Plant and Soil **170:** 167–181, 1995.

Measures of nematode community structure and sources of variability among and within agricultural fields

Deborah A. Neher [1,2], Steven L. Peck [1,2], John O. Rawlings [1,3] and C. Lee Campbell [1,2,4]
[1]*Environmental Monitoring and Assessment Program – Agroecosystem Resource Group, 1509 Varsity Drive, Raleigh, NC 27606, USA,* [2]*Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616, USA,* [3]*Department of Statistics, North Carolina State University, Raleigh, NC 27695-8203, USA and* [4]*Collaborator, U.S. Department of Agriculture, Agricultural Research Service, 1509 Varsity Drive, Raleigh, NC 27606, USA*

Key words: diversity index, maturity index, power curve, semi-variogram, variance component

Abstract

Whole nematode communities, extracted from soil samples taken from agricultural fields, were enumerated by taxonomic family and trophic group (i.e., bacterivores, fungivores, omnivores, plant-parasites, and predators) to evaluate nematode community structure as an indicator for monitoring ecological condition of soil. No differences were found in mixing treatments or methods of packing or shipping samples. However, extraction using Cobb's sifting and gravity method, followed by sucrose centrifugation, gave greater recovery of free-living nematodes than elutriation followed by sucrose centrifugation. Population means and variance of the sampled area were similar when sampled using different strategies for collecting soil samples within fields, including several patterns, directions and repetitions of transects. Components of variation associated with ratios among the five trophic groups of nematodes and selected indices of community structure were quantified as variation among regions, among counties, among agricultural fields (2-ha area), among transects within agricultural fields, and within composite soil samples. The variance component for 'within composite soil samples' was relatively large compared to the other components of variance. Variation within composite soil samples was less for maturity indices (based on life-history strategy characteristics), ratio of bacterivores to plant-parasites, sum of bacterivores and fungivores, populations of plant-parasites, and populations of bacterivores than for trophic diversity indices, populations of fungivores, populations of omnivores, populations of predators, or the ratio of fungivores to bacterivores. With a single composite sample per field, the ability to differentiate ecological condition of soils among fields within a region improved if the variance among and within fields exceeded the variance within composite samples. Given the variance components, power curves indicated that detection of a 10% change (with 0.8 power) in the ecological condition of soils within a region between two time periods would require sampling a minimum of 25 and 50 fields with one composite soil sample analyzed per field for the maturity and trophic diversity index, respectively. More than 100 fields per region would be required to detect temporal change in populations of individual trophic groups. Biplots of maturity indices, but not of trophic diversity or populations of individual trophic groups, identified clear differences among fields. Thus, maturity indices, which differentiated among sampling sites better and more efficiently than trophic diversity indices or measures based on populations of individual trophic groups, may be appropriate for use in a regional and/or national monitoring program.

Introduction

The Agroecosystem component of the Environmental Monitoring and Assessment Program (EMAP), initiated by U.S. EPA (Kutz and Linthurst, 1990; Messer et al., 1991), is being developed as an interagency monitoring and assessment program to estimate the current status, trends, and changes in selected indicators of the condition of the Nation's agroecological resources on a regional basis with known statistical confidence. A primary question to be answered by this monitoring and assessment effort is: What proportion of agroecosys-

tems in the United States are ecologically sustainable? Critical to answering this question is the development and evaluation of a suite of indicators to assess the condition of the fundamental components of agroecosystems. Biotic or abiotic indicators provide evidence of the condition of a resource at the organism, population, community or ecosystem level of organization (Heck et al., 1993).

Soil is a critical component in the structure and function of agroecosystems and the condition of soil biological communities is important to both the structure and function of soils. Soil organisms are responsible for essential processes such as decomposition and nutrient mineralization. The detritus food web contains primary decomposers (fungi and bacteria), herbivores (plant-parasitic nematodes), consumers of bacteria and fungi (protozoa, bacterivorous and fungivorous nematodes, Collembola), intermediate predators (amoebae, predaceous nematodes) and top predators (predaceous mites) (Moore and de Ruiter, 1991).

An indicator of soil ecological condition would be helpful to measure the current status of vital ecological processes in soil and changes in function through time. For a monitoring effort such as the Agroecosystem component of EMAP, a biological indicator of soil condition must meet several criteria. The indicator should 1) reflect the structure and/or function of ecological processes in soils no matter what soil series or geographic location is sampled, 2) respond to changes in soil condition, 3) be measurable with only one or two sampling periods per year, 4) have available methodologies, and 5) be interpretable. For practical purposes in a national program, it is also desirable that samples be taken by a non-scientist at a reasonable cost.

Nematodes (free-living and plant-parasitic) possess several attributes that make them useful ecological indicators (Freckman, 1988). Soil nematodes occupy important positions in the detritus food web, e.g., they graze on bacteria and fungi in soil (Ingham et al., 1985; Sohlenius et al., 1988), and thus may regulate decomposition (Yeates and Coleman, 1982) and nitrogen mineralization (Seastedt et al., 1988; Sohlenius et al., 1988) in soil ecosystems. Nematodes are relatively small animals with short generation times that allow them to respond quickly to changes in food supply (Bongers, 1990b); however, fluctuations in populations of nematodes are not as rapid as those of microbial populations (Nannipieri et al., 1990). Unlike earthworms, nematodes are ubiquitous, even in polluted or disturbed areas, and certain species are frequently the last animals to die (Freckman, 1988; Samoiloff, 1987), partly because they have the ability to survive desiccation and revive with moisture. With a permeable cuticle, nematodes respond with a range of reactions to pollutants (Bongers, 1990b; Wasilewska, 1989) which reflects the restorative capacity of soil ecosystems (Gorny, 1976; Saly and Ragala, 1984; Wasilewska, 1979; 1989). Changes due to an environmental perturbation, such as tillage, can usually be seen in changes of trophic structure (Hendrix et al., 1986; Parmelee and Alston, 1986; Saly and Ragala, 1984; Wasilewska, 1989). Relative to other soil microfauna, trophic or functional groups of nematodes can be separated easily, primarily by morphological structures associated with various modes of feeding (Bongers, 1990b; Freckman, 1988; Yeates and Coleman, 1982). The relative abundance and size of nematodes also makes sampling and extraction easier and less costly than for other soil fauna.

Although few studies have quantified spatial patterns of mixed communities of plant-parasitic and free-living nematodes in agricultural soils (e.g., McSorley et al., 1985), some of the variability within fields is most likely attributable to aggregations of nematodes around plant roots and organic debris (Barker, 1985a; Barker and Campbell, 1981; Francl, 1986b; Goodell and Ferris, 1980; Noe and Campbell, 1985). The spatial pattern of nematode populations is also influenced by soil properties such as sand content, clay content, sodium concentration, and copper concentration (Goodell and Ferris, 1980; Maggenti, 1991; Noe and Barker, 1985).

Horizontal patterns of population abundance of plant-parasitic nematodes are often described by a negative binomial distribution (Barker, 1985a; Goodell and Ferris, 1980) or Taylor's power law (Barker and Noe, 1988; Ferris et al., 1990). Traditionally, variance of nematode populations associated with spatial pattern within fields and within samples has been described as using coefficients of variation which provide less resolution than alternative expressions of variability such as components of total variance. Partitioning of total variance into variance components has been used occasionally in studies of nematode communities (McSorley and Parrado, 1982).

