NICHE DIFFERENTIATION OF FUNGIVOROUS ACARI AND COLLEMBOLA IN JAPANESE BEECH FOREST SOILS

Anzilni Fathia AMASYA

Abstract  Acari and Collembola are the two most abundant microarthropods in the soil and are found in a wide range of soil habitats. Both animals relatively share the same trophic level as fungivorous microarthropods which directly influence the soil fertility by stimulating the microbial activity, play important roles in fungal dispersions and its relations to plant nutrient uptake. With their high abundance in the soil, the niche differentiation between both animals towards fungal communities in soil remains uncertain. Many studies have been conducted to understand the niche differentiation of fungivorous Acari and Collembola sharing the soil fungal communities as resources, such as through gut content analyses and fungal food preference experiments. Nevertheless these methods may not reflect feeding processes in the field. The community profiling method, Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis was offered as a molecular technique performed to describe the fungal community structure associated with both animals to understand the niche differentiation in the field. Four Japanese Beech forest soils in Northeast (Akita, Choukai, and Iwaki) and Central (Nagano) Japan were chosen as study sites. Results suggested that even though Acari and Collembola showed associations with relatively different fungal communities, overlapping niches were also identified. Among the studied area, the southernmost area (Nagano) had the highest animal abundance in the soil and the least niche overlap between Acari and Collembola.

Key words: Acari, Collembola, soil fungi, niche differentiation, T-RFLP

1. Introduction

Soils are compact environments where life is concentrated in a porous space that typically comprises 30% to more than 60% of the soil volume in the upper horizons (Lavelle 2012: 7-21). Soils provide an immerse array of habitat, and harbors a huge variety of organisms, ranging from <100um body width for the microbes and microfauna, to mesofauna with body width between 100um and 2 mm, macrofauna with body widths >2 mm and up to megafauna with body width >2 cm (Wurst et al. 2012: 28-41).

Across soil ecosystems, Acari and Collembola, which represent the vast majority of the microarthropods, are the most species rich and abundant groups within the mesofauna (Petersen and Luxton 1982). These animals contribute significantly to decomposition processes and nutrient turnover (Visser 1981).

Acari inhabit air-filled soil pores and litter layers, and like spiders, have a hard body and eight legs. Along with direct feeding on dead plant material and the resulting comminution of it, species
of Acari contribute to decomposition processes and nutrient cycling in the soil system by feeding on microorganisms and by the dispersal of microbial propagules (Behan and Hill 1978; Seastedt 1984; Moore et al. 1988). Acari are also important decomposers in almost all habitats; their distribution ranges from arid coniferous forests over floodplain forests to salt marshes (Usher 1975; Mitchell 1979).

Collembola or springtails on the other hand are primitive insects, with six legs, antennae and no wings that represent one of the most abundant groups in soils (Cragg and Bardgett 2001). The number of known Collembolan species is much lower than that of the Acari, but they may reach the same abundances (Petersen and Luxton 1982). One of their main contributions to the soils is the regulation of fungal populations (Warnock et al. 1982), and also in establishing relationships with mycorrhizae (Gange 2000).

With both mesofauna being predominant in soil habitats and at the same time sharing the soil fungal community resources within a given area, it is intriguing to understand how both species that potentially compete for the same resources can coexist in a small spatial scale. Information on the proportion of fungal species shared by both animals in a soil community at a given time would be crucial in understanding the niche differentiation between Acari and Collembola.

Previous studies used indirect methods in gaining fungal community information from Acari and Collembola through gut content analysis and laboratory-based feeding preferences methodologies (Mitchell and Parkinson 1976; Walter and Kaplan 1991; Jorgensen et al. 2005; Schneider 2005; Hishi et al. 2007). Although these techniques provided valuable insights on feeding behavior, they have not been able to explain actual field feeding processes in the soil.

With the advent of molecular techniques, community profiling methods such as Terminal Restriction Length Polymorphism (T-RFLP) enables to depict the overall community composition of microbial communities in any given substrate. Using this advantage, a description of soil communities found in Acari and Collembola may be obtained to further reflect how both animals share the fungal resources in the soil.

This study aims to understand niche differentiation between fungivorous Acari and Collembola through T-RFLP. In this study we chose 4 Japanese Beech (*Fagus crenata*) forests soils which have relatively similar altitude, soil type, and understory vegetation.

