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ORIGINAL PAPER

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Does collembolan grazing influence nitrogen fixation by cyanobacteria in the high Arctic?

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Abstract A field experiment was performed to test whether cyanobacterial nitrogen fixation rates increase or decrease when exposed to low and moderate microarthropod grazing intensities. The densities of naturally occurring Collembola, i.e. *Hypogastrura viatica*, were manipulated on a salt marsh covered with cyanobacteria in the high Arctic, Svalbard. Nitrogen-fixation rates in grazed cyanobacterial crust were measured and used as an indirect measurement of cyanobacterial biomass on three dates during one summer. After 30 days, a second order polynomial regression gave a good fit to the data, indicating an increase in nitrogen-fixation rates at low/intermediate grazing pressures and a decrease at high grazing pressures. Thus, grazing collembolans may influence the nitrogen-fixation rates in an arctic salt-marsh community. Although based on a small set of data, the study indicates a compensatory fixation at low grazing pressures followed by a reduction at high grazing pressures.

Introduction

The grazing optimisation hypothesis states that net primary production in plants increases with increasing grazing pressure before it reaches a maximum at a moderate rate of herbivory (McNaughton 1979; Hilbert et al. 1981). The fact that herbivory may increase plant production is supported by a number of investigations (McNaughton 1983; Alward and Joern 1993; Ouellet et al. 1994; Wegener and Odasz 1997; Paige 1999), although it may vary considerably between species, timing of herbivory and growth conditions (Deangelis and Huston 1993; Hicks and Reader 1995; Semmartin and Oesterheld 1996; DeMazancourt et al. 1998). Several mechanisms may account for this increase in plant productivity (Trumble et al. 1993), including ecological mechanisms such as nutrient cycling and physiological mechanisms such as increased photosynthetic activity and hormonal redistribution in the plants.

Similarly, soil fungi also increase in biomass at intermediate grazing pressure in laboratory experiments (Bengtsson et al. 1993; Hedlund and Augustsson 1995), a result partly explained by a physiological change in fungal growth (Hedlund et al. 1991). Nutrient cycling by microarthropod grazing is also important for growth of soil microflora (Fitter et al. 1985).

The high Arctic is an excellent study system for interactions between soil microarthropods and microflora. The food webs are simple and the two-dimensional habitat (i.e. areas covered with a crust of filamentous cyanobacteria) allows direct observation of grazing microarthropods. In arctic salt marshes at Svalbard, Collembola graze at large densities on this cyanobacterial crust (Hertzberg 1997; T. Birkemoe, personal observation).

Cyanobacteria are the major source of nitrogen input in polar environments (Alexander and Schell 1973; Davey 1983; Henry and Svoboda 1986; Chapin et al. 1991) and interactions between microarthropods and cyanobacteria may be important for the N-dynamics. It is not known whether Collembola feed on the cyanobacteria or the mucilaginous sheath material in which the bacteria are embedded.

We first tested whether the collembolans were actually feeding on the cyanobacterial cells. As this was indeed the case, we wanted to test whether the grazing optimisation hypothesis was applicable to the interaction between collembolans and cyanobacteria at a salt marsh covered with a cyanobacterial crust. A field experiment

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manipulating collembolan-grazing intensities was performed. The dominant collembolan in the habitat moves around in large aggregations, which ensures high local grazing intensities over short time periods. In our experiment, however, constant grazing pressures were applied throughout the experimental period. The densities used were therefore chosen to be considerably lower than the densities in the aggregations, simulating low and medium grazing pressure through time. As it is difficult to measure cyanobacterial biomass directly in the field, nitrogen fixation was used as an indirect measurement. At one location, nitrogen-fixation relates directly to biomass when abiotic factors (i.e. temperature, moisture and time of season) are kept constant.

Materials and methods

Site description

The experiment was undertaken at a salt marsh close to the marine shoreline in Bjørndalen, Svalbard, a high-Arctic site with an annual mean air temperature of −6.7 °C and a mean precipitation of 190 mm/year (data from Svalbard Airport, Førland et al. 1997). The area is covered by an orange cyanobacterial crust and scattered individuals of the salt-marsh graminoid *Puccinellia phryganodes*. *Hypogastrura viatica* is the dominant springtail (Sveum 1987; Hertzberg et al. 1994; Hertzberg 1997) in this habitat.