The objectives of our study were to 1) quantify components of variance associated with ratios among five trophic groups (bacterivores, fungivores, omnivores, predators, and plant-parasites) of nematodes, selected indices of nematode community structure, and soil properties and 2) determine the relative ability of these ratios and indices to serve as indicators of soil

ecological condition to differentiate among agricultural fields. North Carolina was selected as a study area because of the ecological variation in soils and climate among its three ecoregions (Mountains, Piedmont, Coastal Plain). Variance components examined were 'among regions', 'among counties', 'among agricultural fields', 'within agricultural fields', and 'within composite soil samples'. Variance associated with different strategies for collecting soil samples within fields, including several patterns, directions, and repetitions of transects, was quantified to determine the relative ability of a composite soil sample collected from single and repeated transects to estimate population means of the sampled area. Methods of mixing soil and extraction of nematodes were compared within samples to separate the variation due to handling and processing of samples from the true biological variation among individual soil cores within a composite soil sample. With estimates of variance components, power curves were used to determine the ability of each index to detect changes in condition through time.

Materials and methods

Four separate experiments were conducted in North Carolina. Although no single experiment addressed all components of variation, the individual studies could be summarized in an overall random effects model: $Y_{ijklm} = \mu + R_i + C_{j(i)} + F_{k(ij)} + T_{l(ijk)} + D_{m(ijk)} + \varepsilon_{n(ijklm)}$ where μ is the overall mean and R_i, $C_{j(i)}$, $F_{k(ij)}$, $T_{l(ijk)}$, and $D_{m(ijk)}$ are, respectively, variance among regions, among counties in regions, among fields in counties, among independent transects within fields, and for repeated transects of the same pattern; $\varepsilon_{n(ijklm)}$ is the random error which was assigned as the variance component 'within composite soil samples' and includes variance associated with mixing and shipping the sample and laboratory analysis. The four experiments, jointly, allowed for estimation of the variance components of this model. Components of variance from all experiments were estimated using the Mixed Procedure (SAS, 1992). Two experiments were statewide surveys and provided data to estimate the variance among regions, counties and fields. The other two experiments were performed to estimate variability within fields and composite samples.

Among region, county, and field variability

Two surveys of the state of North Carolina, one in 1990 and the other in 1991, were conducted to estimate the proportion of total variance attributed to among regions, counties and fields. Counties within a region were selected randomly, without replacement, with probability of inclusion in the study proportional to total hectarage of the three crops selected for that year. The number of fields selected per county and per crop was determined by the distribution of the harvested hectares of the selected crops among counties (NCASD, 1990, 1991; USDC, 1987). County extension agents were consulted for selection of individual fields. Multiple fields of the same crop within a county were scattered across the county, but fields of different crops were sometimes located on a single farm. To give the largest possible range of differences among sampling sites, however, adjacent fields were not sampled on an individual farm.

In December 1990, soil from fields of three annual crops [soybean (*Glycine max*), corn (*Zea mays* L.), and wheat (*Triticum aestivum* L.)] were sampled. The mountain region was was not included in the 1990 survey because only 7% of the cropped hectarage in North Carolina of the three annual crops selected were in this region and thus estimates for the region would be unreliable. In December 1991, soils from fields of one annual crop (soybean) and two perennial crops [alfalfa (*Medicago sativa* L.) from fields at least five years old, and permanent pastures at least 10 years old with tall fescue (*Festuca arundinacea* Schreb.) alone or tall fescue plus legume (usually white clover, *Trifolium repens* L.)] were sampled in all three regions (Table 1).

Soil was sampled by taking 1 core (2-cm diam, 20 cm deep) at each of 20 equally spaced sites along a serpentine transect of a random 2-ha area in each field (Barker and Campbell, 1981). Cores were mixed thoroughly by hand to form a composite sample. All soil samples were stored at existing field moisture levels and 15 C to minimize changes in nematode populations (Barker et al., 1969). Nematodes were extracted within 14 days after sampling using a semiautomatic elutriator followed by sucrose centrifugation (Barker, 1985b). Nematodes were classified into five trophic groups (Table 2): 1) plant-parasites, 2) bacterivores, 3) fungivores, 4) omnivores, and 5) predators. Algal-feeding nematodes were classified as omnivores because they often feed on a variety of food sources such as algae and fungi. Numbers of nematodes in each taxonomic

Table 1. Number of fields in which sampling of soil was conducted for each crop in each region of North Carolina in 1990 and 1991 Surveys

Year	Region	Crop	No. Fields
1990	Piedmont	Soybeans	11
		Corn	5
	Coastal Plain	Soybeans	36
		Corn	31
		Wheat	9
1991	Mountains	Alfalfa	8
		Pasture	10
	Piedmont	Soybeans	5
		Alfalfa	17
		Pasture	18
	Coastal Plain	Soybeans	22

family of plant-parasites and numbers of nematodes in each trophic group were counted in 500 cm^3 soil and were not corrected for extraction efficiency.

In the 1990 survey, a single soil sample and single laboratory determination were obtained for each field. Consequently, the variance component 'among fields' contained all components of variance associated with F (among fields), T (among independent transects) and ε (within composite soil samples) in the general model. In the 1991 survey, three subsamples were taken from the composite soil sample from 21 of the 80 fields and exposed to different methods of packaging (with and without ice) and shipping (mailing or carrying insulated containers). Because there were no significant effects of the packing and shipping treatments on total nematode populations or individual trophic groups, triplicate observations were treated as subsamples and used to estimate variance within composite soil samples. Treatment effects, although not statistically significant, could account for some of the variability within composite samples. In terms of the general model, the variance component 'among fields' in the 1991 survey included the variance components F and T, and the variance component 'within composite samples' was included in the estimate of ε.

Soil physical and chemical properties were measured on additional soil subsamples from the composite samples. These samples were air-dried and crushed using a hammer mill to a diameter $\leq$ 2 mm. All soil was dried at 90 C for 48 hours before laboratory analyses were performed. Soil properties measured included organic matter, extractable phosphorus, pH, cation

Table 2. Nematode genera and families identified in agricultural soils collected in North Carolina. Taxa are classified by trophic groups and their respective *c-p* (colonizer-persister) value as defined by Bongers (1990b) for calculation of the Maturity Index separately for free-living and plant-parasitic nematodes. Higher and lower *c-p* index values represent persisters and colonizers, respectively. Classification of trophic groups determined following Yeates et al, (1993) and taxonomy according to Maggenti (1982, 1991) and E. M. Noffsinger (pers. comm.)