2. Materials and Methods

   **Sampling of soil**

   Soil and mesofauna samples were taken from Japanese Beech (*Fagus crenata*) forests in four areas across Japan (Fig. 1). The areas are: a plateau near Lake Tazawa of Akita Prefecture (Fig. 2.1), Mt. Choukai located between Akita and Yamagata Prefecture (Fig. 2.2), Mt. Iwaki of Aomori Prefecture (Fig. 2.3), and Shinshu University Research Forest near Mt. Kisokomagatake in Nagano Prefecture (Fig. 2.4).
The location description and characteristics of the study areas is listed on Table 1. Japanese Beech is native to Japan and grows on light (sandy) and medium (loamy) soils and prefers well-drained soil, with pH of acid, neutral and basic (alkaline) soils. All soils sampled had relatively low pH, ranging from 3.96 – 4.6.

Table 1  Location and characteristics of the study area

<table>
<thead>
<tr>
<th>Study Area</th>
<th>Akita</th>
<th>Choukai</th>
<th>Iwaki</th>
<th>Nagano</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinates</td>
<td>Lake Tazawa Plateau</td>
<td>Mt. Choukai</td>
<td>Mt. Iwaki</td>
<td>Mt. Kisokomagatake</td>
</tr>
<tr>
<td>Vegetation</td>
<td>Fagus crenata, Quercus crispula</td>
<td>Fagus crenata, Viburnum furcatum, Lindera umbellate</td>
<td>Fagus crenata, Quercus serrata, Betula ermanii</td>
<td>Fagus crenata, Quercus serrata, Pinus pumila</td>
</tr>
<tr>
<td>Floor vegetation</td>
<td>Sasa kurilensis</td>
<td>Sasa kurilensis</td>
<td>Sasa kurilensis</td>
<td>Sasa kurilensis</td>
</tr>
<tr>
<td>Soil type (FAO, 1988)</td>
<td>Light colored Karobou soils (Fulvic Andosols)</td>
<td>Brown forest soils (Cambic Podzols)</td>
<td>Brown forest soils (Dystric Cambisols)</td>
<td>Brown forest soils (Dystric Cambisols)</td>
</tr>
<tr>
<td>Av. O Horizon thickness (cm)</td>
<td>5.1</td>
<td>6.1</td>
<td>5.3</td>
<td>8.0</td>
</tr>
<tr>
<td>Av. soil pH (H₂O)</td>
<td>4.10</td>
<td>4.60</td>
<td>3.96</td>
<td>4.20</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>760</td>
<td>730</td>
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<td>780</td>
</tr>
<tr>
<td>Annual Rainfall (mm)*</td>
<td>2260</td>
<td>2362</td>
<td>1570</td>
<td>1070</td>
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<tr>
<td>Mean annual temperature(°C)*</td>
<td>7.3</td>
<td>6.8</td>
<td>5.7</td>
<td>7.2</td>
</tr>
</tbody>
</table>

*data obtained from The Japan Meteorological Agency
Fig. 2.1 Topographic map (left) and pictures of *Fagus crenata* forest landscapes at Lake Tazawa Plateau, Akita Prefecture (right). Star (★) indicates the sampling location.

Fig. 2.2 Same as in Fig. 2.1 except at Mt Choukai, Yamagata Prefecture.
Nine repeats of soil samples were collected within a 10x10 m$^2$ from *F. crenata* forest in Akita, Iwaki, and Nagano prefectures, Japan, in July – August 2011 and from Choukai in July 2012. These nine sub plots were approximately located in the same distance with each other as described in Fig. 3 (left).

In each sub plot, O horizons (L, F, H layers) were removed, litter was weighed in the field and A horizons were collected using a cylinder with a capacity of approximately 800 cm$^3$ volume (Fig. 3 (right)).
Soil sampling methodology. Soil samples were taken within a 10x10 m$^2$ plot with approximately same distance between each sub plot (left) using a cylinder (right).

Isolation of Acari and Collembola from soil

Acari and Collembola were collected by modified Tullgren method (Macfadyen 1953) and kept for 7 continuous days under a 5 watt light bulb with approximately 15 cm from the surface of the sampled soil, kept in a constant 24°C room temperature. Collection of animals were stored in 70% ethanol for surface sterilization and identified under a digital high density video microscope (VH-7000, Keyence, Osaka).