Observations of feeding

To test whether *H. viatica* feeds on cyanobacteria, ten animals were collected from the cyanobacterial crust at the *Puccinellia* salt marsh and the presence of cyanobacteria in their dissected guts determined by fluorescence microscopy.

Grazing experiments

A total of 45 open plastic cylindrical enclosures (7 cm high, inner area 50.0 cm^2) with nylon mesh (0.2 mm) covers were pushed into the soil. As *H. viatica* does not go deep into the soil, the enclosures represent a reliable barrier for the collembolans. The cylindrical enclosures were placed randomly within the study site and 2 treatment densities of Collembola (40 and 80 animals per 10 cm^2, 15 cylinders of each) and a control (no animals, 15 cylinders) were randomly allocated to the enclosures.

Manipulated densities were 10 and 5 times lower than in aggregations found in the field (T. Birkemoe and T. Liengen, personal observation). As *H. viatica* moves around in groups, as is common to several *Hypogastrura* species (Leinaas 1983a,b), the experiments were set up while the majority of individuals were feeding outside the experimental area. Thus, grazing pressure on controls was insignificant.

Temperatures were recorded inside and outside the enclosures (Grant Squirrel datalogger), with mean temperatures inside the enclosures slightly higher (average 0.4 °C) than outside.

The experiment started on 7 July 1997 and soil from five enclosures from each treatment and control was collected on 12 July, 21 July and 6 August. Nitrogen fixation was measured in a 9.6-cm^2 sub-sample taken in the centre of the 50.0-cm^2 sample. Collembola were extracted from the remaining sample with a slightly modified high-gradient extractor (MacFadyen 1961) into benzoic acid. All animals were counted and identified.

Nitrogen fixation

Nitrogen fixation was measured using the acetylene reduction method (Stewart et al. 1967). Samples were placed in 160-ml modified serum bottles with modified ground-glass stoppers, immediately after collection. The joint bottles were sealed by high-vacuum grease and rubber septa. Air 10% (v/v) was replaced with 10% (v/v) acetylene gas in the serum bottles, and the samples incubated at 19 °C and 100 μE m^{-2} s^{-1} (PAR quantum sensor, Li-Cor, model LI-185B). Gas samples were taken with a syringe after 0.5 and 1.0 h incubation and analysed for ethylene by a gas chromatograph (Shimadzu GC-14A, Shimadzu, Kyoto, Japan) equipped with a flame ionisation detector and a 1.8-m column, packed with 50/80 mesh Porapak T (Supelco, Bellefonte, Pa.). Nitrogen was used as carrier gas; the column was maintained at 60 °C, the injection port at 200 °C and the detector at 240 °C.

There was no detectable natural production or consumption of ethylene in the soil, a prerequisite for the acetylene reduction method to provide a reliable measurement of the nitrogen-fixation activity on the cyanobacterial crust (Postgate 1998). Soil moisture was calculated from the weight difference of the sample before and after drying at 105 °C, and calculated as percent moisture of the samples' dry weight.

Statistics

The densities of *H. viatica* were transformed by log(x + 1) prior to statistical analyses to homogenise the variance sufficiently. The relationship between nitrogen-fixation rate and animals' density was established by choosing the regression model with the best fit. However, due to the low number of data-points, a second-order polynomial regression was the most complex model applied. All analyses were performed using JMP 3.2.2, SAS Institute.

Results and discussion

H. viatica was found in large numbers at the salt marsh. Eight of the ten investigated specimens had their guts filled with cyanobacterial cells of the genus *Nostoc*, the dominant cyanobacteria in the crust (Liengen and Olsen 1997a,b). Thus, neither the mucilaginous sheath material nor the filamentous growth forms of the cyanobacteria, which have been suggested as defence towards invertebrates grazing in freshwater systems (Kirk and Gilbert 1992; Goarant et al. 1994; Dodds et al. 1995), prevented collembolans from feeding.

There was no significant difference in nitrogen-fixation rate between the control and treatments in our experiment (Table 1, Fig. 1). However, there was a significant relationship between the observed densities of *H. viatica* and nitrogen-fixation rate at the end of the experiment (Fig. 2) when the difference between the treatment densities had disappeared (Fig. 3).

