The influence of grazing by *Hypogastrura viatica* (Insecta: Collembola) on microbial activity in decomposing kelp on Spitsbergen

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The influence of three different grazing levels (5–20 ind.) by *Hypogastrura viatica* (Collembola) on microbial activity in a Spitsbergen kelp *Laminaria* has been evaluated during a 10 day laboratory experiment under oxic conditions.

Respiration has been measured by infra-red gas analysis in the form of CO_2-emissions. The total and metabolically active fungal and bacterial biomass has been estimated by direct microscopical counts and epifluorescence techniques.

Despite the results of other similar experiments, an increase in microbial numbers and biomass was recorded following the introduction of grazing.

Grazing increases the mean respiration between 0.6 to 1.4 times. A corresponding increase in the microbial biomasses was also recorded. Although a trend towards decreased mean bacterial cell size was found, the fungi were more influenced by grazing than the bacteria. Bacteria constituted the predominant part of the microbial biomass in all the experimental vessels.

The results indicate that Collembola plays an important functional role by regulating the microbial activity, probably by nutrient mobilization.

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Meiofauna has normally a limited direct influence on the turnover of organic matter in ecological systems (Anderson et al. 1981; Petersen & Luxton 1982). Only a small fraction of the total CO_2 respired, and thus carbon mineralized, is metabolized directly by the faunal component of the ecosystem.

In many systems, however, the meio- and microfauna are known to enhance the mineralization/nutrient cycling and carbon mineralization through their consumption of microbial biomass, i.e. fungal hyphae and bacteria (e.g. Johannes 1965). As Anderson (1975) pointed out, the study of the interaction between microfauna and microflora is one of the most intractable problems of ecology.

There have been few studies dealing with the microbial ecology of decomposing kelp heaps to date. As Griffith & Stenton-Dozey (1981) noted, little is known about the fate of stranded kelp. The aim of this study was to evaluate the influence of one type of heterotrophic interaction, i.e. grazing by Collembola on the decomposing dynamics. At the same time more general effects on decomposition and microbial activity by micrograzers can also be evaluated. The present experiments were carried out as a part of a study of the ecology of seaweed heaps on Spitsbergen. This substudy has been very much influenced by similar investigations where comparable experimental designs and background philosophies have been employed, (see Hanlon & Anderson (1979) and Andrén & Schnürer (1985)).

Stranded kelp is initially rich both in energy and nutrients, and microbial activity is supported very well. As the seaweed loses its initial structural support through water loss and decomposition, a more or less solid layer of variable depth is formed, which is associated with a very limited exchange of gas. This enhances the formation of anoxic conditions dominated by anaerobic microbial activity.

Koop et al. (1982b) estimated the total amount of carbon mineralized by microbes to be about 70%. However, less than 4% of the nitrogen wrecked in macrophytal biomass is recorded as leaching. This implies a very high degree of microbial immobilization of nitrogen although ammonia is the principal end product of microbial decomposition both in aquatic and terrestrial habitats (cf. Fenchel & Blackburn 1979) and might be lost to the atmosphere under airy conditions. Sveum (in prep.) found very high fractions of the total microbial biomasses in kelp beds on Spitsbergen sea shores to be metabolically inactive.

Seaweed heaps on Spitsbergen differ from those found in central and southern Norway because of their lower biological activity, and consequently they have a heterogeneous and more aerobic gas composition than the anaerobic temperate varieties (Sveum, in prep.). Only one species of Collembola, *Hypogastrura viatica* was found in any number in the seaweed heaps (Sveum, in prep.), and this species was selected as the microbial grazer for the experiment. *Hypogastrura* has earlier been found to graze on fungal biomass (Addison 1977).

Koop et al. (1982a) reported that kelp (i.e. *Eklomia maxima)* is primarily decomposed by bacterial activity rather than by fungi.

The Spitsbergen kelp beds were composed of two more or less distinct layers; an upper one where aerobic conditions predominated and a lower more or less anoxic layer. The kelp studied and reported in this paper is of the first type. Here *H. viatica* dominated among the invertebrates. The protozoa have not yet been studied in these kelp beds.

Material and methods

Experimental design

Decomposing seaweed *(Laminaria)* was collected at Kvadehuken in the outer part of Kongsfjorden on the western coast of Spitsbergen. The decomposing seaweed had been on the shoreline for approximately 12 months.