Fungal community profiling from Acari, Collembola, and soils

To investigate the fungal communities associated with both soil mesofauna, 3-5 individuals of each animal from each sub-plot were initially crushed and fungal DNA was isolated using Prepman™, and this process was repeated 3 times for each location, and further taken the average. The soil fungal DNA was extracted using ISOIL for Beads Beating Soil DNA Extraction Kit (Nippon Gene Co., Ltd). The ribosomal DNA Internal Transcribed Spacer (ITS) regions were then amplified by the PCR using primer pair ITS-1F/ITS-4 and DNA Taq Polymerase enzymes (Applied Biosystems) with conditions as follows: a hot start at 96°C for 30 seconds, then 35 cycles consisting of 10
seconds at 96°C, 30 seconds at 58°C, and 30 seconds at 72°C. Aliquots of the amplified DNA were digested with restriction endonucleases to obtain ITS-RFLPs; each sample was digested with Hae III and HhaI (Promega).

To obtain the fungal community profiles both in soil and in soil mesofauna bodies, a quenching fluorescence primer was used for real-time quantitative PCR (qPCR) assay to monitor the PCR amplification and then used for terminal-restriction fragment length polymorphisms, or T-RFLP (Liu 1997) using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and read on an Applied Biosystems 3130xl Genetic Analyzer.

Shannon-Wiener ($H'$) Diversity Index (Hill 1973) was calculated to quantify the diversity of fungal communities in soil and mesofauna, with the T-RF fragment sizes representing fungal species richness and the T-RF peak heights represent the fungal species abundance. The Shannon-Wiener index was calculated as follows, where $p_i$ is the proportion of characters belonging to the $i^{th}$ species:

$$H' = -\sum p_i \ln(p_i)$$

To describe the similarity between locations, the Sorensen index ($QS$) was used and calculated below, where A is the number of species found only in location A, B is the number of species found only in location B, and C is the number of species found in both locations $A$ and $B$:

$$QS = \frac{2C}{A + B}$$

Nucleotide sequences were determined with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) according to the instructions supplied and with a PE Applied Biosystems Automated DNA Sequencer (model 3130xl, Applied Biosystems). The double-stranded DNA sequences were assembled and analyzed using Genetyx (version 11.0) and Genetyx-ATSQ (version 4.0) software (Genetyx, Tokyo, Japan), respectively, and compared with similar DNA sequences retrieved from the DDBJ/EMBL/GenBank databases using the NCBIBLAST and DDBJ-BLAST programs.

3. Results and Discussions

Acari and Collembola abundance

The Order Oribatida of Acari, identified by having fungivorous mouth parts, were selected, while Acari with other types of mouth parts (e.g. having chelate or sub chelate pedipalps) were not included in this study. Most of the Collembola found belonged to the Family Entomobrydae (69%) and the rest belonged to the Order Poduromorpha (31%). Acari collected had the length of 0.6 – 0.9 mm, while Collembola were about 1.3 – 1.5 mm in length, as shown in Fig. 5.

Sampling site of Nagano showed the highest average weight of litter layer, and had the highest mesofauna abundance (Fig. 5). The number of individuals responded to the average weight of litter layer, whereas the more litter piled up the more abundant both animals tend to be extracted. The mean abundance of Acari and Collembola in this study which was conducted in temperate deciduous forests, translates into a density of 11,043 individuals/m² and 10,774 individuals/m², respectively. This is lower than those reported in most temperate areas of central Japan (Hiji 1994),
and some tropical sites (e.g., Seastedt 1984, Gonzalez et al. 2001). In Choukai and Iwaki, Acari were found more abundant. Similar findings of Acari having higher abundance compared to Collembola in forests of central Japan was reported, and was said to be related with the characteristics of the forest having heavy rainfall, acidic soils, a large amount of litter accumulation, and slow decomposition rates (Takeda and Abe 2001; Lin et al. 2002). Acari was found less abundant compared to Collembola in Akita and Nagano, which is in accordance with the findings by Hijii (1994) who reported ratio of springtail to mites (ca. 1.12) in coniferous forests of Japan.

Fig. 5 Mean number of Acari and Collembola captured in each sampling area per gram soil, as compared to the average litter layer weight. Left-above: Acari; left-below: Collembola.

Fungal community profiles

In an attempt to understand how both Acari and Collembola differentiate their fungal community resources in the soil, the T-RFLP was conducted and results are shown in Fig. 6. The peak heights in the y-axis show the intensity, or in other words describing the abundance of fungal communities in mesofauna. The x-axis on the other hand shows fragment sizes of restricted fungal DNA, with each base pair length representing a specific fungal species. From the peak heights alone, samples from Nagano showed the least fungal abundances compared to other areas (peak height of 2696), while Iwaki showed the highest (peak height of 8261). However based on the fragment sizes, samples from Nagano showed the highest number of species (a total of 198 species) among other sampled areas. By using both fungal richness and abundance information, the Shannon-Wiener Diversity Index ($H'$) was calculated for soil, Acari and Collembola in all four locations (Table 2).

