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## High dimensionality of stoichiometric niches in soil fauna

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## Abstract

The ecological niche is a fundamental concept to understand species coexistence in natural communities. The recently developed framework of the multidimensional stoichiometric niche (MSN) characterizes species niches using chemical elements in living organisms. Despite the fact that living organisms are composed by multiple elements, stoichiometric studies have so far mostly focused on carbon (C), nitrogen (N), and phosphorus (P), and therefore a quantitative analysis of the dimensionality of the MSN in living organisms is still lacking, particularly for animals. Here we quantified ten elements composing the biomass of nine soil animal taxa (958 individuals) from three trophic groups. We found that all ten elements exhibited large variation among taxa, which was partially explained by their phylogeny. Overlaps of MSNs among the nine soil animal taxa were relatively smaller based on ten elements, compared with those based on only C, N, and P. Discriminant analysis using all ten elements successfully differentiated among the nine taxa (accuracy: 90%), whereas that using only C, N, and P resulted in a lower accuracy (60%). Our findings provide new evidence for MSN differentiation in soil fauna and demonstrate the high dimensionality of organismal stoichiometric niches beyond C, N, and P.

**Keywords:** biogeochemical; elemental content; stoichiometry; hypervolume

## Introduction

Determining the niche volumes of coexisting species is key to understanding their interactions and functions in natural ecosystems. The elemental composition of living organisms is of fundamental importance for individual fitness, population dynamics, food web structures, and nutrient cycling (Sterner and Elser 2002; Hall 2009). The recently developed hypothesis of “multidimensional stoichiometric niche” (MSN, González et al. 2017) or “biogeochemical niche” (Peñuelas et al. 2008, 2019) characterizes species niches based on the multiple elements composing living organisms. These hypotheses are rooted in the idea that during long-term evolutionary processes, species can develop various morphological and physiological traits and functionality, which govern the allocation of elements to their body tissues and organs. The MSN hypothesis predicts that species differ from each other in their elemental composition, and therefore in their stoichiometric niches, and that such differences increase as their phylogenetic distances increase (Peñuelas et al. 2008; González et al. 2017).

All organisms are composed of approximately 25 elements (Frausto da Silva and Williams 2001), which provide common currencies to estimate MSN similarities or differences among species or taxonomic groups. However, research has so far largely focused on the body contents of carbon (C), nitrogen (N), and phosphorus (P) of living organisms. Two recent studies used these three elements to distinguish different taxa and trophic groups in terrestrial and marine food webs (González et al. 2017; Andrieux et al. 2021). However, research on terrestrial plants suggested that C, N, and P might not be sufficient to characterize the high-dimensional volume of MSNs and distinguish taxa effectively (Peñuelas et al. 2019; Sardans et al. 2021). A quantitative evaluation on the dimensionality of the MSN within and across multiple species (i.e., beyond C, N, and P) is still lacking, particularly for animals.

According to ecological stoichiometry theory, the elemental content of living organisms is a proxy for their nutritional demands, and plays an important role in driving

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consumer-mediated nutrient cycling (Vanni et al. 2002). Unlike autotrophs (e.g., terrestrial plants) that exhibit a large plasticity in elemental composition, animal consumers usually show a more confined stoichiometry and relatively higher nutrient contents (Elser et al. 2000; Persson et al. 2010). Moreover, compared to primary consumers (e.g., detritivores), higher trophic levels (e.g., omnivores and predators) are faced with lower stoichiometric mismatches between them and their resources, and thus can better maintain their elemental composition (González et al. 2011, 2018; Elser et al. 2000). Quantifying the elemental content of different taxa across and within trophic groups is crucial for understanding the size and overlap in their MSNs, and predicting their ecological functions in elemental cycling.