Trophic group	Family	Genera	C-p
Bacterivores	Alaimidae	*Alaimus*	4
		Amphidelus	4
	Bastianidae	*Bastiania*	3
	Bunonematidae	*Bunonema*	1
	Cephalobidae	*Acrobeles*	2
		Acrobeloides	2
		Cervidellus	2
		Chiloplacus	2
		Eucephalobus	2
		Zeldia	2
	Cylindrolaimidae	*Cylindrolaimus*	3
	Diploscapteridae	*Diploscapter*	1
	Isolaimidae	*Isolaimium*	4
	Leptolaimidae	*Aphanolaimus*	3
	Microlaimidae	*Microlaimus*	3
		Prodesmodora	3
	Monhysteridae	*Monhystera*	1
		Monhystrella	1
		Theristus	1
	Panagrolaimidae	*Panagroiaimus*	1
	Prismatolaimidae	*Prismatolaimus*	3
	Plectidae	*Anaplectus*	2
		Plectus	2
		Wilsonema	2
	Rhabditidae	*Bursilla*	1
		Cruznema	1
		Mesorhabditis	1
		Poikilolaimus	1
		Rhabditis	1
		Rhitis	1
		Teratorhabditis	1
	Rhabdolaimidae	*Rhabdolaimus*	3
	Teratocephalidae	*Euteratocephalus*	3
		Teratocephalus	3
Fungivores	Anguinidae	*Ditylenchus*	2
		Pseudhalenchus	2
	Aphelenchidae	*Aphelenchus*	2
	Aphelenchoididae	*Aphelenchoides*	2
	Diphtherophoridae	*Diphtherophora*	3
		Tylolaimophorus	3
	Paraphelenchidae	*Paraphelenchus*	2

Table 2. continued

	Tylencholaimidae	*Enchodelus*	4
		Longidorella	4
		Tylencholaimus	4
	Tylencholaimellidae	*Doryllium*	4
		Tylencholaimellus	4
Omnivores/algal feeders	Belondiridae	*Axonchium*	5
		Belondira	5
	Cyatholaimidae	*Achromadora*	3
	Dorylaimellidae	*Dorylaimellus*	5
	Dorylaimidae	*Dorylaimus*	4
		Drepanodorus	4
		Eudorylaimus	4
		Labronema	4
		Mesodorylaimus	4
		Pungentus	4
		Thornenema	4
	Leptonchidae	*Dorylaimoides*	4
		Leptonchus	4
		Proleptonchus	4
	Oxydiridae	*Oxydirus*	4
	Plectidae	*Chronogaster*	2
	Tripylidae	*Tobrilus*	3
		Tripyla	3
Predators	Anatonchidae	*Anatonchus*	4
		Miconchus	4
	Carcharolaimidae	*Carcharolaimus*	4
	Chromadoridae	*Chromadorita*	3
	Diplogasteridae	*Butlerius*	1
		Mononchoides	1
		Pristionchus	1
	Dorylaimidae	*Aporcelaimus*	4
		Discolaimus	4
	Iotonchulidae	*Iotonchus*	4
	Ironidae	*Ironus*	4
	Mononchidae	*Mononchus*	4
		Prionchulus	4
	Mononchulidae	*Oionchus*	4
	Mylonchulidae	*Granonchulus*	4
		Mylonchulus	4
	Nygolaimidae	*Nygolaimus*	5
		Sectonema	5
	Seinuridae	*Seinura*	2
Plant-parasites	Belonolaimidae	*Merlinius*	2
		Tylenchorhynchus	2
	Criconematidae	*Criconemella*	3
		Hemicriconemoides	3
	Heteroderidae	*Heterodera*	3
		Meloidogyne	3

Table 2. continued

	Hoplolaimidae	*Helicotylenchus*	3
		Hoplolaimus	3
		Rotylenchulus	3
		Rotylenchus	3
		Scutellonema	3
	Longidoridae	*Xiphinema*	5
	Pratylenchidae	*Pratylenchus*	3
	Trichodoridae	*Paratrichodorus*	4
		Trichodorus	4
	Tylenchidae	*Aglenchus*	2
		Atylenchus	2
		Basiria	2
		Boleodorus	2
		Coslenchus	2
		Ecphyadophora	2
		Filenchus	2
		Psilenchus	2
		Tylenchus	2
	Tylenchulidae	*Paratylenchus*	2

exchange capacity (CEC), exchangeable cations, percent base saturation, electrical conductivity, and trace metals (copper, zinc, aluminum) (Brookside Farms Laboratory Association, Inc., New Knoxville, OH). Soil texture was measured by the authors for two subsamples from each composite sample using a hydrometer method (Gee and Bauder, 1986).

Within field variability

Four strategies of collecting the soil samples were compared in six fields in the Piedmont and Coastal Plain regions of North Carolina during October 1991 to estimate the variance component 'within fields'. Fields were selected with contrasting slope and soil map units to achieve a range of variation among and within fields. Slope ranged from 2-10% in three fields and no obvious slope was observed in the other three fields. Soil sand content for the six fields was not significantly different with a median value of 65.4%. Soils represented in the six fields were Cecil gravelly sandy loam and sandy loam, Norfolk sand and coarse sandy loam, and Portsmouth sandy loam.

A randomly chosen 2-ha area was sampled in each field. Within that 2-ha area, a 5 × 5 grid pattern was established (with grid points 30-m apart) to sample nematode communities and to investigate spatial variability of nematode communities and soil properties.

Twenty soil cores of 20-cm depth were collected within 1-meter of each point of the grid. The 20 cores collected from each point of the grid were mixed thoroughly and submitted as a single, composite sample for a single laboratory determination. Three transect patterns were compared for obtaining soil samples to estimate communities of nematodes in each field: 1) a diagonal transect across the grid, 2) a horizontal transect across the grid, and 3) a serpentine transect in one quadrant of the grid. To estimate the variance component 'among independent transects within fields', two transects of the same pattern were oriented in opposite directions or in opposing quadrants. Each transect soil sample was a composite sample of soil cores taken at 20 equally spaced sites along the transect and a single, mixed, composite sample was sent for laboratory analysis. To estimate the variance component 'among repeated transects', the second transect direction was repeated. For the second transect, two soil cores were taken at each sample site. After pooling and mixing, the soil sample was subdivided to provide two samples for laboratory determination. Soil chemical properties were analyzed and nematodes extracted and enumerated as described previously.

The statistical model used to estimate the variance component 'within fields' was: $Y = \mu + \phi_i + T_{j(i)} + D_{k(ij)} + \varepsilon_{1(kij)}$ where μ is the overall mean and ϕ_i, $T_{j(i)}$ $D_{k(ij)}$, and $\varepsilon_{1(kij)}$ were, respectively, effects among fields, direction of transect within fields, repeated transects of the same direction, and within composite soil samples (which includes effects associated with mixing and shipping the sample and laboratory analysis). The ϕ term was a fixed effect. Data were pooled across transect patterns because there were no effects ($p > 0.05$) of pattern on nematode community indices and soil chemical properties. Data from the 5×5 grid were not used in variance component estimations.

Semi-variograms were constructed using an isotropic model to quantify the spatial structure of the 5×5 grid using GEO-EAS for personal computers (Englund and Sparks, 1991). Semi-variograms illustrate the rate of decrease in correlation between measures of a variable taken at increasing distances from each other in the field. Grid data were analyzed separately for each of the six fields, soil chemical properties, and nematode community parameters.

