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PRIMARY RESEARCH PAPER

Bottom-up effects on freshwater bacterivorous nematode populations: a microcosm approach

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Abstract Nutrient enrichment may alter population dynamics of species in different ways depending on their life strategies. The aim of this study was to test the effect of different nutrient concentrations on the population development of two bacterivorous freshwater nematodes, *Bursilla monhystera* and *Plectus aquatilis*. Microcosms with autoclaved natural sand from a pristine stream (Fuirosos, NE of Spain) were enriched with different levels of phosphate, nitrate and ammonia as inorganic nutrients and glucose as a biodegradable dissolved organic carbon source. Although leaching of carbon and nutrients from the detritus fraction in the sediment initially may have overruled differences between treatments, later samplings revealed bottom-up control, with *Bursilla monhystera* abundances positively correlated to bacterial abundances at high nutrient concentrations. Nevertheless, there were several indications that nematodes in turn affected microbial abundance, most likely through excretion of ammonia and through grazing. In contrast to *B. monhystera*, *Plectus aquatilis* at high nutrient concentrations showed a unimodal abundance curve, while not increasing in abundance at low nutrient concentrations. Glucose enrichment did not have any stimulatory effect on either microbial or nematode abundances, probably as a result of unfavourable C:N:P stoichiometry. P enrichment, by contrast, stimulated microbial and *Bursilla* abundances. Our results indicate that episodic nutrient enrichment may affect populations of bacterial-feeding nematodes in the short term. Their longer-term dynamics may, however, be more dependent on leaching of carbon and nutrients from the pools of sediment-bound detritus.

Keywords Eutrophication · Nematodes · Bacteria · Bottom-up · Top-down control

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Introduction

Nematodes are often the most abundant metazoans in the soft sediments of freshwater bodies. Their ecological significance in terms of productivity, food-chain relationships and the remineralisation of organic substances may be substantial (Ingham et al., 1985). Compared with soil and marine habitats, relatively little is known about the ecology of freshwater

nematodes, especially those in lotic systems (Traunspurger, 2002; Gaudes et al., 2006; Hodda, 2006; Lopez-Doval et al., 2010).

Bacterial-feeding nematodes can affect bacterial communities at different levels, influencing bacterial activity, either positively (Findlay & Tenore, 1982; Ingham et al., 1985; Alkemade et al., 1992a, b; Traunspurger et al., 1997) or negatively (De Mesel et al., 2003). This may be a direct effect of grazing (especially at low nematode numbers; Ingham et al., 1985), but bioturbation (Abrams & Mitchell, 1980; Alkemade et al., 1992b), the production of faeces and mucus secretions (Traunspurger et al., 1997; Riemann & Helmke, 2002; Moens et al., 2005) by nematodes are also important. In addition to affecting microbial activity, nematode activity may alter the composition and diversity of bacterial communities (De Mesel et al., 2004, 2006).

Bottom-up effects such as nutrient availability on bacterial and nematode populations have been extensively studied in soil environments due to the importance of these animals in nutrient mineralisation and consequently in soil fertility (e.g. Ferris et al., 1995; Yeates, 2003). Again, however, there is a paucity of similar studies in freshwater systems. There is a large amount of literature on the direct effects of nutrient enrichment on the periphyton, focusing on the autotrophic compartment, while less attention has been paid to the heterotrophic bacteria, fungi, protozoa and meiofauna inhabiting these complex biofilms (Hillebrand et al., 2002). Recently, studies have shown that nutrient enrichment in oligotrophic systems may increase the rate of organic matter decomposition, stimulating associated microbial activity and reducing the amount of litterfall remaining in streams (Gulis & Suberkropp, 2003; Gulis et al., 2004; Greenwood et al., 2007). The effects of enhanced nutrients on detritus decomposition may extend to consumers, both to their community composition and productivity (Cross et al., 2005, 2007; Gaudes et al., 2012).

Rhabditidae and Plectidae are two nematode families that are common in a variety of freshwater and moist soil habitats (Abebe et al., 2008). They are both mainly bacterivorous, yet they exhibit substantial differences in life history and autecology. Rhabditidae are generally characterised by a very short generation time, high fecundity, high tolerance to various kinds of disturbance and pollution, and high food availability thresholds for feeding and reproduction (Schiemer, 1983; Hodda et al., 2006). As a result, they are often considered enrichment opportunists: they rapidly develop under nutrient-enriched conditions since these favour high bacterial populations, and form metabolically dormant dauer larvae under conditions of crowding and food depletion (Bongers, 1999). Their population dynamics are characterised by successive colonizations and extinctions in a patchy environment (Derycke et al., 2007). Although many Plectidae may also behave as colonizers, they are characterised by comparatively longer generation times (3–4 times longer than Rhabditidae), lower food thresholds and a lower tolerance to pollution and disturbance (Schiemer, 1983; Kammenga et al., 1994; Martinez et al., 2012). They also lack a dauer stage. They are classified as general opportunists sensu Bongers (1999) and often replace Rhabditidae in succession when the food supply (i.e. bacterial abundance) decreases (Schiemer, 1983).

Our main objectives were to study the bottom-up effects of different levels of nutrient and dissolved organic carbon enrichment (N, P and glucose in different combinations and at different concentrations) on the populations of bacterial-feeding nematodes in terms of density and biomass. We focused on the rhabditid *Bursilla monhystera* (Buetschli, 1873) which is characterised by a short generation time and rapid population development at high food (=bacteria) availability (Postma-Blaauw et al., 2005). We ran parallel incubations with *Plectus aquatilis* (Andrássy, 1985), which is characterised by a slower reproduction and growth and lower food requirements (Schiemer, 1983; Kammenga et al., 1994). The latter experiment focused on the most nutrient-enriched treatments and the non-enriched control only. We hypothesised that *Bursilla* populations would show a more rapid response to, and a stronger dependence on nutrient enrichment than *Plectus* populations.