Juvenile *H. viatica* hatch in mid- to late summer (Hertzberg et al. 1994, in press) and the animals added to the enclosure on 7 July were dominated by pre-adults/adults. By 6 August (day 30 of the experiment) however, recruitment had taken place and a large number of juveniles were present inside the enclosures. The lack of a significant grazing effect of nitrogen-fixation rate during the first 14 days of the experiment when the treatment densities were still applicable could be due to

Table 1 ANOVA analyses testing for the effect of time and treatment on nitrogen fixation (ethylene production). The r^2 of the model was 0.296. Interaction with moisture was included in the first model, but excluded by backwise elimination procedure as it did not have any significant effect on the model ($P > 0.2$)

	df	*F*-ratio	*P*-value
Time	2	5.53	0.008
Treatment	2	0.55	0.580
Time $*$ Treatment	4	0.75	0.567

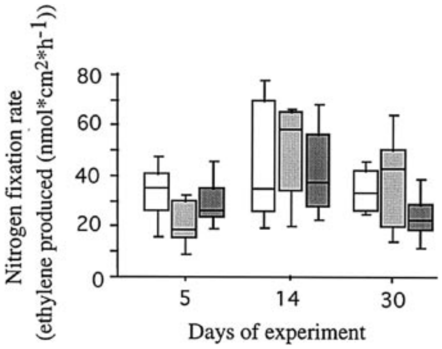

Fig. 1 Nitrogen-fixation rates expressed as ethylene produced by the cyanobacteria in the controls (*unshaded*) and the treatments [40 (*light shading*) and 80 (*dark shading*) *Hypogastrura viatica* added/10 cm^2]. Medians, 25 and 75% quartiles and 10 and 90% percentiles are presented. No values were found outside the 10 and 90% percentiles

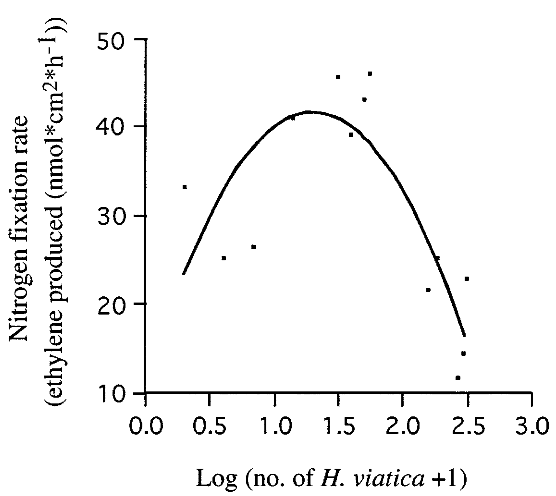

Fig. 2 The polynomial regression with the best fit (i.e. the highest r^2-value) between the number of *Hypogastrura viatica* and nitrogen-fixation rate (ethylene produced) after 30 days of increased grazing pressure. Regression statistics: $r^2 = 0.69$, $P = 0.003$. One outlier, not shown in the plot, was excluded prior to the analysis by comparing Mahalanobis distances from the mean

the short time period of grazing, or possibly, the age structure of the individuals. Rapidly growing juveniles are likely to have a larger energy demand than adults and may have caused more intense grazing in the latter part of the experimental period.

The nitrogen-fixation rate increased at low/intermediate grazing intensities at day 30 of the experiment (Fig. 2). Protozoan grazing has been shown to increase nitrogen fixation of cyanobacteria in a study carried out in desert soils (Ghabbour et al. 1980). Ghabbour et al. (1980) argued that the Protozoa fed on the vegetative cells only and were unable to ingest the heterocysts (i.e. the cells in which nitrogen fixation take place). The much larger size of Collembola as compared to Protozoa, however, makes discrimination between these cell-types unlikely and another mechanism is needed to explain the increase in nitrogen-fixation rates, e.g. compensatory growth.

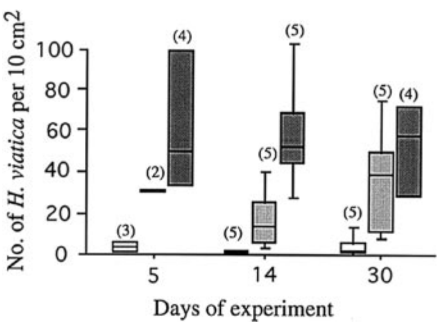

Fig. 3 Number of *Hypogastrura viatica* recovered from the enclosures in the two treatments [40 (*light shading*) and 80 (*dark shading*) *H. viatica* added/10 cm^2] and controls (*unshaded*). The number *in parentheses* denotes the number of samples from which the animals were successfully extracted, i.e. seven samples in total were damaged prior to extraction. Medians, 25 and 75% quartiles and 10 and 90% percentiles are given. No values were found outside the 10 and 90% percentiles

