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Nematode succession and microfauna–microorganism interactions during root residue decomposition

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Abstract

The quality of plant material affects the vigor of the decomposition process and composition of the decomposer biota. Root residues from hairy vetch (*Vicia villosa* Roth), rye (*Secale cereale* L.) and vetch + rye, packed in litterbags were placed in pots of soil at 15 C and the content of the bags was analyzed after 2, 4, 8 and 12 weeks. Bacterial biomass did not differ between residues with contrasting composition. Among bacterivores groups of nematodes that require high bacterial production dominated in fast decomposing resources whereas flagellates with smaller requirements prevail in slower decomposing resources. Biomass of bacterial feeding nematodes correlated positively with early phase (0–2 wk) decomposition that increased in the order: rye < vetch + rye < vetch. Bacterial biomass therefore seems to be under top–down (predation) control during early decomposition. In contrast, the fungal biomass differed between resources with highest values for rye. Moreover, this increase in fungal biomass occurred later during succession and was correlated with decomposition activity for rye in that period. Fungal biomass therefore seems to be under bottom–up (resource) control. The composition of the nematode assemblages (composed of 25 taxa) showed a clear relationship to initial plant resource quality as well as decomposition phase. Early successional microbivorous nematodes vary according to resource quality with demanding bacterivores + predators (Neodiplogasteridae) dominating in vetch and less demanding bacterivores (Rhabditidae) and fungivores (*Aphelenchus*) being equally common in vetch and rye. Later in the succession (2–4 wk) bacterivorous Cephalobidae and fungivorous *Aphelenchoides* prevailed similarly on the different root materials whereas bacterivorous protozoa and the amoebal fraction thereof dominated in rye. At week 12 no species dominated the nematode assemblages that were similar between the resources. The differences between nematode assemblages among plant resources at 2 week were similar to the results of a field study sampled after 6 weeks with the same soil and plant resources. This lends support to the relevance of the successional patterns observed in this incubation study.

Keywords: Nematode; Protozoa; Succession; Bacterivorous; Fungivorous; Microfauna; Litter decomposition; Soil; Rhabditidae; Neodiplogasteridae; Flagellate

1. Introduction

The bacteria feeding microfauna (protozoa and nematodes) is claimed as a better indicator of the activity of bacterial populations than the bacterial biomass itself (Bååth, et al., 1978; Clarholm, et al., 1981; Sohlenius, 1990; Christensen, et al., 1992a; Griffiths, et al., 1992). Griffiths (1994) suggests that nematodes may be useful indicators of substrate quality and nutrient release during decomposition. Data on the composition of the nematode assemblages and their succession during decomposition of plant residues in soil are available from field studies (Wasilewska, et al., 1981; Sohlenius and Boström, 1984; Wasilewska and Bienkowski, 1985; Wasilewska, 1992; Lenz and Eisenbeis, 1998; van Vliet et al., 2000; Ferris and Matute, 2003; Wang et al., 2004). To exemplify, adding high quality organic material (lucerne) resulted in an early stimulation of enrichment opportunistic bacterivorous nematodes (e.g. Rhabditidae) whereas addition of lower quality material (wheat straw) resulted in a stimulation of fungivorous nematodes (e.g. Aphelenchoididae) later during decomposition (Ferris and Matute, 2003), and addition of sunn hemp in litterbags resulted in early dominance of Rhabditidae among bacterivores and *Aphelenchus* among fungivores, followed by

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Acrobeloides and *Eucephalobus* among bacterivores and Aphelenchoides among fungivores, and *Plectus* among bacterivores during 2–10 weeks of decomposition (Wang et al., 2004). Information on nematode succession under controlled conditions is scanty, however. The relationship between bacterial feeding nematodes and protozoa and its importance for the decomposition processes also needs more attention (Bouwman and Zwart, 1994). Bacterial feeding nematodes and protozoa that compete for the same food resources and have a predator–prey relationship to each other may enter complex intraguild predator–prey interactions (Polis, et al., 1989; Polis and Holt, 1992).

The objective of this study was to investigate the succession of nematodes and their interaction with protozoa during decomposition at controlled environmental conditions of N rich and N poor plant residues that previously developed markedly different decomposer communities in the field (Georgieva et al., 2005).