Materials and methods

Experimental design

A factorial design was prepared using two biodegradable dissolved organic carbon (BDOC) conditions (basal and 20-fold higher). Each BDOC treatment comprised three levels of nutrient availability (basal, 3-fold and 10-fold) for nitrogen, phosphorus or both.

The nutrient concentrations in our experiment were based on long-term bio-monitoring in the Fuirosos, a third-order forested Mediterranean stream located in the NE of Spain (Artigas et al., 2009; Sabater et al., 2011). The reagents used for nutrient enrichment were common standards for D-glucose, $N–NO_3$ and $P–PO_4$. Each treatment was destructively sampled with four replicates for each treatment, with the exception of the basal nutrient treatment, which had six replicates. The experiment thus contained × 3 (N, P and N + P nutrient combinations) × 2 (nutrient levels: × 3 or × 10) × 2 (BDOC conditions) × 5 (sampling dates) × 4 (replicates) + 1 (basal nutrient level) × 2 (BDOC conditions) × 5 (sampling dates) × 6 (replicates) = 300 microcosms for *Bursilla*. For *Plectus*, only the basal nutrient treatment and the 10-fold NP were analysed at the two different BDOC conditions at the three dates described above = 60 microcosms.

Sediment characterisation

In autumn 2005, natural sandy sediments were taken from the Fuirosos riverbed. The granulometry of these sediments (mean grain size of 740 μm, Table 1) was assessed using a Hydro 2000G particle size analyser (Malvern, UK). The organic matter content was ca. 0.5%. Natural detritus in Fuirosos contains an annual average of 11% carbon and 0.61% nitrogen (Gaudes, unpublished data). Sediments were autoclaved prior to the start of the experiment in order to eliminate natural fauna and microbiota.

Table 1 Sediment characterisation

Particle size (μm)	% ($n = 5$)
<4	0.14 ± 0.06
≥4 < 38	2.22 ± 0.26
≥38 < 63	0.89 ± 0.09
≥63 < 125	1.29 ± 0.22
≥125 < 250	5.49 ± 1.26
≥250 < 500	24.46 ± 5.15
≥500 < 800	28.11 ± 0.89
≥800 < 1,000	13.42 ± 1.48
≥1,000 < 1,600	20.29 ± 4.25
≥1,600	3.69 ± 1.02

Median grain size of 656.27 μm. Volume means and standard deviations are shown

Isolation and culturing of organisms

Natural stream water from Fuirosos was filtered through a sterile GF/F Whatman filter to remove all particles >1.2 μm. The filtrate was used as the bacterial inoculum, a few drops of which were inoculated into Erlenmeyer flasks containing 50 ml liquid broth medium including peptone and beef extract. These cultures were further inoculated with bacteria growing in the nematode stock cultures (see below) to ensure that nematode inoculation would not introduce any bacterial strains not present in the control or other nematode treatment. The cultures were renewed every 4–5 days; no fungal or protozoan proliferation was observed. Inevitably, this culture procedure yields a microbial assemblage that is both very different from, and less diverse than, natural microbial assemblages, so caution is due when extrapolating our microcosm results to a field situation.

The freshwater nematode *Bursilla monhystera* (Rhabditidae) was isolated from a small pond in the botanical garden at Ghent University, Belgium, in 1999. *Plectus aquatilis* (Plectidae) was isolated from the shores of Lake Zürich, Switzerland, in spring 2001. Following isolation both species were kept in permanent agnotobiotic culture on 1% agar media composed of bacto and nutrient agar in a 4:1 ratio. Unidentified bacteria from the natural habitat served as food (Moens & Vincx, 1998). The pH of the agar medium was buffered at 7.4 with TRIS-HCl at a final concentration of 5 mM. Stock cultures were maintained at 18°C in the dark. Nematode cultures were boosted prior to harvesting by the addition of a frozen-and-thawed *E. coli* suspension (Moens and Vincx, 1998). Nematodes were then harvested by rinsing them off the surface of the agar layers with sterile distilled water (*B. monhystera*), or by extracting them from the agar through density centrifugation in sucrose (*P. aquatilis*) (Moens & Vincx, 1998).

Microcosm design and sampling procedure

The microcosms were prepared as follows. A thin layer of sterile (autoclaved) sediment was spread on the bottom of 9 cm diameter Petri dishes, and hydrated with 10 ml of the corresponding nutrient solution (see above). They were then inoculated with 20 μl of bacterial inoculum and kept at 20°C in the dark. Bacteria were allowed to grow in the microcosms for 5 days prior to nematode inoculation. The microcosms

were then inoculated with a 100 µl drop of an aqueous suspension containing 126.6 ± 20.0 individuals + 17.7 ± 4.9 eggs of *B. monhystera*, and 186.5 ± 28.9 individuals + 71.8 ± 11.5 eggs ($n = 10$ in both cases) of *P. aquatilis*.

Sampling started 7 days after nematode inoculation and was further performed after 15, 21, 36 and 49 days for *B. monhystera* and after 13, 29 and 42 days for *P. aquatilis*.

A subsample of sediment (4.6 cm^2) was taken from each microcosm with a metal borer and fixed in formalin (2%) for bacterial counting. Pore water was carefully extracted from the sediments to perform nutrient analyses, and the remaining sediment was transferred to plastic pots and fixed in 4% formalin for later extraction and quantification of nematodes.

Sample processing for nutrient concentration and bacterial abundance

Water from each microcosm was extracted with pasteur pipettes, centrifuged for 15 min at 4,000*g* and the supernatant was filtered through pre-combusted glass-fibre filters (Whatman GF/F) before analysis. The pellet obtained after centrifugation contained some nematodes and was hence re-suspended in 5 ml MilliQ water and added to the remainder of the sample used for nematode quantification (see below). Ammonium, nitrate and dissolved phosphorus were analysed using standard methods (APHA, 1992) on a SKALAR San Plus (Breda) automatic chain.