Soil biota play fundamental roles in regulating ecosystem processes such as plant litter decomposition and nutrient mineralization (Bardgett and van der Putten 2014). Despite their large amount and essential roles in nutrient cycling, soil organisms are among the least known groups of invertebrates in terms of MSN patterns (Maaroufi and De Long 2020; Andrieux et al. 2021). Recent studies revealed that the biomass of different soil animal taxa is limited by distinct combinations of multiple elements in litter stoichiometry, including not only N and P, but also calcium (Ca), sodium (Na), and sulfur (S) (Kaspari and Yanoviak 2009; Ott et al. 2014; Jochum et al. 2017). These studies suggest that soil animals likely differ in their MSN and thus may respond to environmental changes idiosyncratically.

To test the MSN hypothesis in soil fauna, we collected soil animals of nine taxa across three trophic groups (i.e., predators, detritivores, omnivores) from 15 forest plots in a temperate mountain and analyzed the contents of ten elements, including C, N, P, potassium (K), Ca, Na, magnesium (Mg), zinc (Zn), copper (Cu), and manganese (Mn). We investigated how these elements differed among taxa and whether they provide sufficient information to distinguish each of them. We hypothesized that (1) soil animal taxa differ in their elemental concentrations and thus MSNs, and such differences might be explained by their phylogeny;

(2) MSN information based on all ten elements is better at differentiating among soil animal taxa than that based on C, N, and P only. Our study provides new evidence for the MSN hypothesis in soil fauna and demonstrates the high dimensionality of MSNs beyond C, N, and P.

## Material and Methods

### Study area and sample collecting

This study was conducted on the Dongling mountain (115°29'48.2"E; 40°01'48.5" N), located in the west of Beijing city, China. The region is characterized by temperate semi-humid climate, with a mean annual precipitation of 612 mm that mostly fall between June and August. The mean temperatures of the whole year, and the warmest and coldest months are 6.3°C, 18.3°C and -10.1°C, respectively. The original forests in the region have been cleared and the current trees recovered naturally or were replanted since 1960s. We chose three forest types: one secondary deciduous and broad-leaved forest dominated by *Quercus liaotungensis* Koidz., and two ~60-year-old artificial plantations that are dominated, respectively, by deciduous coniferous species *Larix principis rupprechtii* Mayr. and evergreen coniferous species *Pinus tabulaeformis* Carr. For each forest type, we chose five plots from different valleys as replicates. Soil fauna samples were mainly collected by sieving leaf litters and soil layer in 50 m × 50 m plots in each sampling site between August and September in 2020. We also used pitfall traps (plastic cups, 7.5 cm in diameter and 15 cm in depth), which contained 20 ml clean water, to collect Carabidae (ground beetle) and Opiliones (harvestman). All individuals were cleaned using tap water, and Oligochaeta (earthworm) were kept in tap water for three days to evacuate their gut contents. Then all animals were killed and stored in -20°C freezers until chemical analyses.

## Soil fauna identification, sample preparation and element determination

We identified all individuals to the lowest taxonomic level possible (i.e., order or family level) using a dissecting microscope. We included nine taxa from three trophic groups: (i) predators, including Araneae (spiders), Geophilomorpha (soil centipedes), Lithobimorpha, Carabidae, and Staphylinidae; (ii) detritivores, including Diplopoda (millipedes) and Oligochaeta; and (iii) omnivores, including Formicidae (ants) and Opiliones. We constructed a phylogenetic tree based on nucleotide sequences of 18S rRNA gene. Sequences of these nine taxa were retrieved from GenBank (Accession numbers: KP271656, Z83753, DQ517970, EF012911, KP419112, AF012509, EU368621, LC460301, and AY509007), and were aligned using MEGA X (Kumar et al. 2018). Phylogenetic reconstruction was performed using maximum-likelihood (ML) method, with the GTR+I+ $\Gamma$  model and partial deletion for gaps in MEGA X. We managed the nucleotide sequences and selected the best fitting substitution model using the Akaike information criterion with R packages *ape* and *phangorn*. For the ML analysis, we input a tree in which Oligochaeta was used as the basal group and the topology of other taxa was defined according to the phylogenetic relationships of arthropods (Regier et al. 2010).