2. Materials and methods

2.1. Experimental design

The soil used in the experiment was sampled in September 1997 in an experimental field of Årslev Research Centre from plots cropped to grass-clover on which vetch (*Vicia villosa* Roth), rye (*Secale cereale* L.) and inter crop of vetch and rye were used as previous catch crops. The field was under an organic farming regime for 8 years. The soil was a sandy loam with 1.7% C, 0.17% N, 14.9% clay, 26.9% silt, 54.8% sand and pH of 7.0 in the upper 25 cm. Soil was sieved through a 1.5 mm sieve and roots were removed. Roots from vetch and rye were gathered on 1st April 1997 from plants in active growth. The roots were washed free from soil, air-dried and cut into 2–3 cm pieces. Thirty-six nylon litterbags (7×7 cm, mesh size 0.1 mm) were packed with 0.5 g d.w. roots from vetch, rye or a 1:1 mixture of vetch+rye. Each litterbag was moistened with 1.5 ml water, placed horizontally in a pot on 300 g soil (fresh wt.), and covered with 900 g soil (fresh wt.). The soil was packed at a density of 1.2 g cm^{-3}. Pots were placed at 15 C and were loosely covered by polythene to minimize evaporation. The soil moisture was maintained at 17–18% and three litterbags of each type were sampled after 2, 4, 8 and 12 weeks.

2.2. Plant material and soil analyses

Sub samples of soil (10 g f.w.) and plant residues (0.2–0.3 g f.w.) were oven dried for 72 h at 105 C for moisture determination. There was no obvious soil contamination of the decomposing residues in the litterbags probably due to their small mesh size. Sub samples (0.2–0.3 g f.w.) of the initial and the decomposing root materials were ground and used for total carbon and nitrogen analyses on a Carlo Erba Nitrogen Analyser (NA 1500).

For measurement of water-soluble compounds, the plant material (0.25 g oven-dried and ground) was placed on a rotary-shaker in 37.5 ml of de-ionized water at 23±2 C for 48 h. Both sample and extract were transferred to a Whatman No. 1 filter paper. The remaining sample on the filter paper (non-soluble compounds) was dried at 60 C and the weight recorded (TAPPI, 1978). The content of soluble compounds was calculated as the weight loss of the sample. The C and N content was calculated on the basis of the C and N content in the original sample and in the non-soluble compounds that remain on the filter. Cellulose and lignin content were measured in accordance with the methods of Goering and van Soest (1970).

2.3. Estimation of bacteria and fungi

Bacteria and fungi were determined in suspensions after being used for enumeration of protozoa (see below). The suspensions were subsequently fixed in 4% formalin and kept at 5 C prior to analyses. Bacteria were counted in a fluorescence microscope after acridine orange staining. An Olympus BH2 microscope equipped with a light reflection fluorescent attachment was used. Twenty times the bacteria in 30 fields were counted at 1250× magnification and scored into biovolume classes using a Porton graticule (May, 1965). The bacterial biovolume was converted into biomass using the conversion factor of 0.28 pg μm^{-3}.

The fungal length was determined by fluorescence microscopy following staining with calcofluor white using the grid intersection method (Olsen, 1950). Two groups of fungi were separated on the basis of their hyphal diameter. The hyphal crossings of the thin fungi with a diameter of 2.4±0.006 μm were counted at 500× magnification either in 60 fields or up to a maximum of 200 crossings. Hyphal crossings of the thick fungi with a diameter of 7.5±0.01 μm were counted at 125× magnification. The fungal biovolume was calculated using the measured lengths and diameter of the hyphae and biomass estimation was based on the dry weight per volume ratio of 0.33 pg μm^{-3} (van Veen and Paul, 1979).

2.4. Protozoa enumeration

The number of heterotrophic flagellates and naked amoebae was estimated by the most probable number method (Darbyshire, et al., 1974; Rønn et al., 1995). Subsamples of 0.2–0.3 g (f.w.) residue were mixed with 20 ml of amoeba saline (Page, 1976) and homogenized for 1 min in a household blender. From these suspensions eight three-fold dilution series of 12 dilutions were prepared in Costar micro titer plates. As growth medium 1/300 TSB (Tryptic Soy Broth: Difco) was used. The micro titer plates were stored in the dark at 10 C and examined for the presence of protozoa after one and three weeks.