The bacterial abundance in each microcosm was estimated after sonicating the samples (2 + 2 min, 40-W power, 40-kHz frequency; Ultrasons, Selecta, Abrera, Spain). After appropriate dilution, fixed samples were stained for 5 min with 4, 6-diamidino-2-phenylindole (final concentration 2 µg/ml) and passed through 0.2-µm irgalan black-stained polycarbonate filters (Nuclepore; Whatman, Maidstone, UK). Bacteria were counted in 25 fields/filter under a fluorescence microscope (Eclipse E-600; Nikon, Tokyo, Japan), corresponding to 300–500 cells per filter, at ×1,250 magnification.

Nematode counts and measurements

Samples for nematode quantification were washed through a 32-µm sieve and the nematodes extracted by flotation using Ludox TM50 (Heip et al., 1985). After staining with Rose Bengal, all nematodes were counted and classified as 'Small' (J1 and J2 juveniles) or 'Large' (J3 and J4 juvenile stages and Adults) (Large/Small ratio) facilitating a rough measure of biomass and yielding information on the age structure of the population. For detailed measurements, a minimum of 100 individuals per sample were picked out, dehydrated, transferred to anhydrous glycerol and mounted on slides. The body length and maximal width of all nematodes were measured under a microscope (Eclipse E-600, Nikon, Tokio, Japan) using an image analyser (analySIS®, Olympus, Münster, Germany). Nematode volume was calculated according to the following formula (Warwick & Price, 1979).

$$\text{Biovolume (nL)} = 530 \times \text{Length (µm)} \times \text{Width}^2\ \text{(µm)}/16 \times 105,$$

and this was converted to dry weight (W) assuming a specific gravity of 1.13 (Wieser, 1960) and a dry-to-wet weight ratio of 0.25 (Feller & Warwick, 1988).

Nematode bioenergetics

Nematode samples were divided into five size classes, roughly corresponding to the four juvenile (J1, J2, J3 and J4) stages and clearly reproductive adults; some young, not yet reproductive adults may have ended up in the J4-class (modified from Ferris et al., 1995). The numbers in the different size classes were used to estimate respiration (R, in $nLO_2\ h^{-1}$) according to the power equation $R = a \cdot W^{b}$ (Schiemer, 1982, 1983). Values for 'a' and 'b' were based on data for the rhabditid *Caenorhabditis briggsae* fed on *Escherichia coli* at 20°C (Schiemer, 1982). The amount of C respired was determined from the total O_2 respired by all size classes using a conversion factor of 4.43 nLO_2 µg (fresh weight)$^{-1}$ h^{-1} described for *Bursilla labiata* (Ferris et al., 1995) and converted to carbon following standard conversions (Waters, 1977).

Production was calculated from respiration according to efficiencies described in the literature for *B. labiata* (a production efficiency of 59%; production efficiency = 100 × production/(production + respiration)) and other rhabditids (Marchant & Nicholas, 1974; Ferris et al., 1997). The sum of production and respiration is assimilation. Based on these values of assimilation, we estimated the potential contribution of nematodes to nitrogen cycling in our microcosms. When consuming sufficient bacteria to support their C requirements, nematodes assimilate more N than

necessary, because the C:N ratios of nematodes exceed those of bacteria (Ingham et al., 1985), and this excess is excreted mainly as ammonia (Lee & Atkinson, 1977). We used a C-to-N ratio of 5.89 for nematodes (mean for eight nematode species cultured on agar with *E. coli* as food) (Ferris et al., 1997) to calculate the weight of N required for secondary production by the nematodes. We calculated the weight of bacterial N associated with the assimilated bacterial C, using a mean C-to-N ratio of 4.12 (Ferris et al., 1997). The difference between the two nitrogen assimilations is the amount of N that can be excreted by the nematodes. We then compared our estimates of ammonia released by nematodes between any two sampling moments with the increase in ammonia observed during that same time interval, to assess whether nematodes could have contributed significantly to N cycling in our microcosms.

Statistical analysis

In both experiments, the normality of the sample distribution and homogeneity of variances were assessed to determine whether data transformation ($\log(x + 1)$) was required. A two-way ANOVA (using time and treatment) was used for *Plectus* and a three-way ANOVA (using time, NP treatment and glucose treatment) was used for *Bursilla* to detect significant differences between treatments over time. Post-hoc comparisons between means were analysed using a Tukey Honest Significant Differences (HSD) test.

Spearman multiple correlation was applied to real nutrient concentrations (ionic N forms in water) in the microcosms and bacterial and nematode density, biomass and Large/Small ratio. We applied a false discovery rate correction (FDR; Benjamini & Hochberg, 1995) because of the large number of comparisons made. All the statistical procedures were applied using the STATISTICA package (version 8.0; StatSoft, Tulsa, Oklahoma, USA).

Results

Effect of nutrients on nematode and bacterial abundances

Surprisingly, bacterial abundances in the *Bursilla* experiment were fairly similar across treatments (Figs. 1, 2), even though some significant differences were observed between nutrient treatments ($P < 0.05$). A significant Time × Nutrient treatment effect (see Table I in the Supplementary Material) highlighted that in general, in basal and 3-fold nutrient treatments (Fig. 1), bacterial abundances remained fairly constant over time, while in the 10-fold additions (Fig. 2), they started off very low but then increased strongly. An effect of glucose was only observed in the non-enriched treatments with a slight decrease in bacterial abundances on the second compared to the first sampling date, except for the 10-fold P and NP treatments, that showed a constant growth. In general, glucose addition did not significantly alter bacterial abundances ($F = 0.71$, $P = 0.4$; see supplementary material).