The experimental period of 20 days was divided into two periods of 10 days each. The decomposing samples of seaweed were incubated at 5 °C in gas tight, 100 ml glass vessels. Then the seaweed was extracted to remove the meiofauna and was later supplied with an equal amount of demineralized water to compensate for the water lost by evaporation during extraction. The vessels were allowed to acclimatize for 48 hours at the incubation temperature before they were closed. Incubation was carried out in a thermo-incubator, in total darkness. A total of 40 experimental vessels was used.

After 10 days, 3 different levels of grazing by the Collembola species *Hypogastrura viatica* were supplied to 30 of the incubation vessels (i.e. 10 replicates of each grazing level).

Analytical techniques

The CO_2 content of the vessels was measured every second day. To avoid hypertension and anaerobiosis in the vessels, fresh outdoor air was supplied by complete aeration. The CO_2 content of the gas samples was measured with an infrared gas analyzer (Siemens Ultramat 1), modified for septum injection and calibrated with gas mixtures of known CO_2 concentration. The total CO_2 emission for each vessel was calculated by the numerical integration of the cumulative CO_2 curve.

As the respiration rate of *Hypogastrura viatica* was not measured in the present study, the rate given by Aunaas et al. (1983) for *Onychyurus groenlandicus* from a nearby site on Spitsbergen, was applied.

Conversion from oxygen consumption to carbon dioxide production was done using $RQ = 0.8$ (Andrén & Schnürer 1985), and from microlitre to microgram carbon by the general gas law. The collembolan respiration for each vessel was calculated as equal to the integrated animal respiration, assuming a linear growth curve from the number added at day 10 to the number present at day 20. The fresh weight of the Collembola was 3.33 times the dry weight (Persson 1983).

At the end of the experimental period, bacterial counts and fungal measurements by epifluorescence microscopy were carried out on the content of each vessel. The total bacterial number was determined by staining with acridine orange (AO) (Trolldenier 1972) and by staining the metabolically active bacteria with fluorescein diacetate (FDA) (Lundgren 1981). The total of

fungal hyphae was measured after staining with methylene blue (Jones & Mollison 1948), while the length of the metabolically active fraction was measured with FDA (Söderstrøm 1977). The intersection method described by Olsson (1950) was used in both cases.

Results and discussion

Extensive studies of the feeding habits of *H. viatica* have not yet been carried out. However, microscopical examination of a few guts of the species obtained from the kelp beds on Spitsbergen showed that fungal hyphae were part of its content. It has been shown earlier that Collembola consume hyphae (Christiansen 1964; Petersen & Luxton 1982). *H. viatica* is a typical surface-living species in this habitat. According to Bödvarson (1970) surface-living species have a greater tendency to be fungal feeding than the strictly soil-living species. *H. viatica* occurs in several very different habitats on Spitsbergen. It is not known how far the predominant diet varies according to local adaptations within various habitats.

The total respiration of the vessel communities for the first 10 days, with and without animals (days 11–20) is given in Table 1. The estimated Collembola respiration is also given in the same table.

There is an increase in the total community respiration in the grazed vessels. The increase is linear with the rise in grazing pressure (r = 0.97, p <0.01). The differences between all combinations of grazed treatments are found to be statistically significant. No significance is found between the treatment groups in the ungrazed initial period. The estimated grazing by Collembola does not account for the increased respiration activity, as it only comprises 0.3% to 0.7% of the total respiration. The total increase ranges from 64% to 145% compared to the control.

Bacteria constitute the predominant part of the microbial biomass in all the vessels. This is consistent with the results of Koop et al. (1982a).

The abundance and masses of biota in the incubation vessels are given in Tables 2, 3 and 4.

Although the total length of fungal hyphae does not increase linearly with grazing pressure (i.e. hyphae are longer in an ungrazed state than at a low grazing pressure), a linear increase is found in the metabolically active fraction (r = 0.97, p < 0.01). The increased metabolical activity of fungal hyphae is probably the result of the increased availability of nutrients due to grazing by the Collembola. The relative fraction of metabolically active hyphae in the vessel with grazers (41-45%) is significantly different from the ungrazed (27%) (x^2 = 4.97, p < 0.01), although no significant increase is found with increasing grazing pressure (r = 0.52, n.s.).

