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Spatial association between entomopathogenic and other free-living nematodes and the influence of habitat

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ABSTRACT

Spatial association between entomopathogenic and free-living nematode populations in soil were analyzed at the landscape scale. GPS coordinates were obtained for 479 locations where soil samples were collected to extract nematodes. Habitats sampled included vegetable and agronomic crop fields, grassy borders adjacent to fields, residential lawns, meadow and forested wetlands in a vegetable growing region in northwest Ohio. Free-living nematodes were classified according to trophic level (bacterivores, fungivores, carnivores, and omnivores) and life history characteristics (*r*-selected colonizing versus *K*-selected persisting species on a 1–5 scale). Spatial associations based on spatial analysis of distance indices (SADIE) were analyzed and compared among entomopathogenic nematodes and free-living nematode functional guilds defined by the classifications described above. Spatial aggregation indices (I_a) revealed that each functional guild's spatial pattern varied among habitats. Considering all data regardless of habitat, spatial aggregation indices showed that functional guilds with *K*-selected persisting life history traits were less aggregated, whereas those with *r*-selected colonizer life history traits were more aggregated. The spatial aggregation index of entomopathogenic nematodes was similar to that of the *r*-selected colonizer type free-living nematodes, which share several life history traits including bacteriophagy, high reproductive rates, insect phoresy, and greater abundance in grassy borders, where spatial associations between entomopathogenic and *r*-selected colonizing functional guilds of free-living nematodes were particularly strong. The spatial aggregation patterns of entomopathogenic and free-living nematodes, suggest that these species associate over larger areas than previously measured and that the extent of these spatial associations might be predicted by the nematode life history traits.

1. Introduction

Nematodes are the most abundant metazoa and an evolutionarily successful group of organisms in many ecosystems (Ferris et al., 2001). Nematodes exist as a diverse and highly speciated group in the soil environment. They occupy a central position in the soil food web, occurring at multiple trophic levels (Yeates et al., 1993) including as bacterivores, fungivores, plant parasites, predators and omnivores. Therefore, nematodes can serve as useful ecological indicators and have the potential to provide insights into the structure and function of the soil food web (Freckman, 1988; Ferris et al., 1999; Ritz and Trudgill, 1999). In addition to their characterization into trophic or feeding groups, the free-living nematodes can also be separated on a colonizer–persister scale (*cp* scale 1–5) that represents the degree of *r* (colonizer, *cp* 1) vs. *K* (persister, *cp* 5) life history strategy (Bongers, 1990; Bongers and Bongers, 1998; Ferris et al., 1999, 2001). Colonizer species display a suite of traits that favor rapid population growth and colonization of new habitats, whereas persister species are adapted to competition in saturated habitats (Kokko and Lundberg, 2001) with limited opportunities for niche access or expansion (Ferris et al., 2001; Reznick et al., 2002). Ferris et al. (2001) defined nematode functional guild as a combination of the trophic group and colonizer–persister class.

Spatial aggregation and association analyses of nematodes in the soil can contribute new knowledge about soil ecological processes including population dynamics, biological control, and soil food web structure and function. The spatial distributions of free-living nematodes have been found to vary with genus (McSorley et al., 1985; Ettema et al., 1998; Park et al., 2013), trophic group

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(Robertson and Freckman, 1995; Park et al., 2013), life history strategy (Park et al., 2013), and functional guild (Park et al., 2013). Using Taylor's power law analysis, Park et al. (2013) showed that bacterivore and plant-parasitic groups were more highly aggregated than omnivorous and predatory nematodes. They also showed that nematodes in *cp*-classes 1 and 2 tended to be more aggregated than those in higher *cp*-classes and the functional guilds were generally more highly aggregated than individual genera, suggesting a higher degree of aggregation at the functional guild level. Although functional guild is a basis of many soil nematode community analyses, each functional guild contains species differing in the components of their life-history strategies: reproductive capacity (Ferris et al., 1996); colonizing ability (Bongers, 1990; Ettema and Bongers, 1993); and preference for temperature and moisture conditions (Sohlenius, 1985). These different life history strategies may result in different spatial patterns.

Most nematode spatial distribution studies conducted on plant-parasitic nematodes show that they are highly aggregated in soil ecosystems (Noe and Campbell, 1985; Ferris et al., 1990), with frequency distributions that are well described by the negative binomial distribution. Such aggregation could influence the net effects of nematodes on ecosystem functioning. For example, the spatial heterogeneity of trophic interactions in soil food webs has been identified as an important determinant of soil trophic dynamics (Parmelee and Alston, 1986; Moore and de Ruiter, 1991). However, little is known of the spatial association between free-living nematodes and entomopathogenic nematodes (EPNs) belonging to Heterorhabditidae and Steinernematidae, which feed on symbiotic bacteria but are obligate insect parasites (Grewal et al., 2005). All available studies on EPN spatial distribution reveal a patchy distribution even in relatively uniform environments (Stuart and Gaugler, 1994; Campbell et al., 1996, 1998; Taylor, 1999). Comparative studies among nematode species reveal that EPNs are associated with soil physical, chemical, and biological characteristics that are most similar to those associated with bacteria-feeding free-living nematodes based on soil sampling, and Baerman funnel extraction and visual identification of nematode species (Hoy et al., 2008) as well as centrifugation and molecular identification by q-PCR (Campos-Herrera et al., 2012).

