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Copper effects on soil nematodes and their possible impact on leaf litter decomposition: A microcosm approach

Joey Genevieve Martinez [a, b, *], Glyde Paulyn Paran [b], Rowena Rizon [b], Nele De Meester [a], Tom Moens [a]

[a] Ghent University, Biology Department, Marine Biology Lab, Krijgslaan 281/S8, 9000, Ghent, Belgium
[b] Mindanao State University-Iligan Institute of Technology, College of Science and Mathematics, Department of Biological Sciences, Bonifacio Ave., Iligan City, Philippines

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ABSTRACT

Scientists and policy makers have to establish criteria to distinguish 'acceptable' from 'harmful' levels of pollution. Earlier studies have shown that even amounts of heavy metal pollutants well below LC50 or EC50 concentrations, can affect the fitness of individual bacterivorous nematode species, as well as the balance of the horizontal interactions between them. Species interactions are critical in shaping community structure and promoting ecosystem functions like organic matter decomposition - a key process that drives the flow of energy and nutrients in ecosystems.

In this paper, we exposed two bacterial feeding soil nematodes, *Plectus acuminatus* and *Acrobeloides nanus*, to different Cu concentrations in monospecific and two-species microcosms containing leaf litter of the common grass species *Urochloa mutica* for a period of 60 days. We demonstrate that toxicant concentrations well below LC50 not only impair the fitness of the nematodes, but may also affect the interspecific interactions between them as a result of their differential sensitivity to Cu. Both *Plectus* and *Acrobeloides* are bacterial feeders and may thus affect the decomposition of leaf litter by impacting on the abundance and composition of bacteria. We observed Cu effects on the decomposition of *Urochloa*, but in the absence of data on the microbial community, it is not possible to assign these to direct effects of Cu on the bacteria or indirect effects through the Cu impacts on nematodes and their interactions.

1. Introduction

Heavy metals have received considerable attention in ecotoxicological studies due to their propensity to persist in the environment. The movement of heavy metals across trophic levels results in bioaccumulation [1] and may trigger biodiversity loss [2] and disrupt ecosystem functions [3], which in turn can lead to a depletion of ecosystem services [4,5]. In particular, copper (Cu) has pronounced negative impacts not only on natural ecosystems [6,7], but also in the vicinity of mining sites in developing countries. For instance, in Boac river in Marinduque province, Philippines, Cu was identified as the primary pollutant in a massive acid drainage spill, leading to the 'biological death' and the loss of ecosystem functioning of the river in 1996 [8]. While the deleterious environmental effects of high loads of Cu have clearly been illustrated, heavy metal concentrations below the LC50 or EC50 have also been reported to affect populations, species interactions, and ecosystem functioning [9–12], even though such concentrations are often used as a basis to establish 'acceptable pollutant levels' (OECD, 1984; 1995).

Decomposition is a key process that drives the flow of energy and nutrients in ecosystems. However, decomposition rates may decrease due to heavy metal pollution [13], leading to the immobilization of essential nutrients [14] which may have reverberating effects from the lower to the upper trophic levels. While the preponderance of studies has hitherto focused on the effect of high pollution levels on ecosystem processes, studies dealing with the impact of sublethal concentrations on population fitness [15] and species interactions [11], and their concomitant effects on decomposition processes, remain scarce.

Nematodes possess several features which render them very suitable test organisms in pollution impact studies (Höss et al.,

* Corresponding author. Ghent University, Biology Department, Marine Biology Section, Krijgslaan 281/S8, 9000, Ghent, Belgium.
E-mail addresses: joeygenevieve.martinez@msuiit.edu.ph, joeygenevieve.martinez@ugent.be (J.G. Martinez).

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2006 [59]). They are ubiquitous, have relatively short generation times, occupy different trophic levels, and some soil nematode species are easy to maintain in the laboratory. Although decomposition is a largely microbially driven process, nematodes can also play a significant role in organic matter decomposition and nutrient cycling by stimulating microbial activity [16–18]. Previous studies have shown that higher decomposition rates occurred in the presence of nematodes [17,19]. Such decomposition effects of nematodes can be highly species-specific [20,21]. The effects of nematodes on decomposition processes are also affected by interspecific interactions [21,22]. Horizontal interactions such as competition, facilitation and inhibition can affect nematode population development and assemblage composition [23,24]. The outcome of such within-trophic group interactions may be affected by pollutants. Therefore, the effects of nematodes on decomposition may also be affected by exposure to sublethal pollutant concentrations, owing to different nematode species exhibiting differential responses to toxicity [11].

Here, we hypothesized that the effect of pollutants at concentrations below EC50/LC50 on nematode population abundances and interactions may also have a concurrent impact on decomposition process. Generally, *Plectus* species exhibit higher sensitivity to heavy metals than *Acrobeloides* sp. [25]. In non-polluted conditions, the interaction of *A. nanus* and *Plectus parvus* was contramensal (+, −), and such interaction affected bacterial biomass in soils compared to treatments with single nematode species [21]. Hence, exposure to metal concentrations could differentially affect nematode species, and the outcome of their individual or combined response may influence leaf litter decomposition. To investigate this, we performed a microcosm experiment with two bacterial-feeding soil nematodes, *Plectus acuminatus* and *A. nanus*, and exposed them to different Cu concentrations in monospecific and combination cultures. At the same time, we measured the decomposition rate of phytodetritus which was offered as a substratum in the microcosms.