Grazing by amoebae may be a possible explanation of this unexpected ratio. Andrén and Schnürer (1985) pointed out that amoebae feed by puncturing the hyphal cell walls and sucking out the cytoplasma. The grazed hyphae will still be measured as total hyphae. If we assume that the effect of Collembola grazing on bacterial cells indirectly stimulates the amoebal population by increasing bacterial cell growth, the lack of increase in metabolically active fungal hyphal length might be an indirect effect of Collembola

Table 1. Total respiration, microbial respiration and estimated Collembola respiration in the different groups of incubation vessels, after 10 and 20 days, respectively.

Measured or estimated parameter	No. Collembola			
	0	5	10	20
Measured total resp. day 1–10	1.24±0.14	1.47±0.12	1.52±0.14	1.34±0.14
Measured total resp. day 11–20	1.11±0.09	1.69±0.22	2.28±0.27	2.72±0.32
Estimated Collembola respiration	0	0.005	0.011	0.019

grazing. The correlation between respiration in days 11 to 20 and the total microbial biomass at the termination of the experiment is positive but not statistically significant ($r = 0.60$, n.s.). A complete correlation was found between grazing level ($r = 1.00$, $p < 0.01$) and respiration in the grazing level, and respiration in the grazing period and the final active fungal length ($r = 1.00$, $p < 0.01$).

The correlation between the total number of bacteria and grazing pressure is significant ($v = 0.82$, $p < 0.05$). However, the corresponding value for bacterial dry mass and thus carbon is lower ($r = 0.66$) and is not significant. This results from the relative increase in smaller cells (Fig. 1).

The correlation between the metabolically active bacterial fraction and the grazing pressure is higher than for the total number ($r = 0.97$, $p < 0.01$), but the correlation between grazing pressure and the active cells is not significant. In the case of fungal hyphae, the relative fraction of metabolically active cells has increased significantly ($x^2 = 47.37$, $p < 0.001$).
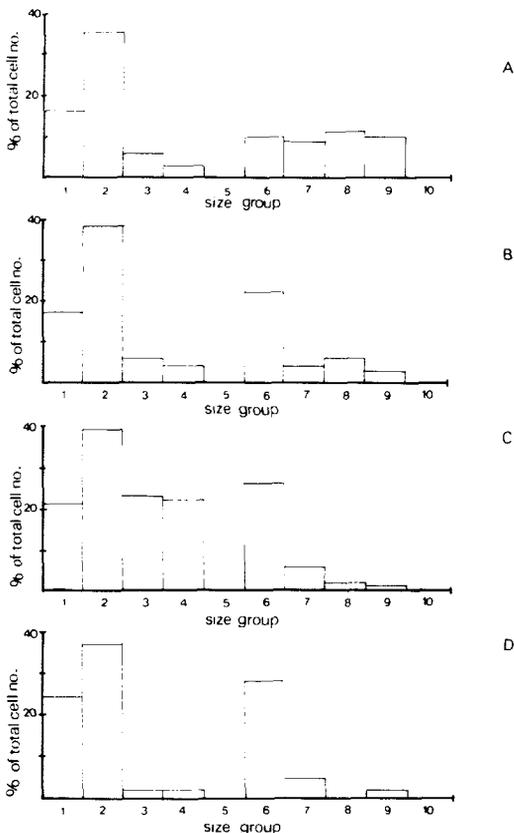
The increase in the number of Collembola within the experimental period is linear to the number added at day 11 ($r = 1.00$, $p < 0.001$). At the end of the experiment all stages of Collembola specimens were found, showing that reproduction had occured also in the later part of the experimental period. The increase in the number of Collembola within the experimental period is linear to the number within any of the vessels below the 'carrying capacity' of the available food source.

Though Andrén & Schnürer (1985) are among those who have reported reduced biomasses in both fungi and bacteria as a result of microbial grazing, the same pattern was not recorded in the present experiment. On the contrary there was an increase both in numbers and biomasses. The biomass increase is lower than the corresponding increase in numbers and length, respectively.

An increased mineralization of macro-nutrients due to the increased grazing pressure of the Collembola seems to give a cell growth beyond the consumption of the Collembola at the population levels established here.

The grazing effect of *H. viatica* can probably not be explained by exclusive feeding on microbial cells. The growth response of the microbial biomass might be due not only to the mineralization of microbially bound nutrients by grazing. The presence of a high microbial biomass might stimulate the general consumption of kelp which is rich in nutrient. Ulber (1983) found that *F. fimetaria* was feeding on living plant roots only when infested by fungi to a certain degree.