Before elemental analyses, each individual animal was freeze-dried for 72 h. Individual body masses (dry weight) were subsequently measured on an electronic microbalance (C30, Cahn Instruments, Cerritos, California) to the nearest microgram ( $\pm 0.1 \mu\text{g}$ ). Specimens larger than 20 mg were grounded individually into a homogenous powder using a bead mill homogenizer (Bead Ruptor 12, Omni International, USA). Approximately 2 mg of powder was used to quantify C and N contents (percentage of dry mass) using a vario EL cube CHNOS Elemental Analyzer (Elementar Analysensysteme GmbH, Germany). Another ~2 mg of powder was digested with nitric acid (2 ml and 70% by weight) and used to quantify the contents of Ca, Cu, K, Mg, Mn, Na, P, and Zn using an iCAP 6301 ICP-OES Spectrometer

(Thermo Fisher, USA). During the grinding process, a fraction of powder cannot be retrieved and thus lost, making it difficult to quantify all elements for small individuals. Thus, for individuals smaller than 20 mg, we used the whole body or pooled a number of individuals from the same sampling plot (thus forming an “aggregated individual”) to measure either the C and N contents or contents of other eight elements. For 12 individuals of Diplopoda and 13 individuals of Oligochaeta (15% and 16% of total numbers of each taxon, respectively) that weigh between 5 and 20 mg, we cut each individual in two halves to measure both C/N and the other eight elements, since these organisms are relatively homogeneous in their body structure. We use the term “C (or other element) content” to describe C (or other element) content as a percent of dry body mass. In total, we measured elemental contents from 958 individuals (including aggregated individuals) of soil animals from nine taxa. Among them, we were able to obtain ten elements for 239 individuals, whereas the rest of the individuals (719) were measured for either C/N contents or the other eight elements due to their small size (Appendix S1: Table S1).

### **Statistical analysis**

To avoid pseudo-replicates, for each taxon and each plot, we first derived plot-averaged samples by calculating the average of each elemental content across all individuals of the focal taxon within the respective plot. This leads to a total of 94 samples, which include 10 samples for Araneae, 14 for Carabidae, 9 for Diplopoda, 11 for Formicidae, 4 for Geophilomorpha, 7 for Staphylinidae, and 13 for each of Lithobiomorpha, Oligochaeta, and Opiliones (Appendix S1: Table S1). Elemental content was log-10 transformed to improve normality and homoscedasticity. To examine the variation in elemental contents among forest types and taxa, we used a two-way analysis of variance (ANOVA, R package *stats*) (note that Geophilomorpha and Staphylinidae were excluded due to their low numbers in plot-averaged

samples).

To further incorporate the effect of phylogeny (in addition to forest type and taxon), we used Bayesian phylogenetic linear mixed-effects models (R package *MCMCglmm*, Hadfield 2010) with forest type as fixed effect, and phylogeny and taxon as random effects (Sardans et al. 2021). We calculated the proportion of variation explained by the forest type, phylogeny (accounted for variability in the shared ancestry), and taxon (taxon-specific variance independent of the shared ancestry) following Sardans et al. (2021). While above ANOVA and mixed-effects models focused on the variation of a single element, we also tested the variation across all elements using a permutational multivariate analysis of variance (PERMANOVA, R package *vegan*).

To quantify the MSNs and explore niche differentiation among the nine soil animal taxa, we calculated the hypervolume based on all ten elements (hereafter; full niche) or C, N, and P (hereafter; reduced niche) using the R package *hypervolume* (Blonder et al. 2018). For these analyses, we used elemental contents of each taxon (total sample size:  $n = 94$ ). For the full niche analysis, we first run a principal components analysis (PCA, R package *stats*) and extracted the first three PCA axes (Blonder et al. 2018). We then calculated the distance among hypervolume centroids and the degree of similarity/overlap, using the Jaccard similarity index, among the nine taxa in multivariate space. For the reduced niche analysis, we directly used the plot-averaged C, N, and P contents ( $n = 94$ ).