Within sample variability

Two fields in the Piedmont region of North Carolina were chosen to determine the variability due to methods of mixing and subsampling composite samples. Fields were selected that were cropped to corn or soybeans the previous year and had not been fumigated, had loam to sandy loam soil, and were $\geq$ 2-ha in area. Within each field, 10 randomly placed, diagonal transects were used. Three cores at each of 20 equally spaced sites along the transect were collected, one core was placed into one bucket (composite of 20 cores) and two into another bucket (composite of 40 cores). Each composite was mixed thoroughly with one of two mixing treatments, either by hand (20-core composite) or by pouring soil through riffler sampler three times (Fisher Scientific, Pittsburgh, PA) (40-core composite). One subsample of the hand-mixed soil and two subsamples from the riffler-sampled soil were submitted to each of two laboratories. Laboratory A extracted nematodes with a semiautomatic elutriator followed by sucrose centrifugation as described previously. Laboratory B used Cobb's sifting and gravity method (Ayoub, 1980; Thorne, 1961) modified by triplicate passes through each 710 μm-, 250 μm-, 150 μm-, 75 μm-, and 45 μm-mesh sieves. The final pass through the sieves was followed by centrifugal-flotation (Caveness and Jensen, 1955), modified by using a 1:1 sugar solution (v:v) and centrifuging for 1 min to extract nematodes from soil. Nematodes extracted from the 710 and 250 μm sieves were pooled as a subsample for centrifuging as were nematodes extracted from 150, 75, 45 μm sieves. The two subsamples were combined after centrifugation for identification and enumeration. Numbers of nematodes in each of the five trophic groups and plant-parasitic families were counted by both laboratories. Personnel at Laboratory B also counted numbers of nematodes in each taxonomic family of free-living nematodes. Voucher specimens were preserved in 10% formalin in 25 mL vials with 0.5 to 1.0 mL of glycerin added and the vial sealed with paraffin wax and stored at room temperature (Daykin and Hussey, 1985).

Components of variance associated with methods of elutriation and mixing treatments were estimated from the following statistical model: $Y = \mu + \phi_i + T_{j(i)} + L_k + M_l + \varepsilon_{m(ij)}$ where μ is the overall mean and ϕ_i, $T_{j(i)}$ L_k, M_l, and $\varepsilon_{m(ij)}$ are respectively, effects among fields, among independent transects within fields, between laboratory extraction methods, between mixing treatments, and within composite soil samples (which includes effects associated with shipping the sample and laboratory analysis). ϕ, L, and M were considered fixed effects and components of variance were estimated in the completely nested, unbal-

anced analysis of variance. Spearman rank correlations (SAS, 1989) were used to compare estimates of trophic group populations and community indices obtained for the composite soil sample by each laboratory and between subsamples of the same composite sample within each laboratory.

The 1990 and 1991 surveys were combined to estimate jointly the variance components among regions and counties from the conceptual model (i.e., R_i and $C_{j(i)}$). Data from the Within Field and Within Sample experiments were pooled to estimate the variance components 'among independent transects', and 'repeated transects' (i.e., $T_{l(ijk)}$ and $D_{m(ijk)}$). $\varepsilon_{n(ijklm)}$ was estimated by pooling data from all experiments except the 1990 survey. $F_{k(ij)}$ was estimated by pooling data from all experiments. Components of variance were weighted inversely with the standard error for each experiment that was pooled.

Biplots (Rawlings, 1988) were constructed from Within Sample experiment data to illustrate the correlative relationship among the five nematode indices and the indices' relative ability to differentiate among fields. Biplots are a projection of the centered and standardized data vectors onto the first and second principal components dimensions which give the best two-dimensional representation of the relationships among those vectors in the original 5-dimensional space. Projected vectors that have length near 1.0 are well represented in the two-dimensional plot. Indices that were highly correlated were bundled roughly in the same direction (positive correlation) or opposite direction (negative correlation). Individual observations were also plotted to visually represent relative similarities of individual samples in the two-dimensional space. Clumping of the individual observations from the same field suggested that the indices identified field differences.

Indices of nematode communities

The structure of nematode communities was expressed with several indices (Table 3). Maturity indices, a measure based on life-history strategy characteristics of nematode taxa, were calculated separately for plant-parasitic (PPI) and free-living (MI) families (Bongers, 1990b). PPI was calculated for all experiments, but MI was calculated only for the Within Sample experiment. Nematode families were classified on a scale of 1-5, with colonizers (short life cycle, high reproductive rates, tolerant to disturbance) = 1, and persisters (long life cycles, low colonization ability, few offspring, sensitive to disturbance) = 5 (Table 2). Maturity indices were calculated as the weighted mean (MI or PPI = $\Sigma\ (v_i \times f_i)/n$ of the values assigned constituent nematode families (and the genera and species they contain) where v_i = the colonizer-persister (c-p) value assigned to family i, f_i = the frequency of family i in a sample, and n = total number of individuals in a sample (Bongers, 1990b).

Trophic (and family) diversity were estimated using the 1) Shannon diversity index (Hill's N1), with N1= exp ($-\Sigma P_i[\ln P_i]$), where P_i is the proportion of trophic group (or family) i in the total nematode community (Ludwig and Reynolds, 1988), and 2) Simpson diversity index (Hill's N2), with $N2 = 1/\lambda = 1/(\Sigma[n_i/N]^2)$, where n_i = number of individuals in trophic group (or family) i, and N is the known total number of all individuals in the community (Heip et al., 1988; Ludwig and Reynolds, 1988).

Other indices compared were simple ratios or sums of two trophic groups (Table 3). A $\ln(x+1)$ transformation was used to normalize the variance of populations of nematodes classified by trophic group before ratio calculations and analyses were performed. Logarithmic transformations are appropriate for normalizing data when the underlying distribution is negative binomial and is the typical transformation calculated for nematode populations (McSorley, 1987).

Power of detection

Power curves were constructed using the variance components from all four experiments to estimate the total number of fields within a region to detect a 10% change between two time periods, within a region, with a power (1-β) of 0.8 and an alpha (α) of 0.1. Power is defined as the probability of detecting true differences when they actually exist. The distribution of two time periods was assumed to be normal. Power was calculated for a sample size ranging from 25 to 200 fields per region with one composite sample and one laboratory determination per field.

Results

Within samples, the modified Cobb's method consistently recovered more nematodes than elutriation. Counts of plant-parasites (r = 0.43) and PPI (r = 0.45) were correlated significantly ($p < 0.05$) between extraction methods, but other trophic groups, diversity indices, or maturity indices were not. Elutriation failed

Table 3. Indices calculated to describe nematode community structure for soils in agricultural fields of North Carolina

Index	Resolution	Expt.[a]	Interpretation	Reference(s)
Maturity Index (PPI)	pl. parasite families	R,F,S	plant-parasitic taxa based on a *c-p* scale of 2-5; higher index reflects increased plant production	Bongers, 1990b
Maturity Index (MI)	free-living families	S	free-living taxa based on a *c-p* scale of 1-5; lower index reflects disturbance	Bongers, 1990b
Shannon Diversity	trophic group	R,F,S	index gives greater weight to rare taxa; higher index indicates greater diversity	Pielou, 1977
	free-living families			
Simpson Diversity	trophic group	R,F,S	index gives greater weight to common taxa; higher index indicates greater diversity	Heip et al., 1988;
	free-living families	S		Pielou, 1977
No. bacterivores + fungivores	trophic group	R,F,S	total primary decomposers; reflects higher index magnitude of decomposition activity	Twinn, 1974
No. fungivores/bacterivores	trophic group	R,F,S	decomposition pathway in detrital food webs; lower ratios associated with higher rates of decomposition and nutrient turnover	Twinn, 1974 Sohlenius et al., 1988
No. bacterivores/pl. parasites	trophic group	R,F,S	decomposition activity relative to reduction of primary productivity	Freckman, 1988

[a]R=Among Region, County, and Field Variability; F=Within Field Variability; S=Within Sample Variability Experiments.

