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Effects of Cadmium on the Interactions between Bacterivorous Nematode Species, *Acrobeloides nanus* and *Bursilla monhystera*

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Abstract

Interactions of species have been recognized to play an important role in the structure and functioning of the environment. However, the exact mechanism is not well understood particularly under shifting environmental conditions like pollution. To determine the effects of pollutants/contaminants on the fitness of two soil nematodes (*Acrobeloides nanus* and *Bursilla monhystera*) under pristine and polluted condition, we performed experimental set-ups (monoculture and combined cultures) and exposed the organisms to varying sublethal cadmium (Cd) concentrations (0 mg/L, 1.4 mg/L and 1.9 mg/L). In monoculture, results showed continuous increase in the abundances of *A. nanus* and *B. monhystera* in non-polluted condition. Abundance of *A. nanus* remained increased in polluted condition, however, *B. monhystera* responded negatively to increasing Cd concentrations. In combination, results in non-polluted condition showed that *B. monhystera* exhibited a negative effect on the abundance of *A. nanu*s, suggesting that *B. monhystera* is a stronger competitor than *A. nanus*. However, a shift in abundance pattern was observed when both species were exposed to pollutants particularly on the positive effect by *A. nanus* on *B. monhystera* which implies that sublethal Cd concentrations can potentially alter species interactions. Thorough assessment of nematode interactions is essential in explaining patterns of community structure in disturbed systems and determining proper functioning in the ecosystem.

Keywords: abundance, community, competitor, monoculture, pollution

Introduction

Nematodes represent a very abundant group of soil organisms, and these non-parasitic species are important for soil quality and in the soil food web (Sochova et al., 2006). They are ubiquitous in aquatic and terrestrial habitats and constitute one of the largest animal phyla. Soil inhabiting nematodes are divided into different feeding groups: plant, bacterial and fungal feeders, predators, and omnivores (Yeates et al., 1993). In recent years, it has been shown that nematodes are appropriate bioindicators of soil condition, and they are also suitable organisms for laboratory toxicity testing (Sochova et al., 2006). Nematodes offer perspectives for ecotoxicological research because of their abundance, species diversity and differences in sensitivity to chemicals (Kammenga et al., 1994) such as cadmium (Alvarez et al., 2006; Kammenga et al., 1994, 1996; Martinez et al., 2012).

Bacterivorous nematodes are said to be the key intermediaries in decomposition processes of organic matter in soil (Freckman, 1988). Studies have shown enhancement of the decomposition process in the presence of nematodes both in terrestrial and aquatic environments (De Mesel et al., 2003; Lillebo et al., 1999).

Like any other organisms, nematodes also interact in several ways. Species interactions are important in shaping the community structure (Ingham et al., 1985; Shannon et al., 1994; van der Heijden et al., 1998; Bongers & Ferris, 1999; De Mesel et al., 2004; Postma-Blaauw et al., 2005) and in the proper functioning of ecosystems (Shannon et al., 1994). For instance, Postma-Blaauw et al. (2005) found that the contramensal interaction between *B. monhystera* and *Plectus parvus* affected the bacterial community structure caused by increased biomass and nitrogen mineralization. Gaudes et al. (2013) explained that 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), which may eventually alter the composition and diversity of bacterial communities (De Mesel et al., 2004, 2006).

However, such balance in interaction can be threatened due to increasing heavy metal pollution levels (Fleeger et al., 2003). Heavy metals influence the food availability and competitive interactions among species of soil biota in an indirect way (Han et al., 2009). A study of Martinez et al. (2012) demonstrated that sublethal pollution level can change the interaction of two nematode species, *P. parvus* and *A. nanus* owing to their differential tolerance - *P. parvus* being the more sensitive species than *A. nanus,* leading to counterintuitive result such as increase in *A. nanus* fitness during pollution.

In this paper, we would like to determine how do the two bacterivorous nematode species, *A. nanus* and *B. monhystera* interact with each other under non-polluted condition, and how do sublethal Cd levels affect the nature of their interactions. To address these questions, we performed a microcosm experiment using the nematodes *A. nanus* and *B. monhystera* and exposed them to varying sublethal cadmium concentrations (0 mg/L, 1.4 mg/L and 1.9 mg/L) for a period of 40 days.

Materials and Methods

Preparation of a nematode culture

Two bacterivorous nematode species belonging to the same trophic group namely *Acrobeloides nanus* and *Bursilla monhystera* were used in this experiment. Cultures of these nematodes were obtained from the Department of Biological Sciences Laboratory of Mindanao State University - Iligan Institute of Technology. Nematodes were subcultured in the agar medium and were fed with *Escherichia coli*, which proved to be a suitable food source of the nematodes (Li et al., 2005). The medium was supplied with sterols due to their important role in nematode reproduction (Vanfleteren, 1980).