To test whether the MSN approach provides sufficient information to distinguish different taxa and quantify its accuracy, we performed a linear discriminant function analysis (LDA, R package *MASS*) using plot-averaged samples of all ten elements ( $n = 94$ ). We determined Squared Mahalanobis distances between taxa centroids (R package *HDMD*) and identified significant differences between taxa using a Hotelling's  $T^2$  test (R package *ICSNP*). We tested the accuracy of the LDA model in discriminating a data set of 239 individuals



across the nine taxa for which all ten elemental contents were available. We then repeated the LDA analysis using only C, N, and P. We compared the accuracy of the full model (LDA using ten elements) and the reduced model (LDA using C, N, and P) in their classification success ratios on the 239 individuals of soil animals.

## Results

Our analyses revealed that soil animal taxa are composed mainly of C (27.5-60%), N (4.5-13%), P (0.4-2.6%), and K (0.3-1.3%), followed by Ca (below 1% in all taxa except Diplopoda), Na, Mg, Zn, Mn, and Cu (Figure 1 and Appendix S1: Table S2). Carbon, N, and P have relatively lower coefficient of variation across taxa (mean of 5.1%, 7.7%, and 13.9%, respectively, Appendix S1: Table S2) compared to other elements (> 18%). Our two-way ANOVA showed that variation in each of the ten elements was mainly explained by taxon, whereas forest type accounted for less than 1% of the variation for all elements (Appendix S1: Tables S3 & S4; Figure S1). By further accounting for the effect of phylogeny, our Bayesian phylogenetic linear mixed-effects models confirmed the weak effects of forest type on each of the ten elements (< 1.5% variation explained). These mixed-effects models also revealed that phylogeny and taxon together explained most of the variation in each element (37% – 63% and 23% – 41%, respectively, see Appendix S1: Table S5). Further, by combining all ten elements, the PERMANOVA revealed significant differences among the nine soil animal taxa ( $R^2 = 0.94$ , pseudo- $F_{8,85} = 158$ ,  $p = 0.001$ ).

Based on the first three PCA axes which explained 83% of the total variations of ten elemental contents (full niche;  $n = 94$ ), the MSN estimates for the full niche model showed low niche overlap (0 – 20%) among the nine soil animal taxa. Overall, the MSN distances between taxa within trophic groups (1 – 6.6) were comparable to those between taxa from different trophic groups (1.2 – 8.3; Table 1). When using only C, N, and P, the reduced niche

model revealed generally larger pairwise MSN overlaps (up to 30%) than those from the full niche model (Table 1). While Formicidae and Oligochaeta displayed the largest niche sizes in both the full and reduced niche models, the rank-niche sizes of other taxa differed between the two models. For example, Lithobiomorpha and Carabidae ranked third and fourth, respectively, in the full niche model, but they ranked seventh and the last under the reduced niche model using only C, N, and P (Table 1).

The full niche LDA model using all ten elements was able to separate the nine taxa (Hotelling's  $T^2$  tests: all  $p < 0.004$ , Figure 2a and Appendix S1: Table S6). Along the first two axes of the LDA analysis, the five predator taxa were grouped together and were positioned far away from the two detritivore taxa. In contrast, the omnivore Formicidae overlapped with some of the predators (Figure 2a). The LDA model (full niche) correctly classified ~90% of the 239 individuals – which included complete information of all ten elements – into their respective taxa. Most of the wrong classifications appeared in Araneae and Formicidae (Figure 2b). The reduced LDA model using only C, N, and P showed higher levels of overlap among all taxa except Diplopoda (Figure 2c), and only correctly classified 60% of the 239 individuals to their respective taxa, with lower classification success ratios in all taxa, compared with the full LDA model (Figure 2d). In particular, the reduced LDA was unable to separate Araneae from Lithobiomorpha, or to separate Formicidae from Carabidae, Geophilomorpha, and Staphylinidae (Hotelling's  $T^2$  test:  $p = 0.26, 0.23, 0.27, \text{ and } 0.17$ , respectively, Appendix S1: Table S6).