Generally, fungal communities were most diverse in Nagano soils ($H' = 4.84$), followed by Iwaki ($H' = 4.50$), Choukai ($H' = 4.25$) and lowest diversity was found in Akita soils ($H' = 4.08$). Interestingly, fungal communities associated with Collembola showed the same pattern. According to MacArthur (1955) more diverse communities will enhance ecosystem stability. Therefore, the high fungal diversity in the soil of Japanese Beech forest in Nagano indicates a soil ecosystem with more stability among other studied areas. Combining diversity with the information given in Fig. 5, Collembola were found most abundant in Nagano (29.85 individuals/gram) which makes it the predominant species in the area. Their predominance may lead to their ability in accessing a wider range of soil fungal communities, resulting in a higher
diversity of fungal communities associated with them as shown through T-RFLP.

Fig. 6  T-RFLP profiles of fungal species isolated from Acari, Collembola, and soils of 4 Japanese Beech forests. Fragments were restricted with HhaI enzymes in this figure.

Most of this association is suggested to have occurred through fungal consumption. However, given the complexity of the soil environment, actual consumption of fungal resources may not reflect collembolan preferences *per se*. For example, the ability of most Collembola to disburse to resources and forage throughout the soil matrix is constrained by structural and architectural characteristics of the soil, including pore spaces (Schrader and Lingnau 1997; Larsen *et al.* 2004), which may result in more opportunistic than selective feeding patterns.

From Fig. 6, fungal communities associated with Acari showed high abundance in Akita and Choukai. Differing from the fungal communities in Collembola, those extracted from Acari showed no specific pattern in diversity across the studied area, whereas the highest diversity was found in Iwaki, then followed by Choukai, Akita and Nagano. Assuming the associations towards fungal communities are mostly consumption related, this may suggest Acari had more specific preferences in the soil regardless the available resources. Another suggestion is the behavior of Oribatida mites itself that may limit their accessibility towards fungal resources. Oribatida mites
are described as sluggish, slow-moving mites, with an aversion to light and a strong tendency to
hide in holes or under bits of detritus (Walter and Proctor, 1997). When disturbed by a larger
arthropod, the armored adults of all three species pulled their legs close to their bodies and ceased
movement. If turned on their back or otherwise annoyed, the mites continued to play dead
(thanatosis) for several minutes after harassment stopped before slowly unfolding their legs,
righting themselves by vigorously waving their legs and slowly walking away (Walter and Proctor,
1997).

### Table 2

<table>
<thead>
<tr>
<th>Site</th>
<th>$H'$ Acari</th>
<th>$H'$ Collembola</th>
<th>$H'$ Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akita</td>
<td>2.50</td>
<td>2.56</td>
<td>4.08</td>
</tr>
<tr>
<td>Choukai</td>
<td>2.85</td>
<td>3.20</td>
<td>4.25</td>
</tr>
<tr>
<td>Iwaki</td>
<td>3.52</td>
<td>3.29</td>
<td>4.50</td>
</tr>
<tr>
<td>Nagano</td>
<td>1.64</td>
<td>4.20</td>
<td>4.84</td>
</tr>
</tbody>
</table>

Schneider et al. (2005) also stated that most species of Acari tend to be considered as ‘choosy
generalists’ because of their tendency to feed selectively when high quality food is available but in
shortage of the preferred food they are also able to feed on other low quality fungi.

**Niche differentiation of Acari and Collembola in Fagus Forest Soils**

Fungal communities extracted from Acari and Collembola through T-RFLP showed both
distinct and overlapped species. To quantify the number of fungal species found specifically in
Acari or Collembola or both in each studied area, a pie chart is presented in Fig. 7. Each slice of
the pie in Fig. 7 represents the species richness of fungal communities extracted from either soil or
animal substrates. ‘Overlap’ slices illustrate the number of fungal species found in both Acari and
Collembola, reflecting how much both animals compete for their fungal resources in the soil. The
slice of ‘soil’ describes the number of fungal species found only in soil, and not in either animal.
Thus the total percentage number of fungal species found in Acari, Collembola and at the same
time available in soil would be a sum of slices ‘Acari’, ‘Collembola’ and ‘Overlap’.

In Akita, 42% (50 species) of fungal communities were associated with Collembola alone,
while 31% (38 species) were with Acari. There were 7% or 8 fungal species shared by both
animals, while the 20% (24 species) of fungal communities lived in the soil, presumably
unfavorable, inaccessible, or inedible by either animal. Compared to samples from Akita, samples
from Choukai had a higher number of shared species (23%, 36 species), while samples from Iwaki
showed the highest overlap (26%, 48 species) among areas.