The experiment was carried out in partitioned and full Petri dishes (8 cm in diameter). These were filled with a 1.5% bacto-agar medium. Cadmium concentrations were added to the Petri dishes and stirred gently to homogenize with the agar. Sublethal Cd concentrations used in the study were 1.4 mg L^{-1} and 1.9 mg L^{-1}. Sublethal concentrations were determined based on the LC_{50} of Cd on both *A. nanus* and *P. parvus* (Martinez et al., 2012). Using a fine copper wire, five *A. nanus* and five *B. monhystera* adults were selected and placed in the partitioned Petri dish (monoculture set-up), and a combination of the nematode species (5 *A. nanus* : 5 *B. monhystera*) was placed in full Petri dish (interaction set-up).

The set-ups were replicated three times, hence the entire experimental set-up included two nematode set-ups (monoculture and combined) and three cadmium concentrations (0 mg/L, 1.4 mg/L and 1.9 mg/L) in four (4) sampling periods, which totalled to 24 plates. All set-ups were sampled destructively after 10, 20, 30 and 40 days. The designed time frame was intended to appropriate at least three generations of each species.

Nematode counts

Adult nematodes were counted every after 10th, 20th, 30th and 40th day. Nematodes were extracted by dissolving the agar in boiling water while stirring constantly. Samples were sieved out using a 75-µm-mesh-size sieve and preserved in 2% formalin solution.

Statistical Analysis

Statistical analyses were performed using Statistica 7.0 and Paleontological Statistics, PAST software. When all assumptions for ANOVA were met, one-way ANOVA was used to test the equality of the means of the abundances of *A. nanus* and *B. monhystera*. A *p* value is provided to indicate how close the mean variances are to one another. A *p* value that is lesser than 0.05 is said to be significant. Exploratory tests via regression line and bar graph with error bars were generated to graphically determine the difference in the abundances of the two nematode species.

Results and Discussion

Non-polluted condition

Figure 1 shows the varying abundances of *A. nanus* and *B. monhystera* between monoculture and combined set-ups in non-polluted condition. In monoculture, there was a continuous increase in the abundance of both *A. nanus* and *B. monhystera* from day 1 to 40. *A. nanus* has a generation time of 11 days at 20^0C (Sohlenius, 1973) and can survive for more than 55 days (Kammenga et al., 1994). *B. monhystera* is characterized by a short generation time and rapid population development at high food availability (Postma-Blaauw et al., 2005). Hence, the increase in the abundances of *A. nanus* and

B. monhystera was expected because the agar plates provided a suitable place for them to thrive. However, in combination set-up, *A. nanus* abundance was statistically lower than *B. monhystera* ($p<0.05$). The result suggests that under pristine condition, *B. monhystera* may be the stronger competitor leading to its higher abundances ($p<0.05$). The general trend in the combination set-up was that the abundance of *A. nanus* was negatively affected by the presence of *B. monhystera* showing asymmetric pattern of intraspecific interaction. This conforms to the asymmetric relationship described by Postma-Blaauw et al. (2005).

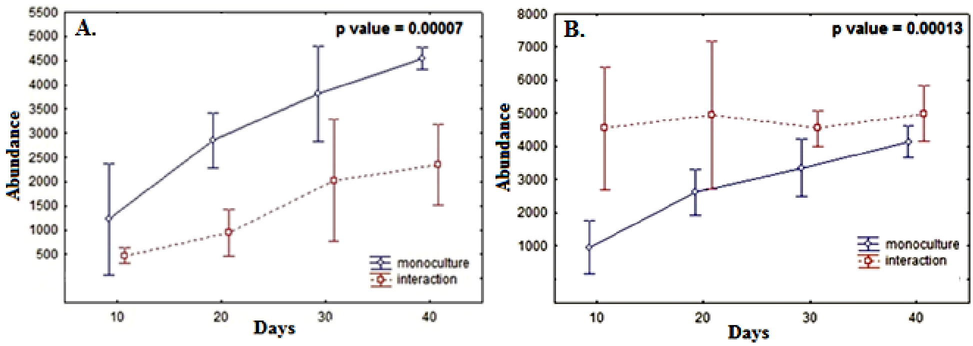

Figure 1. Abundances of adult *A. nanus* **(A) and** *B. monhystera* **(B) between monoculture and combination set-ups in non-polluted condition.**

Asymmetric competition between species is commonly found in aquatic and aboveground ecosystems, especially with species competing by direct interference (Begon et al., 1996). Different responses among genera in the same trophic group are common (Porazinska et al., 1999), and nematode genera within the same trophic group can exhibit asymmetric competition, negatively influencing the abundance of other genera (Postma-Blaauw et al., 2005). Such interaction outcome may be due to the limited similar food source, which resulted to competition and resource depletion, and in turn increased the competition between species (Martinez et al., 2012). Eventually, uninterrupted competition may lead to inhibition where individuals of one species suffer a reduction in growth, survivorship and fecundity as a consequence of resource exploitation or interference of the other species (Begon et al., 1996; Keddy, 2001).