## Discussion

Our study provides new evidence for the MSN hypothesis by demonstrating large variation in the body elemental content and significant niche differentiation among nine soil animal taxa (González et al. 2017; Andrieux et al. 2021). Variation in the body elemental

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content of soil animal taxa was partially (on average 53%) explained by phylogeny, consistent with recent findings in terrestrial plants (Andrieux et al. 2021), filter-feeding freshwater bivalves (Atkinson, van Ee, and Pfeiffer 2020), and terrestrial as well as aquatic invertebrates (González et al. 2011, 2018). Our analyses revealed partially separated MSNs across detritivorous, predatory or omnivorous taxa, but we also observed a considerable degree of overlap between the latter two groups (Figure 2 and Table 1). Several studies suggested that the variation in the stoichiometry of terrestrial arthropods might be explained by trophic group, with higher body N and/or P contents in carnivores (or predators) than in herbivores and detritivores (Fagan et al. 2002; González et al. 2011, 2018). Our results, together with earlier studies, showed that trophic level may play a limited role in determining animal stoichiometry (Martinson et al. 2008). The lack of difference in the elemental composition of organisms across trophic levels might be explained by the fact that consumers at low trophic levels could accumulate N and/or P to regulate body stoichiometry (i.e., homeostasis) through complementary feeding on nutrient-poor food resources (Ott, Rall, and Brose 2012) or feeding on microorganisms (Pokarzhevskii et al. 2003). Our data included only nine taxa, which restricts our inference on the effect of trophic level. Increasing the number of taxa/species in each trophic group may increase the statistical power for evaluating the effects of trophic level, phylogeny, and their interactions on the overall elemental contents of soil animals.

While predators and omnivores differed from detritivores in their MSNs, substantial variation exist among taxa within each trophic group (Bartrons et al. 2018). Predatory taxa in our study differed significantly in their P, Zn, Mn, and Cu contents but not in N content, suggesting that predators are likely to seek different combinations of elements. Previous lab-feeding experiments demonstrated that predators Araneae and Carabidae can forage

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selectively for protein and lipids to balance specific nutritional demands (Mayntz et al. 2005). The distinct MSNs of these predators suggests diverse and complementary top-down effects in terms of multiple bioelemental cycling. Surprisingly, detritivores Diplopoda and Oligochaeta exhibited no niche overlap and differed significantly in all elements except Mn, suggesting distinct decomposing functions on the same detritus food resources. This may also explain why Oligochaeta consumes faecal material of Diplopoda as important food source (Scheu and Wolters 1991). The omnivore taxa Formicidae exhibited the largest hypervolume niche size and the largest niche overlap with all other taxa except Diplopoda, indicating low homeostatic elemental composition of Formicidae, which likely relates to a wider variety of food sources.

Our results highlight the necessity of including elements beyond C, N, and P to characterize the MSN of soil fauna. In line with previous studies (González et al. 2011; Fagan et al. 2002), our analyses revealed relatively low variation in the contents of C, N, and P among soil animal taxa. In comparison, the other seven elements exhibited higher variability among taxa, despite their low content in invertebrate biomass (except Ca in Diplopoda, Figure 1 and Appendix S1: Table S2). Consequently, analyses incorporating all ten elements revealed low niche overlap and had high discriminant ability in differentiating the various soil animal taxa, but those using only C, N, and P revealed higher niche overlap and had much lower discriminant ability (Figure 2). Such a high dimensionality of the MSN of soil fauna provides a plausible explanation for why the abundances of different soil animal taxa are found to be limited by multiple elements in complicated ways (Kaspari and Powers 2016). For instance, the Na content which spans 0.04 – 0.78% in animals (Figure 1 and Appendix S1: Table S2)

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but only 0.002 – 0.013% in leaf litter (data not shown) , play a key role in shaping the spatial distribution and abundance of different soil animal taxa (Kaspari 2020). Previous studies have also shown that forest litter quality, which mainly refers to the ratios of C to N, P, S, Na, and Ca, significantly limit the total biomass of soil animals, either to the whole community or to specific taxa (Kaspari and Yanoviak 2009; Ott et al. 2014; Jochum et al. 2017).