Based on Fig. 7, the area with the least fungal species overlap between Acari and Collembola
was found in Nagano (2%, 4 species). This suggests both animals seem to occupy almost
completely different niches compared to other studied areas. Due to the wide range of fungal
communities in Collembola and the lowest percentage of overlap species, fungal peaks of Nagano
were selected to be sequenced further to obtain information up to the species level.

From sequencing, it was obtained that the most predominant fungal species found in Acari of
Nagano was *Laccaria laccata* (81.26%), followed by *Alternaria alternata* (6.79%), *Aspergillus*
niger (4.33%), and Eurotium amstelodami (3.97%). In Collembola Paxillus obscurosporus was found predominant (77.29%) followed by Cladosporium herbarum (8.32%), Penicillium chrysogenum (6.43%), Penicillium expansum (3.66%), and Absidia glauca (1.25%). The common fungal species detected in both animals were Lanchancea kluyveri (45.19%), Tuber aestivum (26.23%), and Trichoderma viridae (19.26%).

Fig. 7 Proportions of fungal species extracted from Acari, Collembola and Soil from 4 sites of Japanese Beech forests.

The high number of overlapping species shown in areas of Iwaki and Choukai does not necessarily inhibit the coexistence of both animals. However the low number of overlapping species in Nagano positively relates with Acari and Collembola abundance in the soil. Therefore, this study showed how niche differentiation may play an important role in species survival.

To understand how similar or dissimilar each fungal community structure in Acari and Collembola from all studied areas, the Sorensen Similarity Index was estimated and shown in Table 3. This Index, ranging from 0 to 1, was calculated based on the fungal species richness. Indices showing values closer to 0 indicates less similarity between two communities. On the contrary, the higher the value of this index or in this case, closer to 1, indicates higher similarity between two communities.

Based on Table 3, the numbers underlined is the closest to 1, indicating the two most similar community structures which are between fungal communities found in Acari of Iwaki and Acari of Choukai. This may show the similar fungal preferences by Acari in Iwaki and Choukai. Within the same area, fungal communities in Acari and Collembola of Iwaki showed a high similarity, suggesting that both animals have high overlapping niche (see Fig. 7).
Table 3  Sorensen Similarity Index of fungal communities associated with Acari and Collembola in 4 Japanese Beech forest soils of Japan.

<table>
<thead>
<tr>
<th></th>
<th>aA</th>
<th>aC</th>
<th>aI</th>
<th>aN</th>
<th>cA</th>
<th>cC</th>
<th>cI</th>
<th>cN</th>
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</thead>
<tbody>
<tr>
<td>aA</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>aI</td>
<td>0.4386</td>
<td>0.7563</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>aN</td>
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<td>0.2885</td>
<td>0.2105</td>
<td></td>
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<td></td>
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<tr>
<td>cA</td>
<td>0.1569</td>
<td>0.4898</td>
<td>0.4598</td>
<td>0.2626</td>
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<tr>
<td>cC</td>
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<tr>
<td>cI</td>
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<td>0.0904</td>
<td>0.0841</td>
</tr>
</tbody>
</table>

a: Acari, c: Collembola, A: Akita, C: Choukai, I: Iwaki, N: Nagano. The underlined value shows highest similarity, while the bold value shows lowest similarity.

On the other hand, the numbers printed in bold showed the lowest index, indicating both communities tend to be the most dissimilar among other compared community structures. In this study, fungal communities found in Acari and Collembola from Nagano showed the highest dissimilarity, emphasizing how Collembola and Acari in Nagano harbors significantly different fungal communities. Thus the low overlap percentage of niche differentiation between Acari and Collembola in Nagano forest soils enables their coexistence and therefore contributes to their high abundance in the soil.

4. Conclusion

Niche differentiation between two predominant soil animal species in the soil can generally be visualized and relatively quantified through molecular techniques such as T-RFLP. The proportion of fungal communities preferred by Acari in the soil ranges between 31-38%; while for Collembola ranges between 23-63%. Niche overlap showed highest in Iwaki, and lowest in Nagano. Among the studied areas, Nagano showed the highest fungal diversity in the soil and niche differentiation between Acari and Collembola was most distinct.

Although T-RFLP showed to be an effective method through this study, niche differentiation studies should be combined between laboratory observations and direct assessment from the soil to a better understanding of the coexistence of soil fungivorous microarthropods.

Acknowledgements

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