The effect of the microfauna grazing indicates that the growth rate of the microflora is regulated by the availability of nutrients. This is in accordance with the results of Koop et al. (1982b), who found a very high degree of microbial immobilization of macro-nutrients in the kelp debris.

The apparent reduction in the size of bacteria in the 'grazed' vessels (Fig. 1) indicates that *H. viatica* is able to select longer bacteria as food

Fig. 1. Size and shape distribution of bacteria in the incubation vessels at experimental termination. A. 0 Collembola added. B. 5 Collembola added. C. 10 Collembola added. D. 20 Collembola added. Size group 1–5 are cocci of increasing cell size, while size group 6–11 are rods of increasing cell size (cf. Ramsay 1983).

items. Another explanation may be a relative increase in the bacterial species with a rapid adaptation to an improved nutrient supply, and at same time a smaller cell size. As no taxonomic survey of the bacteria present in the experimental vessels has been carried out, nothing is actually known about the growth characteristics of the bacterial species involved. In addition to a possible selective feeding on bacteria above a certain size, accidental ingestion of smaller cells together with selected bacteria and fungal hyphae will certainly occur. Amy & Morita (1983) demonstra-

Table 2. Length of fungal hyphae (total and metabolically active), with dry mass and carbon content calculated.

Measured or estimated parameter	No. Collembola			
	0	5	10	20
Total hyphal length (m)	420 ± 63	375 ± 41	462 ± 44	451 ± 46
Mg dry mass	0.11	0.09	0.12	0.11
FDA-active hyphae (m)	134 ± 14	160 ± 14	182 ± 16	204 ± 19
Mg dry mass	0.03	0.04	0.05	0.05
Active length (%)	27.3	44.4	41.7	45.4

Table 3. Number of bacteria (total number and number of metabolically active cells), with corresponding dry mass and carbon content calculated.

Measured or estimated parameter	No. Collembola			
	0	5	10	20
Total number of bacteria				
No. $\times 10^8$	4.26 ± 0.63	3.43 ± 0.42	4.72 ± 0.46	5.72 ± 0.63
Mg dry mass	29.31	12.49	9.45	8.0
FDA-active bacteria				
No. $\times 10^8$	0.68 ± 0.08	0.91 ± 0.10	0.99 ± 0.11	1.98 ± 0.23
Mg dry mass	4.68	3.31	1.98	4.20
Number active (%)	16.6	33.3	25.0	37.5

Table 4. Number of specimens of *Hypogastrura viatica* at the experimental termination (no. gdw^{-1}).

Measured or estimated parameter	No. Collembola			
	0	5	10	20
Collembola				
No.	0	42 ± 3.2	85 ± 7.3	149 ± 13.6
Mg dry mass	0	0.164	0.331	0.580

ted that bacterial cells which were rod shaped when grown under nutrient-rich conditions became coccoid when grown under nutrient-deficient conditions. Although this tendency is not recognized in this experiment, their results indicate that changes in size group distribution may be a more complex matter than a higher influence of grazing or nutrient changes.

When no changes in the mean hyphal diameter were found, this might be due to a more or less uniform diameter in the hyphae offered to the grazer.

Andrén and Schnürer (1985) found reduced microbial biomass to be a result of grazing by *F. fimetaria*. The biomass found at any time in a grazed decomposer system will be determined by the sum of the microbial production and grazing upon the biomass. The microbial production varies between different habitats and substrates, as does the grazing efficiency of the animals involved. It is thus not unexpected that the standing microbial biomass in two very different decomposer situations deviates. Microbes associated with barley straw decomposition probably inhabit a much more stable environment than those associated with decaying kelp. They might thus not be able to respond to environmental changes as fast as those in the latter habitat. Kelp decomposers, on the other hand, especially those living in the upper exposed layer of the kelp heaps, are exposed to changes that might be rapid: e.g. temperature changes, mixing of kelp due to wave exposure. These changes will be even more pronounced in the Arctic than in the temperate parts of the world.

As mentioned above, only Collembola was considered as a grazer in this experiment. It might, however, be assumed that protozoa like amoebae, ciliates and flagellate species contribute to the grazing.

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