Taken together, our study supports the MSN hypothesis by revealing that soil organisms have different stoichiometric niches, which are partially explained by their phylogeny. Furthermore, our results highlight the importance of incorporating more elements, beyond the commonly studied C, N, and P, to characterize the stoichiometric niches of soil animal taxa. Such a high-dimensional perspective of stoichiometric niches should be adopted in future research to understand the organization and functions of soil animal communities.

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### **Conflict of interest:**

The authors declare no competing interests.

### Author contributions:

BZ and SW conceived the idea and wrote the manuscript; BZ, HC, MD, and JL collected data; BZ analyzed data; ALG contributed with advice on data analyses and editing.

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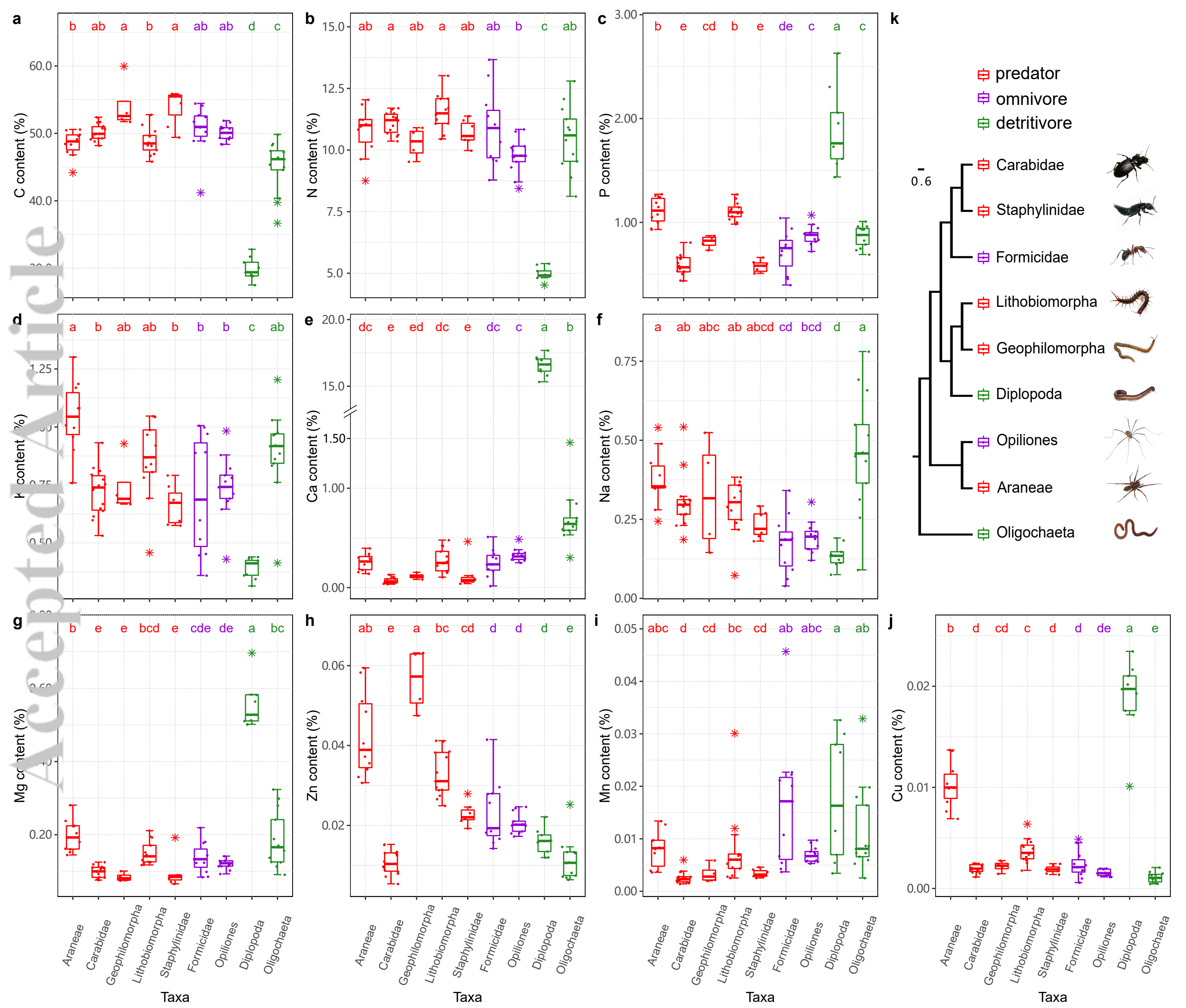
**Table 1.** Hypervolume niche size (the last column), pairwise niche distance (the upper triangular panel of A and B, in italic) and niche overlap (the lower triangular panel of A and B) of nine soil animal taxa. The hypervolume niche sizes were generated using the first three axes extracted from principal components analysis (PCA) based on (A, upper part of table) contents of ten elements (C, N, P, K, Ca, Na, Mg, Zn, Mn, and Cu) and based on (B, lower part of table) contents of C, N, and P. Numbers in bold indicate > 5% niche overlap between two taxa.