Bursilla abundances differed significantly with time, nutrient and glucose treatment, and also exhibited significant first-order interaction effects of glucose × nutrient and time × nutrient treatment (see Table II in the Supplementary Material). Nematode abundance in basal nutrient conditions tended to follow a unimodal curve with initial exponential growth, a plateau and a slight decrease towards the end of the experiment (Figs. 1, 2). Tukey post-hoc comparisons also revealed differences between the basal treatment and the 3-fold NP and 10-fold NP treatments, with densities becoming three times higher in the nutrient-enriched treatments towards the end of the experiment. Treatments enriched only with N showed a similar trend to basal treatments. Conversely, phosphorus- and combined NP-enriched treatments showed highly variable, but continuous population growth patterns of *Bursilla* (Figs. 1, 2).

The significant glucose effect on nematode abundance ($F = 5.03$, $P = 0.026$) was not consistent over time and mainly occurred in combination with a nutrient treatment effect (see Table II in the Supplementary Material). In the 10-fold N treatment without glucose (Fig. 2), the density of nematodes was almost twice the maximum density achieved in the 3-fold N treatment (Fig. 1). In the phosphorus-enriched treatments the pattern was the opposite, with more nematodes in the 3-fold P treatment with glucose compared to the 10-fold P enrichment (Figs. 1, 2). In the combined NP treatments, the highest *Bursilla* abundances were found in the 3-fold and 10-fold NP treatments without glucose (Figs. 1, 2). Tukey post-hoc comparisons revealed differences ($P < 0.01$) between 10-fold N, P and NP treatments with glucose

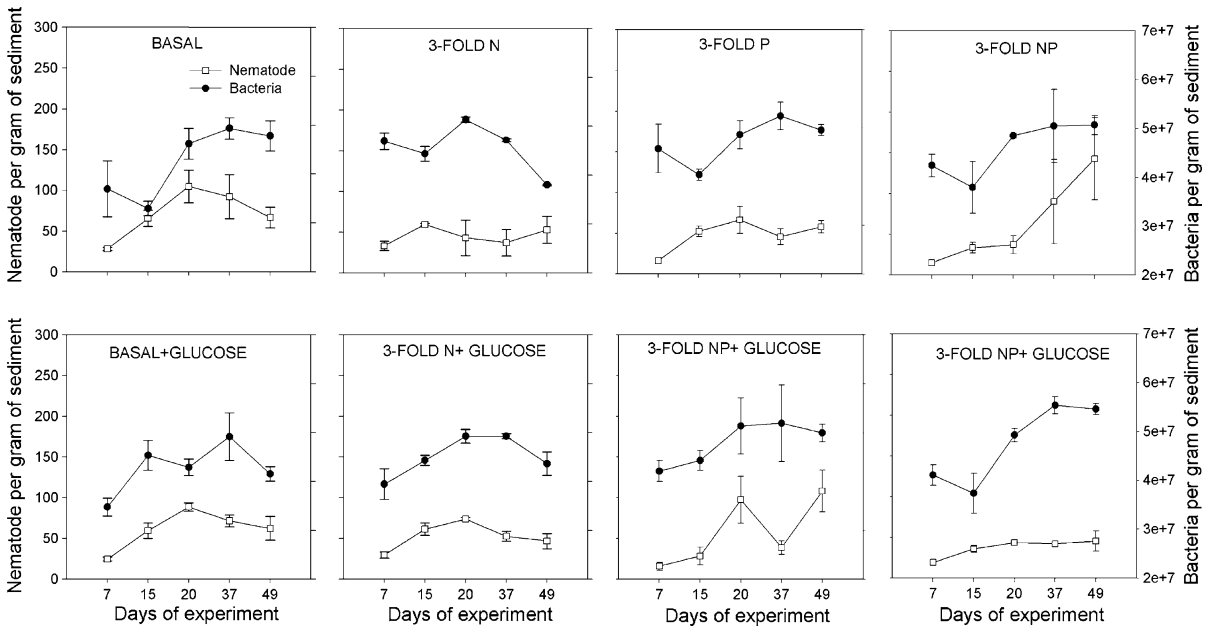

Fig. 1 Mean (±SE) abundances of *Bursilla* and bacteria under basal and 3-fold N, P and NP. *X*-axis represents the days of experiment after nematode inoculation