2. Materials and methods

2.1. Nematode cultures

The bacterial-feeding soil nematodes *P acuminatus* and *A. nanus* were obtained from the Nematology Laboratory of Ghent University, Belgium. Although males have been reported for both species, they are both generally considered parthenogenetic species [10,26], and we never observed any males in our cultures. They are general opportunists with a cp value of 2 [27], and are widely distributed in many soils. They were reared in the laboratory and fed *Esherichia coli* OP50. Both nematode species can be easily distinguished from each other, even under low magnification, by the shape of their tails [28,29]. *A. nanus* have a generation time of ca. 11 days at 21 °C [30], while *P. acuminatus* develop to reproductive adults in 3.5 weeks at 20 °C [10].

2.2. Microbial inoculum

We isolated microbes from a grassland in Iligan, Philippines, where the plant material, *U. mutica* was also collected. A microbial inoculum was prepared by rinsing 10 g of *U. mutica* leaves with 90 mL sterile distilled water. A nutrient broth composed of 3.0 g beef extract and 5.0 g peptone dissolved in 1 L of water was used to maintain the microbial culture. We verified the suitability of the microbial inoculum as a food source for both nematodes in a preliminary test by comparing for each species the population abundances with those of the same nematode species fed *E. coli*. After 2 weeks, both nematode species exhibited very similar population development on both microbial food sources, suggesting that the inoculum was a suitable food source. Note that the microbial inoculum is a mixture of several bacterial species and fungi, both of which participate actively in litter decomposition. Unlike bacteria, fungi probably do not function as a food source to *Acrobeloides* and *Plectus*. We measured the microbial densities with a spectrophotometer and standardized the initial abundance of the inoculum by always adding 100 μL and 200 μL of a suspension of 9.0×10^7 bacterial cells mL^{-1} to the single-species (SSE) and the combination experiments (CE), respectively.

2.3. Main experiment

The main experiment was performed in 3.5 cm and 5.5 cm diameter Petri dishes with 2.5 and 5.0 mL, respectively, of 1.5% Bacto agar (DIFCO). The differences in size of the Petri plates and the volume of the agar were designed to provide (nearly) equal space and resources *per capita*. Hence, single-species experiments (SSE) were carried out in the smaller plates while the combination experiments (CE) in the larger plates. For single-species experiments (SSE), ten adults of *A. nanus* or *P. acuminatus* were handpicked using a copper wire and added to the smaller plates, and a separate control set-up without nematodes was also prepared. Ten individuals each of both species were inoculated together for the combination experiment (CE). Desired sublethal concentrations of Cu were prepared from $CuSO_4.5H_2O$ (purity = 99.7%, Sigma): 0, 2, 4 and 8 mg L^{-1} or 0, 31.7, 63.5 and 127.9 μM, respectively. These concentrations were based on LC50 estimates for *P. acuminatus* under Cu exposure by Kammenga and Riksen [31]: LC50 in their study equaled 3.6 mg L^{-1} or 56.6 μM after 46 days.

Heavy metals were introduced to the plates and the required agar volumes were poured into the Petri plates, which were then gently shaken to homogenize the mixture. Fresh leaves of *U. mutica*, a common grass species, were washed with distilled water and air-dried [20] at 20 °C for 24 h. The leaves were cut in pieces approximately 1 cm in length, weighed and distributed to each of the small Petri plates [17]. Two equally sized pieces of leaves were added to the larger Petri plates. Afterwards, aliquots of the natural bacterial inoculum were added and incubated for 48 h at 25 °C. Microcosms were replicated four times per treatment and time (15, 35, 60 days) and were sampled destructively (4 replicates x 4 treatments x 4 concentrations x 3 moments in time = 252 plates). For an efficient collection of the nematodes, the agar was immersed in hot water (60–80 °C). Nematodes were extracted with a 10 μm sieve and preserved in 4% formaldehyde. Leaf fragments were thoroughly rinsed in tap water over the same sieve to collect nematodes that were present on the leaves. Since population fitness can be assessed from abundance data [32,33], the abundance of vermiform stages of nematodes (juveniles and adults) was counted, and in the combination cultures, they were identified to species level under a stereomicroscope at 60× magnification after 15, 35 and 60 days. The leaves, mostly intact, were carefully picked and dry-weighed using an analytical balance (Mettler Toledo). Leaf litter weight loss was determined after 15, 35 and 60 days by air-drying of the fragments at 20 °C for 24 h, using the formula:

$$\Delta \text{ wt.} = \frac{\text{dry weight}_{\text{initial}} - \text{dry weight}_{\text{final}}}{\text{dry weight}_{\text{initial}}} \times 100$$

Note that we tested differences between our air-drying procedure and a more accurate drying procedure for 48 h at 60 °C on 30 leaf fragments, and found only 2.56% (±0.72%) difference (range from 0.8 to 3.9%).