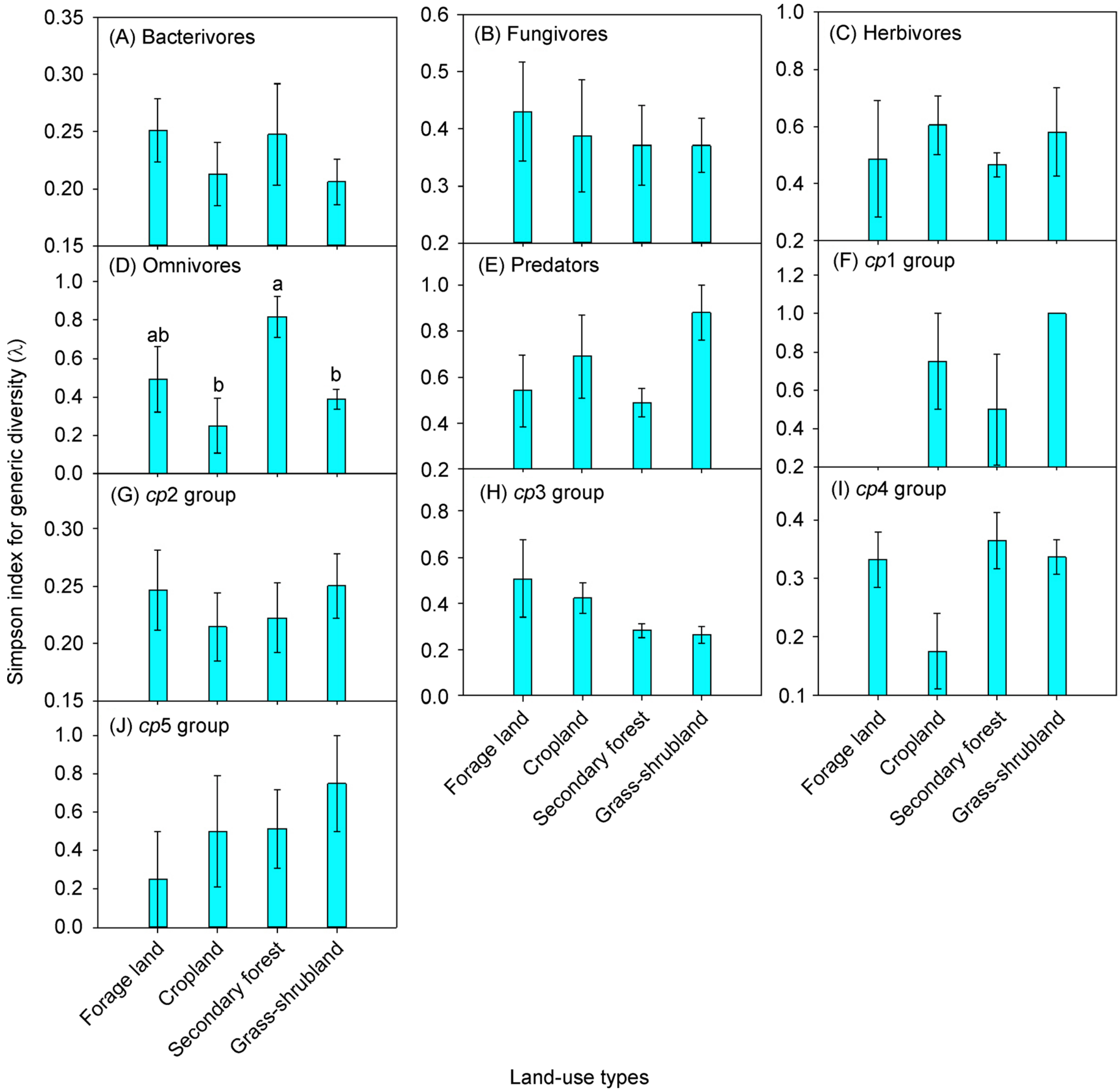

Fig. 4 The Simpson index (λ) for the generic diversity of the bacterivores (A), fungivores (B), herbivores (C), omnivores (D), predators (E), *cp*1 group (F), *cp*2 group (G), *cp*3 group (H), *cp*4 group (I), and *cp*5 group (J) in the forage land, cropland, secondary forest and grass-shrubland. Values are means±SE. Different letters indicate significant ($p < 0.05$) differences among land-use types.

investment in traditional nematode microscopy identification. In addition, application of new methods (e.g., molecular methods) to evaluate the diversity of soil nematodes will greatly improve the time efficiency of the nematode community analysis and may make larger sample size accessible (Porazinska et al., 2009; Darby et al., 2013). However, the molecular identification of nematodes is not mature and application of molecular techniques to nematode community analysis needs to be further tested.

5 Conclusion

In summary, assessment of nematode diversity at a high classification level (i.e., order) was more sensitive at detecting differences among land-use types than assessment of nematode diversity at a low classification level (i.e., genus and family). In addition, the functional group diversity and the taxon diversity for each functional group of nematodes revealed significant differences among land-use types. A basic ranking of nematode diversity for the four land-use types in this study was cropland < forage land < secondary forest < grass-shrubland, which was consistent with the disturbance intensities of these land-use types (Zhao et al., 2015). In other words, disturbance decreased nematode diversity. The results indicate that ecological studies of soil nematode diversity may benefit from systematically analyzing 1) nematode diversity at different classification levels, 2) the diversity of different functional groups of nematodes, and 3) taxon diversity within each functional group of nematodes.

Confict of interest

Jiangnan Li, Peiqin Peng and Jie Zhao declare that they have no conflict of interest.

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