to extract omnivores and predators on average 5.5 and 6.6 times more often, respectively, than the modified Cobb's method for subsamples of the same composite soil sample. For the samples in which both methods extracted omnivores and predators, the abundances were an average of 7 and 3 times higher, respectively, for modified Cobb's method than by elutriation. There were no significant differences ($p > 0.05$) between soils mixed by hand or with a riffler sampler for either extraction method, i.e., elutriation or modified Cobb's extraction methods, between maturity indices, diversity indices, or trophic group abundances, except for bacterivores. Despite the relatively high variance of nematode trophic groups 'within composite samples' (Table 4), indices calculated for two separate subsamples from one composite sample were significantly correlated ($p < 0.06$) for PPI ($r = 0.59$), Shannon's diversity index ($r = 0.43$), predators ($r = 0.69$), and omnivores ($r = 0.63$) but not MI, Simpson's diversity index, populations of bacterivores, populations of fungivores, and populations of plant parasites when extracted by modified Cobb's method. In contrast, two separate subsamples

from one composite sample were correlated significantly ($p < 0.05$) for plant-parasitic nematodes ($r = 0.58$) and PPI ($r = 0.54$), but not for trophic diversity, populations of bacterivores, populations of fungivores, populations of omnivores, or populations of predators when extracted by elutriation.

Biplots using both measures of maturity and diversity indices to describe a nematode community showed a clear separation between the fields (Fig. 1). When index types were plotted separately, there was still clear separation between fields with the maturity indices, MI and PPI, but there was considerable overlapping of the fields using only the diversity indices (data not shown). The ability to distinguish fields is associated with the variability within composite soil samples. Within sample variability comprised 38.9%, 49.5%, and 51.2% of the total variance for PPI, Shannon diversity index, and Simpson diversity index, respectively (Table 4).

The largest component of variance was 'within composite samples' for the trophic diversity indices, populations of fungivores, populations of omnivores, populations of predators, and the ratio of fungivores to bacterivores (Table 4). The proportion of total variance attributed to 'within composite soil samples' was a minimum of 25.2% for all the indices of nematode community structure. When the variance component 'within composite samples' was less than 40% of the total variance, the variance components associated with either among fields and/or among independent transects within fields attributed a relatively large portion to the total variance (Table 4). For example, the largest component of variance for the maturity index was 'among independent transects'. The variance among regions was the largest component for populations of plant-parasites and the variance among fields was the largest component for populations of bacterivores, the sum of bacterivores and fungivores, and the ratio of bacterivores to plant-parasites.

The proportion of total variance attributed to, within composite soil samples, was much less for soil chemical properties than for nematode community indices, i.e., 9% for extractable aluminum and less than 2% for the remainder of the soil properties measured (Table 5). The largest component of variance was 'among regions' for pH, percent base saturation, percent clay content, and percent sand content. Soil texture and acidity differed more among regions than among fields. The component 'among fields' accounted for a large proportion of the total variance for organic matter, electrical conductivity, exchangeable potassium, exchangeable calcium, exchangeable magnesium, exchangeable sodium, extractable phosphorus, cation exchange capacity, extractable aluminum, extractable copper, and extractable zinc. The variability among counties was similar in magnitude to variability among fields for cation exchange capacity, exchangeable calcium, and exchangeable magnesium. Properties such as organic matter, electrical conductivity, exchangeable cations, phosphorus, cation exchange capacity, and trace metals were influenced more by management on a relatively local scale than by regional differences in geology.

Within fields, the horizontal scatter of points between distance and semi-variance provided no evidence of spatial dependence of nematode trophic groups or community indices at the scale of sampling (Isaaks and Srivastava, 1989; Oliver, 1987). The semivariograms were similar for soil chemical properties, including soil texture. Means and variances calculated for individual transects traversing the 2-ha area were not different ($p > 0.05$) than grid estimates and, therefore, a transect was assumed to estimate adequately the "true" population of the sampled area within a field.

Power curve analysis suggested that maturity and trophic diversity indices of nematode communities may reduce the variance sufficiently to meet data quality objectives for a national monitoring program (Fig. 2). Based on power curves, detection of a 10% change (with 0.8 power) in the ecological condition of soils within a region from one time period to another would require sampling a minimum of 25 and 50 fields, with one sample analyzed per field for a maturity and trophic diversity index, respectively. Only values of the Shannon diversity indices are illustrated because the Shannon index weights less abundant taxa more than the Simpson index (Camargo, 1992), which is appropriate for trophic group diversity where ecologically important groups such as omnivores and predators are commonly low in abundance due to their position in the detritus food web (Wasilewska, 1979). The detection ability of Shannon's diversity index was better than that of either fungivores or plant-parasites (Fig. 2). Variability associated with omnivores and predators (data not shown) was much greater and, therefore, the ability to detect a 10% change in condition with a power of 0.8 would require too many samples within a region to be practical.

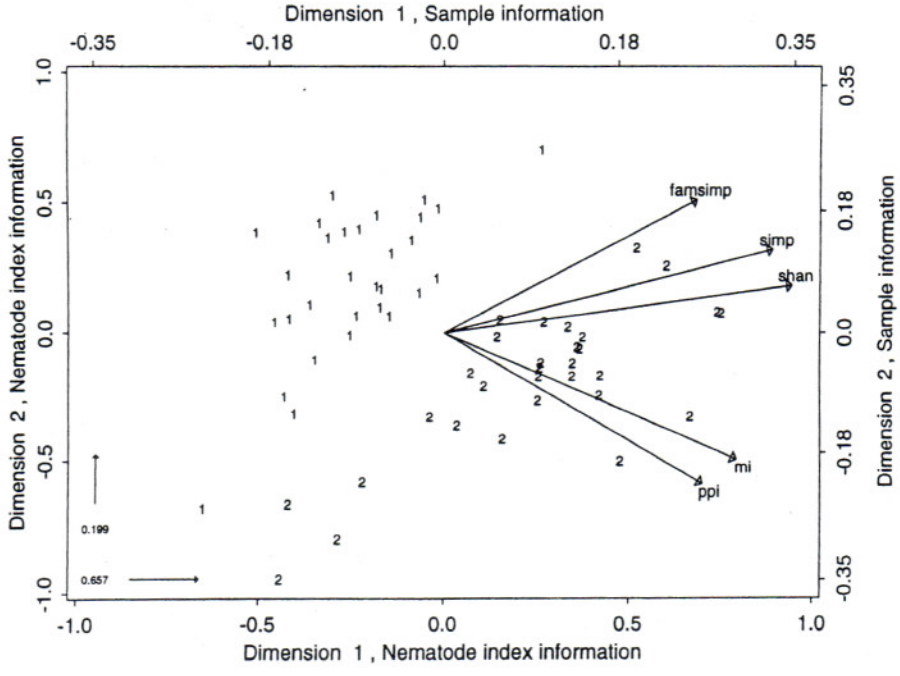

Fig. 1. Biplot of dimension one and dimension two of nematode community indices. Smaller angles between vectors indicate greater correlations between indices: maturity indices (mi and ppi), diversity free-living nematode families (famsimp) and diversity of nematode trophic groups (simp, shan). Points with similar numbers represent individual composite soil samples enumerated for nematodes within a field. Within Sample experiment data where nematodes were extracted from soil with the modified Cobb's sifting and gravity method followed by sucrose centrifugation were used to construct the biplots.