Polluted condition

Figure 2 compares the abundance of *A. nanus* and *B. monhystera* between the monoculture set-up and interaction set-up in different cadmium concentrations. Results showed that the abundance of *A. nanus* in the monoculture set-up was increasing exponentially across sublethal Cd concentrations (Figure 2A). The significant increase in abundance of *A. nanus* monoculture may be attributed to the high tolerance of *A. nanus* to cadmium pollutant ($p<0.05$) (Martinez et al., 2012). Increase in *A. nanus* fitness was evident as Cd concentration increased, but non-existent in the combination cultures. A reduced trend was observed in the combined set-up, however the difference in the abundance of *A. nanus* in all Cd concentrations was not quite substantial. Statistical value confirms the significant difference (p=0.00034) observed in the abundance of *A. nanus* between two experimental set-ups. The increase in fitness of *A. nanus* even under polluted condition in the monospecific set-up has also been observed in several organisms. According to Calabrese and Baldwin (2001), this is an adaptive response of organisms to low pollutant levels, causing an increase in fitness for a certain period of time. However, such phenomenon was not observed when *A. nanus* was combined with *B. monhystera*.

On the other hand, *B. monhystera* responded negatively to increasing Cd concentrations particularly at the highest concentration of 1.9 mg/L (Figure 2B), which suggests that *B. monhystera* is more sensitive than *A. nanus*. However, when combined with *A. nanus*, the higher sensitivity of *B. monystera* towards Cd disappeared. Particularly, at 1.9 mg/L, the abundance in the combination culture exceeded significantly than that of the monoculture experiment ($p<0.05$). The rapid increase in the abundance of *B. monhystera* in the combined set-up may be attributed to the high competitory ability of *B. monhystera* where *Bursilla* populations would show a more rapid response to, and a stronger dependence on nutrient enrichment (Gaudes et al., 2013).

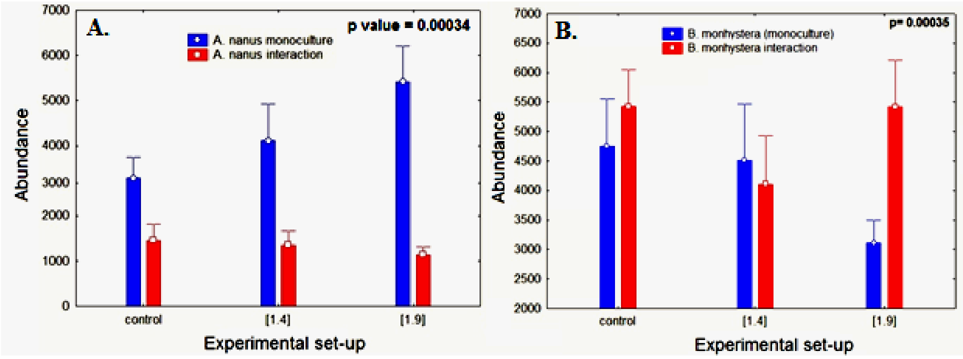

Figure 2. Abundance of *A. nanus* (A) and *B. monystera* (B) between monoculture and combination cultures treated with varying Cd concentrations.

It has been demonstrated in several studies that pollutants can alter species interactions (Fleeger et al., 2003; Martinez et al., 2012). Heavy metal pollutants influenced the food availability and competitive interactions among species of soil biota and finally affected the biota communities in an indirect way (Korthals et al., 1996). This was confirmed in our set-up when the presence of varying concentrations of cadmium had significantly altered the interaction of nematodes in the combined set-up. The negative effect on *A. nanus* by *B. monhystera* was evident from non-polluted to polluted condition, indicating that sublethal Cd level did not alter species interaction. However, there was a shift in the interaction of *B. monystera* as a function of Cd concentration. The positive effect by *A. nanus* on *B. monhystera* at 0 mg/L was lost at 1.4mg/L, but reappeared at the highest Cd concentration. This may be attributed to the nature of the medium used. Depending on the degree of susceptibility of both species to toxicants, pollutants can dramatically shift the balance of their interaction (Martinez et al., 2012). Possible sublethal effects of metals on population dynamics may include affected generation time, metal accumulation in consecutive generations, and impairment of reproduction (Vranken & Heip, 1986). The culture medium was relatively poor in nutrient and was composed of a highly purified agar - sterols. The agar might selectively bind metals (Vranken & Heip, 1986) at higher Cd concentrations and hence reduce their toxicity to *B. monhystera*.

Conclusions and Recommendations

Our results confirmed the asymmetric competition between *Acrobeloides nanus* and *Bursilla monhystera*. Both in polluted and non-polluted conditions, *B. monhystera* exhibited a negative effect on the abundance of *A. nanus*. When the abundance of the stronger competitor *B. monhystera* increases, the relative abundance of the weaker competitor *A. nanus* inversely decreases. The results obtained also confirm the high sensitivity of *B. monhystera* and high tolerance of *A. nanus* to varying sublethal concentrations of cadmium pollutant. Furthermore, our experiment showed that sublethal concentrations of Cd can potentially alter species interactions. Future studies aiming to expand such research may consider determining the differential tolerance of the other bacterivorous nematodes to pollutants like zinc, copper and lead. It is also highly recommended to compare different sensitivity among nematode species to assess which species can be used best as bioindicator for toxicity tests.

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