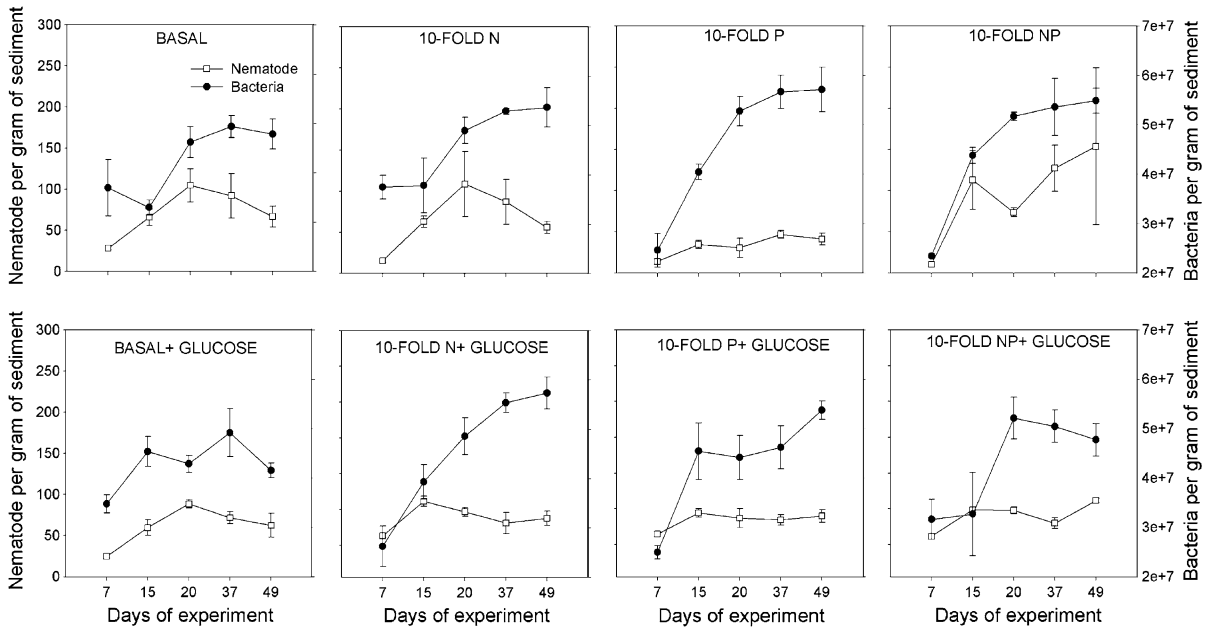

Fig. 2 Mean (±SE) abundances of *Bursilla* and bacteria under basal and 10-fold N, P and NP. *X*-axis represents the days of experiment after nematode inoculation

(with lower densities) and 10-fold NP without glucose (with higher nematode abundances). In terms of biomass, both enriched treatments started with higher biomasses compared to the controls (Fig. 3), but the 10-fold NP treatment with glucose showed a slight decrease in individual biomass towards the end of the experiment compared to the same treatment without glucose (Fig. 3).

Plectus abundance or biomass did not exhibit a clear effect of treatment (basal vs. 10-fold NP) nor of glucose addition (abundance: $F = 0.515$, $P = 0.67$; biomass: $F = 0.65$, $P = 0.59$; see Tables V and VI in the Supplementary Material). Bacteria density was affected by treatment ($F = 4.5$, $P = 0.014$) and time ($F = 12.5$, $P = 0.0003$). *Plectus* density also exhibited a significant change over time ($F = 5.83$, $P = 0.009$), more or less opposite to the bacterial trend (Fig. 3).

Comparison between *Plectus* and *Bursilla* response to nutrient availability

Under basal nutrient treatments at both glucose levels, *Bursilla* populations strongly increased until they reached a plateau (typically after 20 days) and then decreased (Fig. 1), while *Plectus* populations showed no significant increase over the course of the experiment except for a slight increase in the NP amended treatment (Fig. 4, upper two panels). However, in the high nutrient treatment, especially that enriched with glucose, the *Plectus* population did increase (Fig. 4, lower two panels), but the increase in *Bursilla* numbers started earlier, peaking after 15 days and then showing a variable pattern towards the end of the experiment (Fig. 2), whereas *Plectus* populations again decreased between 30 and 42 days of incubation (Fig. 4).

Bursilla bioenergetics and contribution to N cycling

Spearman tests showed significant positive correlations of ammonia and phosphate concentrations with *Bursilla* abundance as well as with the ratio of large to small individuals ($P < 0.01$; see Table III in the Supplementary Material). Ammonia and phosphate concentrations were also positively correlated with bacterial density but only at a significance level that did not meet the FDR cut-off requirements. Nitrite and nitrate concentrations, in contrast, showed a negative correlation with bacteria and nematode biomass. There was also a positive correlation between densities of bacteria and nematodes over the different treatments ($P < 0.01$; see Table III in the Supplementary Material).

Based on the potential excretion values measured for *Bursilla* and the ammonia concentrations measured in interstitial water from the microcosms, a higher proportion of that ammonia was attributable to the metabolic activity of nematodes in the basal treatments along the experiment than in the NP amended microcosms (Table 2).

Nematode respiration in basal nutrient conditions was the lowest on the first sampling date (Fig. 5). However, on the second and third sampling dates there was an increase in respiration concomitant with a larger proportion of J4 and adult nematodes. This increase was much lower in the glucose-enriched treatments (labelled 'Gluc'; Fig. 5). On the last two sampling dates the population was mainly dominated by small and medium sized nematodes (J1, J2 and J3) and there was a concomitant decrease in respiration.

On the first sampling date, respiration in the 10-fold NP treatments was similar to that in the basal treatment (Fig. 5). From the second sampling onwards, there was a variable but increasing respiration in the NP-enriched treatment. The glucose-enriched treatments showed lower nematode respiration.

Discussion

Bottom-up versus top-down dynamics

Heterotrophic bacteria and fungi and their grazers are an important link in the functioning of many ecosystems, particularly for the mineralisation of nutrients. Organic matter provides an energy source for microbial growth, resulting in the release of inorganic nitrogen and other essential nutrients by the microbiota and thereby increasing their biomass (Freckman, 1988). Unlike Coleman et al. (1977), we did not find any clear effect of glucose addition in our experiment, perhaps partly due to an accelerated DOM and nutrient release from bulk detritus as a result of the pre-treatment of the sediments (e.g. detrital carbon content in our microcosms was ca. 11 mg, while only ca. 0.3 mg of glucose was added).