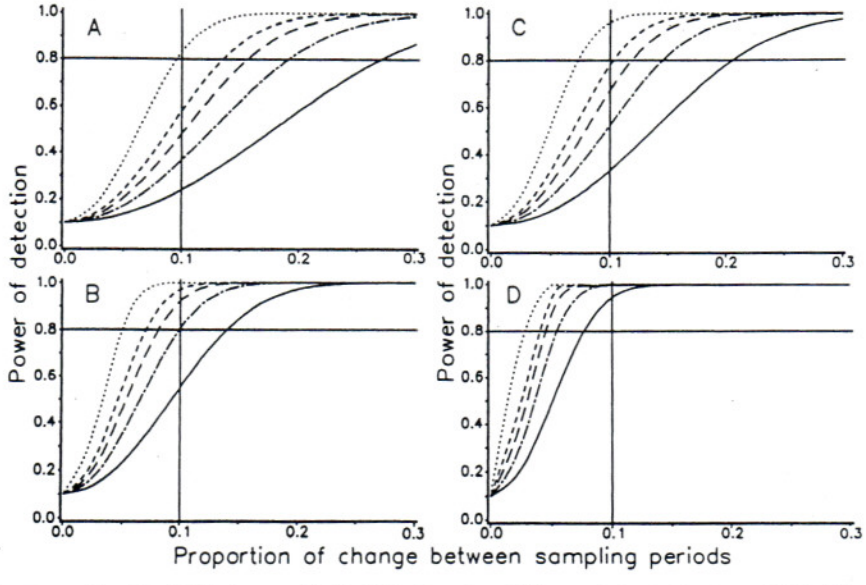

Fig. 2. Power curves representing the statistical power (1-β) of detecting a true difference between the mean of **A**) ln (fungi-feeding nematodes + 1), **B**) Shannon's diversity index, **C**) ln (plant-parasitic nematodes + 1), and **D**) Plant Parasitic Index with a given proportional increase from the one time period to another time period. A single curve can be interpreted as the probability of detecting x proportion of change with y power from one time period to another time period. Contrasting line styles indicate the number of fields sampled in a region (solid=25, long and short dashes=50, long dash=75, short dash=100, dotted=200). These power curves assume that one composite sample was analyzed per field. The variance components from all four experiments were combined to compute the power curves.

Table 4. Total variance of nematode community indices partitioned into components i.e., among regions in North Carolina, among counties within regions, among fields within counties, among independent transects within fields, among repeated transects of the same pattern, and within composite soil samples[a]. Components are expressed as actual variance and sums across rows equal total variance

Index	Lab A[b] Among Region	Among County	Among Fields	Among Trans.	Repeat. Trans.	Within Sample[a]	Lab B[b] Within Sample[a]
Maturity Index (plant-parasites)	0.004	0.019	0.000	0.057	0.000	0.051	0.017
Maturity Index (free-living)	—[c]	—	—	—	—	—	0.024
Trophic Diversity (Shannon)	0.033	0.054	0.079	0.017	0.016	0.195	0.214
Trophic Diversity (Simpson)	0.044	0.039	0.000	0.020	0.047	0.158	0.254
Bacterivores[d]	0.012	0.157	0.353	0.000	0.002	0.222	0.358
Fungivores[d]	0.003	0.093	0.095	0.005	0.027	0.515	0.466
Omnivores/Algal Feeders[d]	0.000	0.213	0.000	0.023	0.065	0.369	0.975
Predators[d]	0.000	0.011	0.116	0.002	0.000	0.270	0.788
Plant-Parasites[d]	0.339	0.000	0.296	0.169	0.000	0.288	0.227
Bact. + Fung.[e]	0.000	0.122	0.270	0.000	0.005	0.224	0.395
Fung. / Bact.[e]	0.008	0.006	0.000	0.000	0.002	0.052	0.006
Bact./Bact + Pl.par.[e]	0.001	0.000	0.003	0.002	5×10^{-5}	0.002	4×10^{-4}

[a]Includes repeated transects, sample mixing, nematode extraction, and nematode enumeration components.
[b]Extraction methods: Lab A = single semiautomatic elutriation followed by sucrose centrifugation, Lab B = modified, triplicate Cobb's sifting and gravity method followed by sucrose centrifugation.
[c]Unable to estimate.
[d]Transformed as ln $(x + 1)$ before analysis.
[e]Transformed as ln $(x + 1)$ before ratio estimation and analysis.

Table 5. Total variance of soil chemical properties partitioned into components i.e., among regions in North Carolina, among counties within regions, among fields within counties, among independent transects within fields, among repeated transects of the same pattern, and within composite soil samples[a]. Components are expressed as actual variance and sums across rows equal total variance

Property	Among Region	Among County	Among Fields	Among Trans.	Repeat. Trans.	Within Sample[a]
pH	0.266	0.042	0.098	0.026	0.000	0.007
% Base saturation	152.990	24.521	55.917	21.559	0.000	5.088
% Organic matter	5.302	26.551	51.974	0.152	0.000	0.070
CEC (meq/100g)	0.000	16.678	17.442	0.356	0.000	0.274
% Clay content	66.136	22.910	15.169	10.064	0.000	0.016
% Sand content	190.615	172.237	136.596	19.142	1.691	2.197
Electrical conductivity (dS m^{-1})	0.000	0.002	0.004	5×10^{-4}	0.000	1×10^{-5}
Exchangeable Ca (meq/100g)	0.000	3.488	4.513	0.117	0.000	0.009
Exchangeable Mg (meq/100g)	0.111	0.591	0.629	0.014	0.000	0.006
Exchangeable K (meq/100g)	0.000	0.004	0.035	0.002	0.00	6×10^{-6}
Exchangeable Na (meq/100g)	2×10^{-5}	3×10^{-4}	8×10^{-4}	7×10^{-5}	0.000	1×10^{-5}
Extractable P (mg kg^{-1})	1112.668	1957.719	3469.302	621.861	0.000	17.716
Extractable Al (mg kg^{-1})	1234.199	5015.333	11649.927	1985.218	0.000	1978.397
Extractable Cu (mg kg^{-1})	0.095	0.250	1.956	0.189	0.000	0.001
Extractable Zn (mg kg^{-1})	0.374	0.986	9.015	1.936	1.059	0.024

[a]Includes repeated transects, sample mixing, nematode extraction, and nematode enumeration components.

Discussion

The variability within composite samples has not been quantified previously and reported as a variance component for indices such as the maturity and trophic diversity indices. Indices with low 'within composite sample' variability will require a smaller sample size to detect change in soil condition than indices with higher variability within samples. Given this criterium in our study, the best candidate index for a national monitoring program to use for detecting changes in soil ecological condition is the maturity index of plant-parasitic nematodes, PPI. These results support those of Bongers (1990a) who found that differences in the maturity index of free-living nematodes (MI) within one field were less than differences in the MI of nematode communities at one time among many locations. The ratio of bacterivorous to plant-parasitic nematodes, sum of bacterivores and fungivores, populations of bacterivores, and populations of plant-parasites, separately, also performed well, because within sample variability was less than the combined variability among regions, counties and fields. Bacterivores and plant-parasites were the most abundant of the five trophic groups identified.