Research to understand the spatial heterogeneity of soil food web structure and function relies on spatial models with realistic properties. Spatial analysis techniques coupled with a variety of statistical tools are currently available to help quantify spatial heterogeneity with specific location information, such as geostatistics and spatial analysis with distance indices (SADIE) (Ettema, 1998; Ettema et al., 1998; Perry and Dixon, 2002; Robertson and Freckman, 1995). Geostatistics uses information from both sample values and sample locations to model spatial dependence as a function of distance among sample points (Cressie, 1993), and can be used to estimate values at points that were not sampled. However, the sampling dimension and measurement scale can affect the performance of geostatistics in describing spatial pattern (Liebhold et al., 1993). In contrast, SADIE (Perry et al., 1999; Perry and Dixon, 2002) allows improved interpretation of the spatial patterns of a single population or the spatial associations between two populations within a given sampling, area because it is designed for data that are distributed in discrete areas with relatively well-defined boundaries, and measures the extent of clustering with subsequent testing for spatial patterns in relationships among sample locations (Korie et al., 2000).

The specific objectives of this study were to characterize and quantify the spatial patterns of EPNs and other free-living nematodes, and to test for spatial associations between EPNs and free-living nematodes at the functional guild level, both within various habitats and at landscape scales using SADIE. Our hypothesis was that similarities and differences in life history strategies between EPN and other guilds would lead to corresponding similarities and differences in spatial structuring of populations, even with sampling taking place over much larger areas than those typical of previous research on spatial patterns of nematodes.

2. Materials and methods

2.1. Study site and sampling

Free living nematodes were extracted from approximately 100 sampling sites selected along transects from each of six habitats in an Ohio muck soil vegetable production area: vegetable crops ('Vegetables'; 102 sample sites), row crops (corn or soybean; 'Row crops'; 101 sample sites), residential lawns ('Lawn'; 101 sample sites), mowed but otherwise unmanaged grass borders of crop fields ('Grass borders'; 101 sample sites), unmanaged meadow area ('Meadow'; 104 sample sites) and forested wetlands ('Forest'; 101 sample sites). Further details on habitats, sites, site selection, sampling, and sample processing can be found in a publication that describes the association of nematode species with soil environmental variables based on these data (Hoy et al., 2008). Soil types were either the same or similar throughout the survey area, consisting primarily of high organic matter "muck" soils. The sites were sampled during September–November of 2004. Sites were marked and a subset of them were georeferenced after sampling was conducted with a global positioning system (GPS) that provided accuracy of within 1 m (Trimble, Sunnyvale, California). Out of the initial 610 sampling locations, sufficiently accurate GPS readings for 479 sampling sites were obtained, the remaining 131 sites were not georeferenced either because the site markers were missing or disturbed, or because we lacked sufficient satellite coverage despite repeated attempts, often due to surrounding trees, foliage or buildings. Distances between sample pairs within the 479 sample sites ranged from 26 m to 7862 m.

At each sampling site, ten soil cores were collected (2.0 cm diameter, 15 cm depth), placed in polyethylene bags and kept at 10 °C. EPNs were extracted from these soil samples using a modified insect baiting technique (Fan and Hominick, 1991). The 10 soil cores from each site were mixed and 200 g of the mixed soil was placed in a plastic container (470 ml). Ten last instar wax moth, *Galleria melonella* L., larvae were released into the soil and the soil samples were incubated in a growth chamber in total darkness, at 25 °C and 88% relative humidity. Three days later, half of the dead larvae were placed in modified White traps (White, 1927) and returned to the growth chambers for an additional 7–10 days to test for emergence of EPN infective juveniles and the other half were dissected to count the numbers of infective juveniles that had penetrated. Emergence of EPNs from modified White traps was verified by inoculating fresh *G. melonella* larvae with the emerging nematodes. Ten larvae were placed in a Petri dish with moist filter paper, 2 ml of a nematode suspension from the White trap was added, and the dish was incubated under conditions described above for 7 days. Larvae or cadavers showing symptoms typical of EPN infection were individually placed in modified White traps as previously described and observed for the emergence of infective juveniles. The infective juveniles emerging from this second round of infection were identified to species level using methods given in Kaya and Stock (1997) and keys by Stock and Hunt (2005). All EPN nematode cultures were maintained and their species were later confirmed using molecular methods (Maneesakorn et al., 2011). For each sample the presence of infected (verified by the second round of infection) larvae and counts of infective juveniles in the initial infected larvae following dissection (see above) were recorded. The EPN species identified in the sites analyzed for this study were *Heterorhabditis bacteriophora* (1072 in 31 sites) and *Steinernema carpocapsae* (127 in 6 sites).