An increase in bacterial and *Bursilla monhystera* abundances and a predominance of larger nematodes were strongly intercorrelated: bacterial densities increased following a similar pattern to nematodes except for the most nutrient-enriched conditions in which bacteria, unlike nematodes, continued to grow exponentially until the end of the experiment,

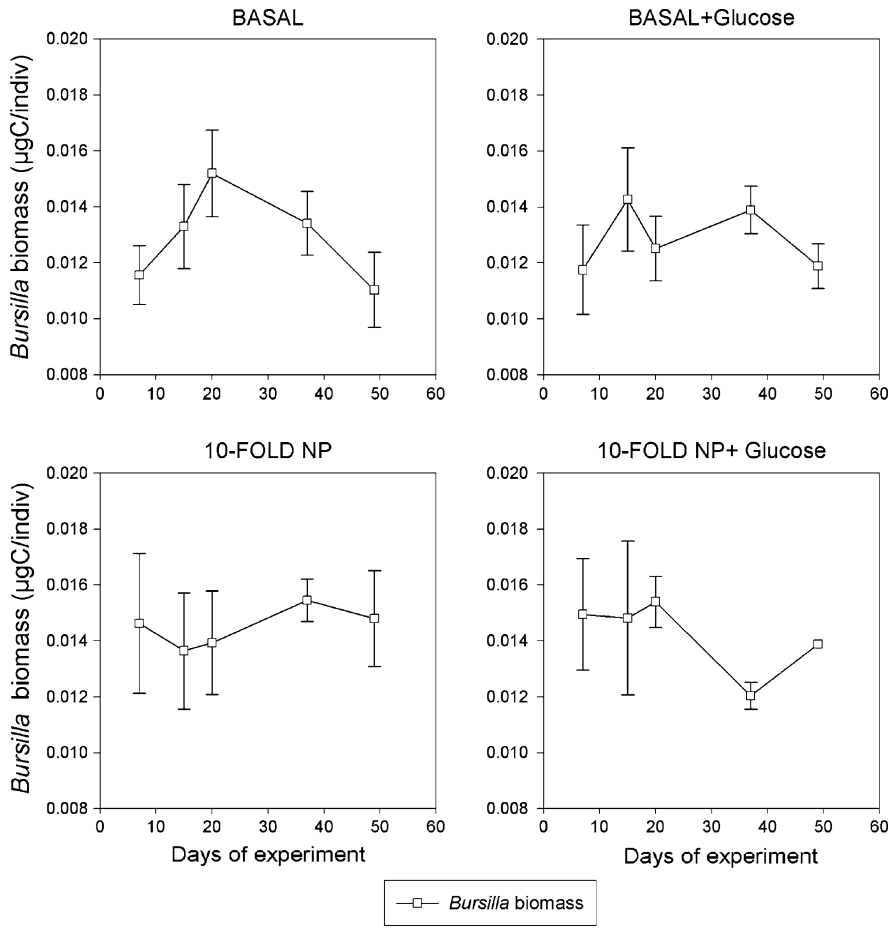

Fig. 3 Biomass of *Bursilla* under low (no glucose addition) and high carbon (glucose amended) and basal and 10-fold NP treatments. *X*-axis represents the days of experiment after nematode inoculation

demonstrating a clear bottom-up control in these microcosms. Some rhabditids have been reported to increase in body length in well-fed conditions (Sudhaus, 1980). *B. monhystera* reacted to changes in their environmental conditions, but if nutrient levels were not high enough, their numbers and mean individual biomass tended to decrease, probably because of retarded or arrested maturation of juveniles; hence, the population then became dominated by small-medium sized juveniles.

In our experiment, only the treatments to which phosphorus was added (mainly the 10-fold addition) maintained a constant increase in the nematode population over the entire experiment. Since phosphorus is a conservative element, the availability and supply of P is a key determinant of the binding, flux and fate of C in freshwater foodwebs (Elser & Hessen, 2005). Changes in P supply can alter the efficiency of trophic transfer by modulating the nutritional match between producers and consumers (Elser et al., 2001). Moreover, glucose- and nitrogen-amended treatments (3-fold NP and 10-fold N and NP) also showed lower nematode abundances than the same treatments without glucose addition, which could be due to a suboptimal stoichiometry. Such an unbalanced stoichometry (C:N:P), largely driven by the potentially high amounts of carbon leached from the detritus, may be sufficient to maintain the basic metabolic rates of non-reproducing individuals (Elser et al., 2001), but not an optimal reproduction. In fact, refractory dissolved organic matter can even reduce nematode fertility (Höss et al., 2001). Thus, stoichiometric imbalance may have contributed to the observation that under nutrient-enriched conditions (10-fold N and 10-fold P), *B. monhystera* increased their numbers over time in spite of not showing a significant increase in the proportion of reproductively active adults. Alternatively, some rhabditids have been found to