The maturity index, MI, has been found to be robust enough to be used among seasons and soil types because variability associated with seasons was less than the variability associated with location (Bongers, 1990a). In our study, an autumn sampling period was selected following cultivation of crops harvested in the fall to minimize within field sampling variation (Francl, 1986a, b). Free-living nematode populations are generally at their peak at this time (Boag, 1977; Sohlenius, 1982) because 1) crop residues are incorporated into soil by cultivation (Kästner and Germershausen, 1989) and 2) temperatures are favorable (15-20 C) (Boag, 1977; Stinner and Crossley, 1982). Surveys are best performed when maximum populations occur (Barker and Noe, 1988).

Although diversity indices are often used in ecological studies, their usefulness as an indicator of ecosystem stability is questionable (Ferris and Ferris, 1974; Platt et al., 1984). Despite the controversy about their interpretation, taxonomic and genetic diversities of microbial and micro-invertebrate communities in Arctic coastal seawater, Arctic freshwater lake, Oregon silt loam soil (Atlas et al., 1991), floodplain forest soils of Czechoslovakia (Jarošík, 1983), and benthic communities of the St. Mary's River (Burt et al., 1991) were found to be lower in disturbed or polluted sites than in undisturbed or nonpolluted sites. Diversity indices are sensitive to a decline in number of taxa found in an area but are insensitive to changes in taxon composition. Therefore, they cannot be used to measure qualitative changes in taxon composition that may result from pollution (Camargo, 1992). However, diversity indices may be more sensitive to recent environmental disturbances than single numbers of species alone (Jarošík, 1983). Trophic diversity indices may be best used as a complement to other ecological indices (Camargo, 1992) such as maturity indices to describe the condition of nematode communities.

One disadvantage of relying on trophic groups for a national monitoring program is that the method of extraction affects the proportion of each trophic group obtained. The modified Cobb's sifting and gravity method with multiple sievings is more time consuming, but retains a higher proportion (up to 90-95% efficiency for both plant-parasitic and free-living nematodes) of total nematodes and a greater representation of the trophic groups present than elutriation with one sieving (McSorley and Walter, 1991). Variance associated with omnivores and predators was greater for samples extracted by modified Cobb's method than by elutriation. However, failure to extract omnivores and predators decreased artificially the variance of omnivore and predator abundances estimated by elutriation.

Differences found between methods of extraction may be confounded by factors associated with different laboratories. Therefore, results should be interpreted as laboratory by extraction method interactions rather than due strictly to extraction method alone. For example, there were significant correlations between subsamples of the same composite sample for populations of plant-parasites and PPI but not other trophic groups or indices within Laboratory A. In contrast, there were significant correlations between subsamples of the same composite sample for most trophic groups and indices within Laboratory B. The correlations may reflect an interaction between extraction efficiency and classification of plant-parasitic and free-living nematodes. The differences between extraction methods is not surprising because the elutriator with a rapid, single-sieve extraction was designed for relatively efficient assays of plant-parasitic nematodes.

There were a number of factors that contributed to the variability of nematode communities within samples. First, bulk sampling contributed to the uncertainty of mean estimation and to total unexplained variation (Francl, 1986b; Schmitt et al., 1990). Second, subsampling error was confounded with errors in handling and

counting (Francl, 1986b). At best, mixing a bulk sample randomizes nematodes so that the variance equals the mean (Francl, 1986b). Procedures for homogenizing soil for chemical properties such as drying and grinding soils would kill nematodes. It is possible that the tremendous variability within samples may have masked differences between experimental treatments of mixing soil. However, we recommend hand-mixing rather than using riffler sampler for mixing soil for future studies because we found no difference in nematode populations mixed in either method and it was discovered hand-mixing was simpler and less expensive.

The sampling patterns within fields used in this study followed recommendations for sampling aggregated or clustered parameters, i.e., populations of nematodes and soil chemical properties (Barker, 1985a; Barker and Campbell, 1981; Lin et al., 1979). Scattering 20 sampling sites across 2-ha sufficiently sampled beyond, not within, natural clusters or aggregates of nematode populations and chemical properties of soil such that the correlation between sampling sites, based on semivariograms, was near zero. Based upon other reports which suggest patch size of nematode clusters in agricultural fields is < 30 m in diameter (Barker et al., 1985; Francl, 1986a; Noe and Barker, 1985; Noe and Campbell, 1985), it is unlikely that the entire grid fell within a single cluster. Although no one transect type (diagonal, horizontal, or serpentine) had greater precision in estimating mean populations of nematodes than another (also Goodell and Ferris, 1981), we chose the diagonal transect for survey purposes because it traversed rows and furrows equally in contrast to the horizontal transect placed along rows and had the potential to represent a wide range of micro-environments in the field (Prot and Ferris, 1992). Composite samples of 20 soil cores per field were sufficient to represent a 2-ha area. Prot and Ferris (1992) found a single 10-core sample collected from an entire field was sufficient to detect the presence of nematode species and estimate relative abundance of plant-parasitic nematodes for an extensive survey.

Based on the results of our study, it would be difficult to differentiate the ecological condition of soils among regions, counties or fields with statistical confidence based upon trophic diversity or individual trophic groups alone. However, the ability to differentiate among fields improved with the use of trophic group ratios and was best with maturity indices of nematode communities. Maturity indices are capable of differentiating effects of tillage, chemical inputs, and different successional stages in agroecosystem soils (Freckman and Ettema, 1993). Before life strategy and trophic ratio indices are applied by national monitoring programs, experiments must be conducted to confirm the appropriate time of sampling during a year and to insure the sampling time is reliable and representative of nematode communities and to establish an appropriate reference baseline for comparison.

Acknowledgements

The authors thank Charles R Harper, Michael J Munster, Mark B Tooley, George R Hess, Julie R Meyer and Karen K Campbell for their help in sampling field soils, and E Mae Noffsinger and Kenneth R Barker for their time and expertise in counting and classifying nematodes. We also appreciate the assistance of the County Agents of the N C Cooperative Extension Service and growers who participated in the study, the assistance of Karen Nauman in analysis of data, and Kenneth R Barker (Dept of Plant Pathology, NCSU) and Marcia L Gumpertz (Dept of Statistics, NCSU) for their reviews of earlier drafts of the manuscript. Although the research described in this article has been funded in part by the U S Environmental Protection Agency's Office of Research and Development through interagency agreement DW12934170 to the United States Department of Agriculture, Agricultural Research Service, it has not been subjected to the Agency's review and, therefore, does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. This work was also supported through a Specific Cooperative Agreement (58-6645-2-016) between the USDA, Agricultural Research Service and the North Carolina Agricultural Research Service.

References

Atlas R M, Horowitz A, Krichevsky M and Bej A K 1991 Response to microbial populations to environmental disturbance. Microbiol. Ecol. 22, 249-256.

Ayoub S M 1980 Plant Nematology, An Agricultural Training Aid. California Department of Food and Agriculture, NemaAid Publications, Sacramento, CA. 195 p.

Barker K R, Nusbaum C J and Nelson L A 1969 Effects of storage temperature and extraction procedure on recovery of plant-parasitic nematodes from field soils. J. Nematol. 1, 240-247.

Barker K R 1985a Sampling nematode communities. *In* An Advanced Treatise on *Meloidogyne* Vol. II: Methodology. Eds. K R Barker, C C Carter and J N Sasser. pp 2-17. North Carolina State University Graphics, Raleigh.