The nitrogen-fixation rate decreased about 50% when the densities of *H. viatica* increased to the highest grazing pressures at day 30 of the experiment (Fig. 2). This effect of high grazing intensity is most likely caused by removal of bacterial biomass beyond a potential compensating capacity. However, a decrease in nitrogen-fixation rate may also be caused by accumulation of collembolan faeces. Nutrient cycling is suggested as one of the mechanisms increasing plant production in response to grazing (Dyer et al. 1986; Holland et al. 1992; DeMazancourt et al. 1998). Similarly, the cyanobacterial growth rate is likely to be influenced positively by nutrient cycling through animal faeces. However, the nitrogen-fixation activity is inhibited rather than facilitated as cyanobacteria decrease the expensive process of nitrogen fixation when combined nitrogen is available (Flores and Herrero 1994; Liengen 1999).

The grazing optimisation hypothesis states that primary production will increase at low and intermediate levels of grazing intensity (McNaughton 1979; Hilbert et al. 1981). Our results indicate a similar effect in the cynaobacteria-collembolan grazing system. It might be argued that our approach reflects a "static" situation; under natural conditions the grazing is patchy in time and space. However, dynamic grazing patterns have proved essential for compensatory growth in plants (Oesterheld 1991; Beaulieu et al. 1996; Semmartin and Oesterheld 1996), as well as in soil fungi (Bengtsson et al. 1993). Thus, if cyanobacteria have a similar response, the compensation in nitrogen-fixation rates, i.e. larger increase in biomass resulting in higher nitrogen-fixation rates, will be stronger with a more patchy grazing regime.

Further studies are needed in order to understand the mechanisms by which grazing collembolans effect cyanobacterial nitrogen-fixation rates. Moreover, as our results are based on a small data set, further investigations should be carried out to verify the existence of a compensatory nitrogen-fixation rate in cyanobacteria. However, our results clearly indicate that grazing collembolans may, dependent on animal densities, increase or decrease nitrogen-fixation rates of cyanobacteria in arctic salt marsh communities.

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References

Alexander V, Schell DM (1973) Seasonal and spatial variation in nitrogen fixation in the Barrow, Alaska, tundra. Arct Alp Res 5: 77–88

Alward RD, Joern A (1993) Plasticity and overcompensation in grass responses to herbivory. Oecologia 95: 358–364

Beaulieu J, Gauthier G, Rochefort L (1996) The growth response of graminoid plants to goose grazing in a High Arctic environment. J Ecol 84: 905–914

Bengtsson G, Hedlund K, Rundgren S (1993) Patchiness and compensatory growth in a fungus-Collembola system. Oecologia 93: 296–302

Chapin DM, Bliss LC, Bledsoe LJ (1991) Environmental regulation of nitrogen-fixation in a high Arctic lowland ecosystem. Can J Bot 69: 2744–2755

Davey A (1983) Effects of abiotic factors on nitrogen fixation by blue-green algae in Antarctica. Polar Biol 2: 95–100

Deangelis DL, Huston MA (1993) Further considerations on the debate over herbivore optimization theory. Ecol Appl 3: 30–31

DeMazancourt C, Loreau M, Abbadie L (1998) Grazing optimization and nutrient cycling: when do herbivores enhance plant production? Ecology 79: 2242–2252

Dodds WK, Gudder DA, Mollenhauer D (1995) The ecology of *Nostoc*. J Phycol 31: 2–18

Dyer MI, DeAngelis DL, Post WM (1986) A model of herbivore feedback on plant productivity. Math Biosci 79: 171–184

Fitter AH, Atkinson D, Read DJ, Usher MB (1985) Ecological interactions in soil: plants, microbes and animals. Blackwell, Oxford

Flores E, Herrero A (1994) Assimilatory nitrogen metabolism and its regulation. In: Bryant DA (ed) The molecular biology of cyanobacteria. Kluwer, Dordrecht, pp 487–517

Førland EJ, Nordli PY, Hanssen-Bauer I (1997) Climate statistics and longterm series of temperature and precipitation at Svalbard and Jan Mayen. Norske Meteorologiske Institutt, Oslo

Ghabbour SI, El Ayouty EY, Khadr MS, El Tonsi AMS (1980) Grazing by microfauna and productivity of heterocystous nitrogen-fixing blue-green algae in desert soils. Oikos 34: 209–218