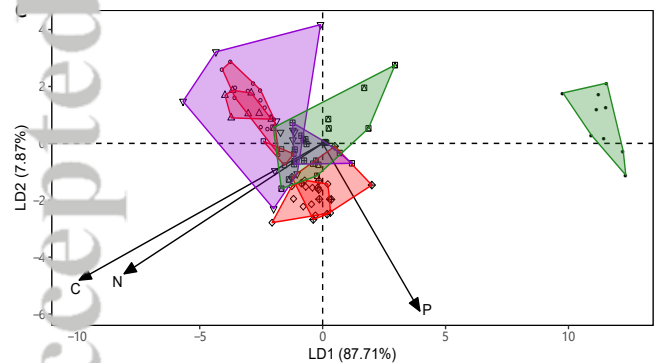
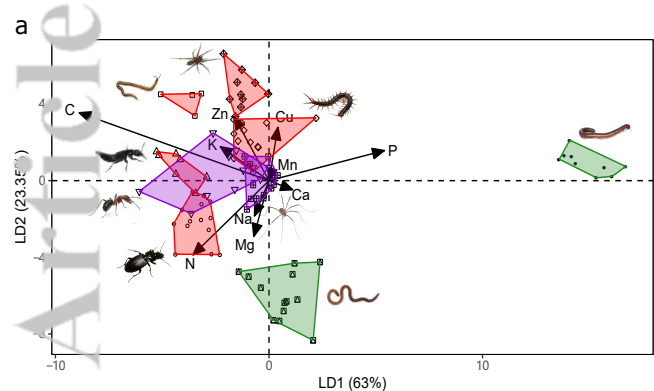
Trophic group	Taxon	Abbreviation	Aran	Cara	Geop	Lith	Stap	Form	Opil	Dipl	Olig	hypervolume
A) Ten elements												
predator	Araneae	Aran		<i>4.189</i>	<i>2.582</i>	<i>1.240</i>	<i>3.627</i>	<i>3.431</i>	<i>2.746</i>	<i>6.900</i>	<i>3.151</i>	10.215
predator	Carabidae	Cara	0.0%		<i>2.770</i>	<i>3.169</i>	<i>1.397</i>	<i>2.144</i>	<i>1.931</i>	<i>8.304</i>	<i>2.427</i>	10.531
predator	Geophilomorpha	Geop	0.0%	0.0%		<i>1.715</i>	<i>1.608</i>	<i>1.903</i>	<i>1.931</i>	<i>8.200</i>	<i>3.511</i>	3.260
predator	Lithobiomorpha	Lith	<b>19.8%</b>	0.5%	<b>6.0%</b>		<i>2.491</i>	<i>2.193</i>	<i>1.557</i>	<i>6.766</i>	<i>2.563</i>	14.853
predator	Staphylinidae	Stap	0.7%	<b>5.9%</b>	0.0%	0.1%		<i>1.192</i>	<i>1.460</i>	<i>8.163</i>	<i>3.005</i>	6.257
omnivore	Formicidae	Form	<b>11.2%</b>	1.8%	1.0%	<b>13.4%</b>	3.8%		<i>1.028</i>	<i>7.126</i>	<i>2.960</i>	83.100
omnivore	Opiliones	Opil	2.5%	3.0%	0.0%	<b>5.5%</b>	<b>11.0%</b>	<b>5.9%</b>		<i>6.792</i>	<i>2.001</i>	4.958
detritivore	Diplopoda	Dipl	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%		<i>6.589</i>	4.400
detritivore	Oligochaeta	Olig	0.1%	<b>6.4%</b>	0.0%	2.6%	0.6%	<b>7.0%</b>	3.5%	0.0%		58.687
B) C, N, and P												
predator	Araneae	Aran		<i>1.798</i>	<i>1.140</i>	<i>0.380</i>	<i>1.836</i>	<i>1.499</i>	<i>0.709</i>	<i>4.439</i>	<i>0.912</i>	2.692
predator	Carabidae	Cara	2.1%		<i>1.106</i>	<i>1.786</i>	<i>0.408</i>	<i>0.307</i>	<i>1.314</i>	<i>5.545</i>	<i>1.337</i>	0.886
predator	Geophilomorpha	Geop	<b>8.9%</b>	<b>6.0%</b>		<i>1.186</i>	<i>0.909</i>	<i>0.869</i>	<i>0.637</i>	<i>5.196</i>	<i>1.292</i>	1.924