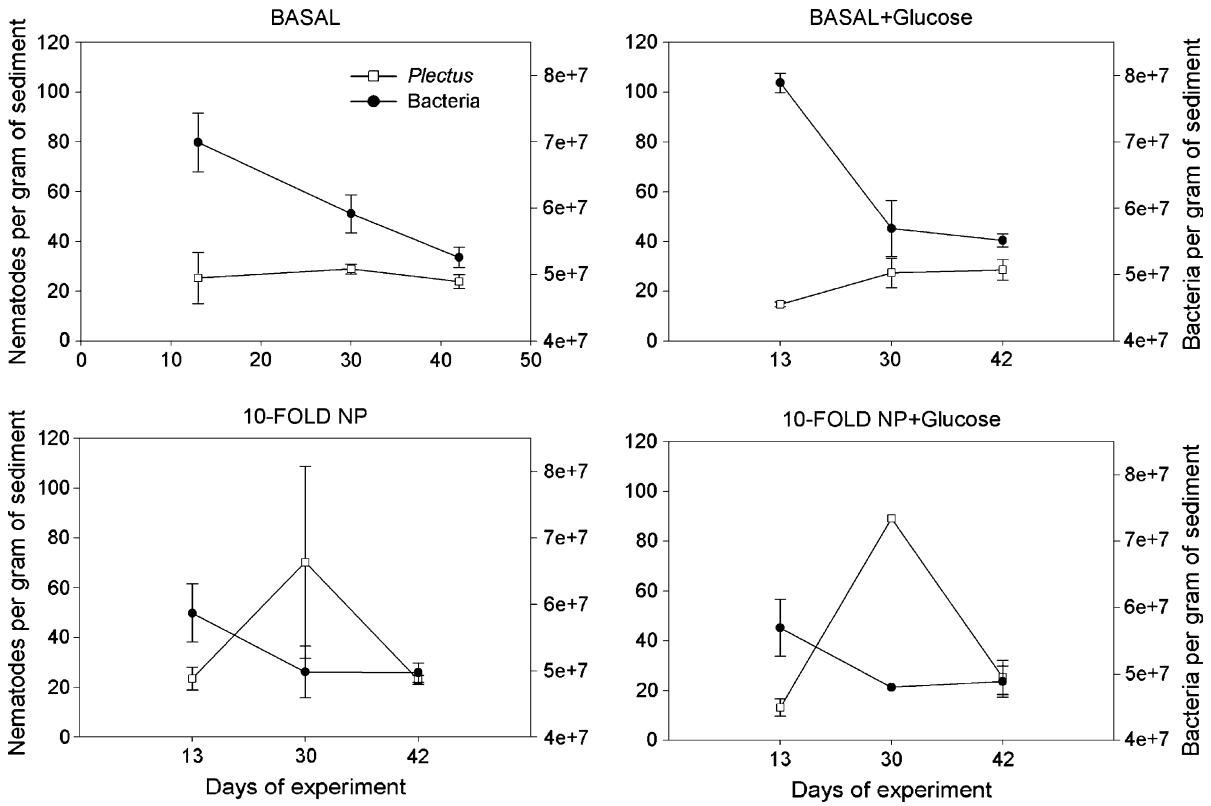

Fig. 4 Abundances of bacteria and *Plectus* under low (no glucose addition) and high carbon (glucose amended) and basal and 10-fold NP treatments. *X*-axis represents the days of experiment after nematode inoculation

increase their colonising ability by an early reproductive effort instead of a constant resource allocation into reproduction (Schiemer, 1983; Woombs & Laybourn-Parry, 1985).

Plectus aquatilis populations significantly increased in abundance in all treatments until the first sampling and under high nitrogen and phosphorus concentrations this increase was fairly constant. However, their response was much slower than that of *Bursilla* individuals. Such a differential increase in abundances has been previously described for *B. monhystera* and another *Plectus* species, *P. parvus* (Postma-Blaauw et al., 2005). It can largely be explained by the considerably shorter generation time of *Bursilla* compared to *Plectus* (Moens, pers. observ.), and by the stronger colonising ability of Rhabditidae at high bacterial abundances, (Schiemer, 1985). On the other hand, *Plectus*, and other cp-2 nematodes combine certain homoeostatic characteristics like a lower standard metabolism (e.g. 2.5 times lower in *P. palustris* than *C. briggsae*; Schiemer, 1985), a prolonged reproductive phase and can endure lower food thresholds and feed on a wider variety of bacteria and bacterial abundances (Postma-Blaauw et al., 2005).

While the results of the *Bursilla* experiment largely support the idea of a significant bottom-up control, the bacterial abundances in the *Plectus* microcosms followed largely opposite abundance patterns with time, showing high abundances (generally higher than in any of the *Bursilla* treatments) after 13 days and then decreasing towards stable values which fell among those in the *Bursilla* treatments. In the basal and basal + glucose treatments, *Plectus* abundances remained low throughout the experiment. In the next paragraph, we suggest that *Bursilla* may have substantially contributed to the ammonia concentrations in our microcosms, thus yielding a more favourable C:N stoichiometry resulting in increasing bacterial abundances with time. At the same time, however, these fast-growing nematodes may have exerted some top-down control on the growth of the bacterial

Table 2 Comparison of potential ammonia (NH_4^+) release by nematodes in microcosms and the measured amounts in porewater

Sampling dates Time (between sampling dates)	2 (192 h)	3 (120 h)	4 (408 h)	5 (288 h)
BASAL				
Potential NH_4^+ excreted by nematodes	391.8	637.3	4,682.2	2,256.1
Ammonia measured in microcosms	2,162.0	1,515.8	4,166.0	2,067.5
Proportion	18.1	42.0	112.4	109.1
Mean nematode density	1,313.3	2,093.7	1,842.3	1,334.0
BASAL + Gluc				
Potential NH_4^+ excreted by nematodes	351.5	634.3	3,375.1	1,798.9
Ammonia measured in microcosms	1,769.0	1,591.0	1,798.5	1,305.3
Proportion	19.9	39.9	187.7	137.8
Mean nematode density	1,194.3	1,771.3	1,433.0	1,247.7
10NP				
Potential NH_4^+ excreted by nematodes	174.7	1,121.0	2,679.2	3,495.1
Ammonia measured in microcosms	2,154.7	2,154.5	3,347.3	8,892.3
Proportion	8.1	52.0	80.0	39.3
Mean nematode density	2,259.7	1,470.0	2,545.3	3,076.7
10NP + Gluc				
Potential NH_4^+ excreted by nematodes	190.3	518.6	2,144.3	680.0
Ammonia measured in microcosms	2,184.7	2,023.8	2,209.7	1,507.8
Proportion	8.7	25.6	97.0	45.1
Mean nematode density	972.0	954.0	598.3	1,230.0

Units in µg/l

community, leading to initially lower bacterial abundances in the *Bursilla* treatments compared to those with the slower growing *Plectus*. Such a top-down control might also explain the good correspondence between the decline in bacterial abundances and the peak in *Plectus* abundances in the nutrient-enriched treatments, where *Plectus* reached similar abundances as *Bursilla*. This population peak of *Plectus* was short-lived and mainly composed of juveniles, which may explain that it did not have any stimulatory effect on bacterial abundances as noted for *Bursilla*. We have, however, no explanation for this sudden increase and decrease of the *Plectus* population. In any case, these results suggest that, while bottom-up control probably dominated in our microcosms, top-down feedback mechanisms by bacterial grazers and their ammonia excretion may have also played a significant role in explaining bacterial dynamics.