Goarant E, Prensier G, Lair N (1994) Specific immunological probes for the identification and tracing of prey in crustacean gut contents – the example of cyanobacteria. Arch Hydrobiol 131: 243–252

Hedlund K, Augustsson A (1995) Effects of enchytraeid grazing on fungal growth and respiration. Soil Biol Biochem 27: 905–909

Hedlund K, Boddy L, Preston CM (1991) Mycelial responses of the soil fungus, *Mortierella isabellina*, to grazing by *Onychiurus armatus* (Collembola). Soil Biol Biochem 23: 361–366

Henry GHR, Svoboda J (1986) Dinitrogen fixation (acetylene reduction) in high arctic sedge meadow communities. Arct Alp Res 18: 181–187

Hertzberg K (1997) Migration of Collembola in a patchy environment. Pedobiologia 41: 494–505

Hertzberg K, Leinaas HP, Ims RA (1994) Patterns of abundance and demography – Collembola in a habitat patch gradient. Ecography 17: 349–359

Hertzberg K, Yoccoz NG, Ims RA, Leinaas HP (in press) The effects of spatial habitat configuration on recruitment, growth and population structure in arctic Collembola. Oecologia

Hicks SL, Reader RJ (1995) Compensatory growth of 3 grasses following simulated grazing in relation to soil nutrient availability. Can J Bot 73: 141–145

Hilbert DW, Swift DM, Detling JK, Dyer MI (1981) Relative growth rate and the grazing optimization hypothesis. Oecologia 51: 14–18

Holland EA, Parton WJ, Detling JK, Coppock DL (1992) Physiological responses of plant-populations to herbivory and their consequences for ecosystem nutrient flow. Am Nat 140: 685–706

Kirk KL, Gilbert JJ (1992) Variation in herbivore response to chemical defenses – zooplankton foraging on toxic cyanobacteria. Ecology 73: 2208–2217

Leinaas HP (1983a) Synchronized moulting controlled by communication in group-living Collembola. Science 219: 193–195

Leinaas HP (1983b) Winter strategy of surface dwelling Collembola. Pedobiologia 25: 235–240

Liengen T (1999) Environmental factors influencing the nitrogen fixation activity of free-living terrestrial cyanobacteria from a high arctic area, Spitsbergen. Can J Microbiol 45: 573–581

Liengen T, Olsen RA (1997a) Nitrogen fixation by free-living cyanobacteria from different coastal sites in a high arctic tundra, Spitsbergen. Arct Alp Res 29: 470–477

Liengen T, Olsen RA (1997b) Seasonal and site-specific variations in nitrogen fixation in a high arctic area, Ny-Ålesund, Spitsbergen. Can J Microbiol 43: 759–769

MacFadyen A (1961) Improved funnel-type extractors for soil arthropods. J Anim Ecol 30: 171–184

McNaughton SJ (1979) Grazing as an optimization process: grass-ungulate relationships in the Serengeti. Am Nat 113: 691–705

McNaughton SJ (1983) Compensatory plant growth as a response to herbivory. Oikos 40: 329–336

Oesterheld M (1991) Effect of stress and time for recovery on the amount of compensatory growth after grazing. Oecologia 85: 305–313

Ouellet JP, Boutin S, Heard DC (1994) Responses to simulated grazing and browsing of vegetation available to caribou in the Arctic. Can J Zool 72: 1426–1435

Paige KN (1999) Regrowth following ungulate herbivory in *Ipomopsis aggregata*: geographic evidence for overcompensation. Oecologia 118: 316–323

Postgate J (1998) Nitrogen fixation, 3rd edn. Cambridge University Press, Cambridge

Semmartin M, Oesterheld M (1996) Effect of grazing patterns on primary productivity. Oikos 75: 431–436

Stewart WPD, Fitzgerald GP, Burris RH (1967) In situ studies on nitrogen fixation using acetylene-reduction technique. Proc Natl Acad Sci USA 58: 2071–2078

Sveum P (1987) The influence of grazing by *Hypogastrura viatica* (Insecta: Collembola) on microbial activity in decomposing kelp on Spitsbergen. Polar Res 5: 71–77

Trumble JT, Kolodnyhirsch DM, Ting IP (1993) Plant compensation for arthropod herbivory. Annu Rev Entomol 38: 93–119

Wegener C, Odasz AM (1997) Effects of laboratory simulated grazing on biomass of the perennial Arctic grass *Dupontia fisheri* from Svalbard: evidence of overcompensation. Oikos 79: 496–502