predator	Lithobiomorpha	Lith	<b>32.9%</b>	0.9%	3.7%		<i>1.832</i>	<i>1.510</i>	<i>0.931</i>	<i>4.756</i>	<i>1.076</i>	1.062
predator	Staphylinidae	Stap	0.0%	<b>25.0%</b>	3.8%	0.0%		<i>0.485</i>	<i>1.287</i>	<i>5.668</i>	<i>1.539</i>	1.358
omnivore	Formicidae	Form	<b>12.4%</b>	4.9%	<b>9.1%</b>	<b>5.9%</b>	<b>7.3%</b>		<i>1.012</i>	<i>5.304</i>	<i>1.080</i>	17.545
omnivore	Opiliones	Opil	<b>19.9%</b>	<b>6.3%</b>	<b>21.9%</b>	<b>6.2%</b>	3.7%	<b>5.3%</b>		<i>4.584</i>	<i>0.802</i>	0.989
detritivore	Diplopoda	Dipl	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%		<i>4.347</i>	3.235
detritivore	Oligochaeta	Olig	<b>15.7%</b>	4.6%	<b>12.0%</b>	<b>9.9%</b>	3.6%	<b>18.8%</b>	<b>10.3%</b>	0.0%		6.858

## Figure captions

Figure 1. Distribution of elemental content ((a)–(j): C, N, P, K, Ca, Na, Mg, Zn, Mn, and Cu, respectively) and phylogenetic relationships among nine soil animal taxa (k). Boxes denote the interquartile range (25–75 percentile) containing the middle 50% of the data. Colors indicate different trophic groups. Different letters above the boxes indicate significant differences based on TukeyHSD multi-comparison tests ( $p < 0.05$ ). The phylogenetic tree was build based on nucleotide sequences of 18S rRNA gene downloaded from GenBank and using the Maximum-likelihood method (for details see text).

Figure 2. Linear discriminant analyses (LDA) of MSNs of nine soil animal taxa (a, c) and the classification accuracy of the LDA for 239 soil animal individuals (b, d). (a) and (b) are based on all ten elements (C, N, P, K, Ca, Na, Mg, Zn, Mn, and Cu). (c) and (d) are based on three elements, i.e., C, N, and P.





◊ Araneae   ◊ Carabidae   ◊ Geophilomorpha   ◊ Lithobiomorpha   ◊ Staphylinidae  
 ◊ Formicidae   ◊ Opiliones  
 ◊ Diplopoda   ◊ Oligochaeta