Effects of nematodes on N-mineralisation

Abundances of bacteria and nematodes were positively correlated to increased nitrate and ammonia availability in the water. This may indicate a general bottom-up control in which higher N-nutrient levels stimulate bacterial growth, which in turn enhances growth of bacterivores. An increase in nutrient mineralisation has been reported in the presence of bacterial grazers such as nematodes and protists (Johannes, 1965; Ferris et al., 1997; Bonkowski et al., 2000). Bacterial-feeding nematodes contribute to N-mineralisation by feeding on and by dispersing bacteria (Anderson & Coleman, 1981; Freckman, 1988; Bouwman et al., 1994; Griffiths, 1994). By grazing on bacteria, bacterial-feeding nematodes participate in organic matter decomposition and N-mineralisation to an extent that is determined by their metabolic and behavioural attributes, by their demography and physiology and by the relative C-to-N ratios of the nematodes and their bacterial prey (Ingham et al., 1985; Ferris et al., 1995, 1996, 1997; Venette & Ferris, 1997). Rhabditid nematodes in particular can strongly contribute to N-mineralisation (Ferris et al., 1998). However, in some cases, like in the most enriched N and P conditions, the high concentrations of ammonia found could not entirely be attributed to excretion. In these cases, alternative explanations may need to be considered.

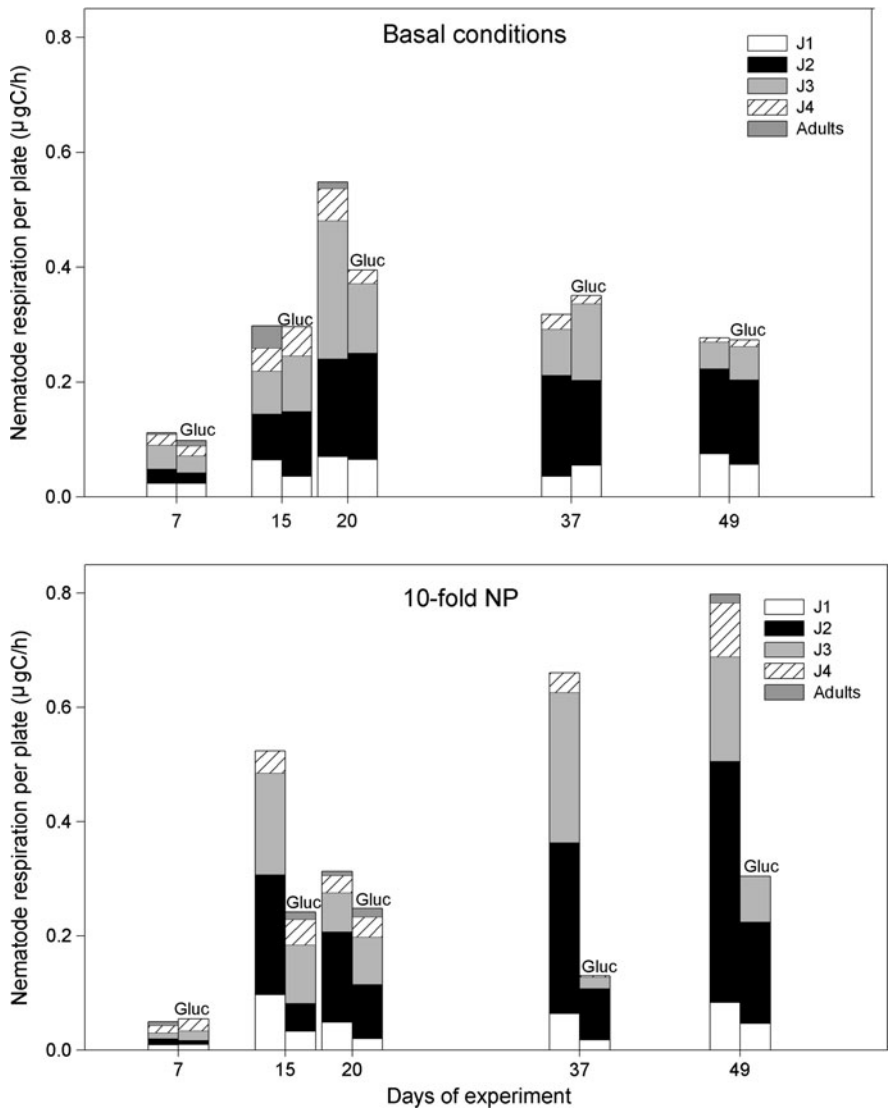

Fig. 5 Estimated respiration (µgC h^{-1}) of nematodes in microcosms: contributions per developmental stage (J1, J2, J3, J4, Adult; see text) on each sampling date. Glucose enrichment is labelled as 'Gluc'

Implications for in situ effects of eutrophication

Human activities (e.g. logging, agricultural practices and industrial waste) are the main sources of organic or inorganic nitrogen (N) and phosphorus (P) that enter aquatic ecosystems. Sabater et al. (2011) and Gaudes et al. (2012) measured the effect of a moderate nutrient addition in a Mediterranean stream focusing on the long-term responses of benthic organisms like changes in stoichometry and annual secondary production. Thus nutrient release in the overlying water would have direct implications on the short-term population dynamics and indirectly on the long-term dynamics through the diffusion of nutrients accumulated in the sediment. Studies conducted in Swedish lakes have shown that thophic status is a major factor structuring benthic nematode communities (Ristau & Traunspurger, 2011) and that meiofaunal abundance and production have a nutrient-related pattern (Schroeder et al., 2012). In that sense, although a decrease of biodiversity is predicted under the climate change scenarios (Sabater & Tockner, 2010), the different traits, energy partitioning and life strategies present in freshwater bacterivorous nematode species could enable different responses to coexist and influence decomposition processes.

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