Barker K R 1985b Nematode extraction and bioassays. *In* An Advanced Treatise on *Meloidogyne* Vol. II: Methodology. Eds. K R Barker, C C Carter and J N Sasser. pp 19-35. North Carolina State University Graphics, Raleigh.

Barker K R and Campbell C L 1981 Sampling nematode populations. *In* Plant Parasitic Nematodes, Vol. III. Eds. E.B M Zuckerman and R A Rohde. pp 451-474. Academic Press, New York.

Barker K R and Noe J P 1988 Techniques in quantitative nematology. *In* Experimental Techniques in Plant Disease Epidemiology. Eds. J Kranz and J Rotem. pp 223-236. Springer-Verlag, Berlin.

Barker K R, Schmitt D P and Noe J P 1985 Role of sampling for crop-loss assessment and nematode management. Agric. Ecosyst. Environ. 12, 355-369.

Boag B 1977 Factors influencing the occurrence and abundance of nematodes in forest soils in eastern Scotland. Ann. Appl. Biol. 86, 446-450.

Bongers A M T 1990a Biologische bodembeoordeling met nematoden. *In* Handboek voor Milieubeheer: Bodembescherming. Eds. F A M de Haan, Ch H Henkens and D A Zeilmaker. pp J2000-1-J2000-17. Samson H D Tjeenk Willink, Alphen aan de Rijn, Deurne.

Bongers T 1990b The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14-19.

Burt A J, McKee P M, Hart D R and Kauss P B 1991 Effects of pollution on benthic invertebrate communities of the St. Mary's River, 1985. Hydrobiologia 219, 63-81.

Camargo J A 1992 New diversity index for assessing structural alterations in aquatic communities. Bull. Environ. Contam. Toxicol. 48, 428-434.

Caveness F E and Jensen H J 1955 Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Proc. Helminthol. Soc. 22, 87-89.

Daykin M E and Hussey R S 1985 Staining and histopathological techniques in nematology. *In* An Advanced Treatise on *Meloidogyne* Vol. II: Methodology. Eds K R Barker, C C Carter and J N Sasser. pp 39-48. North Carolina State University Graphics, Raleigh.

Englund E and Sparks A 1991 GEO-EAS 1.2.1 Geostatistical Environmental Assessment Software-User's Guide. EPA 600/8-91/008. U. S. Environmental Protection Agency, Las Vegas, Nevada. pp 1-1 to 17-2.

Ferris V R and Ferris J M 1974 Inter-relationships between nematode and plant communities in agricultural ecosystems. Agro-Ecosystems 1, 275-299.

Ferris H, Mullens T A and Foord K E 1990 Stability and characteristics of spatial description parameters for nematode populations. J. Nematol. 22, 427-439.

Francl L J 1986a Spatial analysis of *Heterodera glycines* populations in field plots. J. Nematol. 18, 183-189.

Francl L J 1986b Improving the accuracy of sampling field plots for plant-parasitic nematodes. J. Nematol. 18, 190-195.

Freckman D W 1988 Bacterivorous nematodes and organic-matter decomposition. Agric. Ecosyst. Environ. 24, 195-217.

Freckman D W and Ettema C H 1993 Assessing nematode communities in agroecosystems of varying human intervention. Agric. Ecosyst. Environ. 45, 239-261.

Gee G W and Bauder J W 1986 Particle-size analysis. *In* Methods of Soil Analysis, Part 1. Ed. A Klute. pp 383-411. American Society of Agronomy, Madison, Agronomy Monograph No. 9 (2nd edition) 383-411.

Goodell P and Ferris H 1980 Plant-parasitic nematode distributions in an alfalfa field. J. Nematol. 12, 136-141.

Goodell P B and Ferris H 1981 Sample optimization for five plant-parasitic nematodes in an alfalfa field. J. Nematol. 13, 304-313.

Gorny M 1976 Zagadnienie bioindykacjiw glebach biotopów Zdefradowanych. Wiad. Ekol. 22, 224-230 (cited by Wasilewska,1979).

Heck W W, Campbell C L, Finkner A L, Hayes C R, Hess G R, Meyer J R, Munster M J, Neher D A, Peck S L, Rawlings J O, Smith C N and Tooley M B 1993 Environmental Monitoring and Assessment Program (EMAP) - Agroecosystem 1992 Pilot Project Plan. EPA/620/R-93/010. U.S. Environmental Protection Agency, Washington DC.

Heip C, Herman P M J and Soetaert K 1988 Data processing, evaluation and analysis. *In* Introduction to the Study of Meiofauna. Eds. R P Higgins and H Thiel. pp 197-231. Smithsonian Institution Press, Washington DC.

Hendrix P F, Parmelee R W, Crossley D A Jr, Coleman D C, Odum E P and Groffman P M 1986 Detritus food webs on conventional and no-tillage agroecosystems. BioScience 36, 374-380.

Ingham R E, Trofymow J A, Ingham E R and Coleman D C 1985 Interactions of bacteria, fungi, and their nematode grazers: Effects on nutrient cycling and plant growth. Ecol. Monogr. 55, 119-140.

Isaaks E H and Srivastava R M 1989 Applied Geostatistics. Oxford University Press, Oxford, UK. 561 p.

Jarošík V 1983 A comparison of the diversity of carabid beetles (Col., Carabidae) of two floodplain forests differently affected by emissions. Vest. Ceskoslav. Spolec. Zool. 47, 215-220.

Kästner V A und Germershausen K 1989 Struktur und Abundanzdynamik der Nematodenfauna in einem Schwarzerde-Lössboden. Hercynia N. F. 26, 71-93.

Kutz, F W and Linthurst R A 1990 A systems-level approach to environmental assessment. Toxicol. Environ. Chem. 28, 105-114.

Lin C S, Poushinsky G and Mauer M 1979 An examination of five sampling methods under random and clustered disease distribution using simulation. Can. J. Plant Sci. 59, 121-130.

Ludwig J A and Reynolds J F 1988 Statistical Ecology: A Primer on Methods and Computing. John Wiley & Sons, New York. 337 p.

Maggenti A R 1982 Nemata. *In* Synopsis and Classification of Living Organisms. Ed. S P Parker. pp 878-923. McGraw-Hill, New York.

Maggenti A R 1991 Nemata: Higher classification. *In* Manual of Agricultural Nematology. Ed. W R Nickle. pp 147-187. Marcel Dekker, Inc., New York.

McSorley R 1987 Extraction of nematodes and sampling methods. *In* Principles and Practice of Nematode Control in Crops. Eds. R H Brown and B R Kerry. pp 13-47. Academic Press, Orlando.

McSorley R and Parrado J L 1982 Estimating relative error in nematode numbers from single soil samples composed of multiple cores. J. Nematol. 14,

McSorley R and Walter D E 1991 Comparison of soil extraction methods for nematodes and microarthropods. Agric. Ecosyst. Environ. 34, 201-207.

McSorley R, Dankers W H, Parrado J L and Reynolds J S 1985 Spatial distribution of the nematode community on perrine marl soils. Nematropica 15, 77-92.

Messer J J, Linthurst R A and Overton W S 1991 An EPA program for monitoring ecological status and trends. Environ. Monit. Assess. 17, 67-78.

Moore J C and de Ruiter P C 1991 Temporal and spatial heterogeneity of trophic interactions within below-ground food webs. Agric. Ecosyst. Environ. 34, 371-397.

Nannipieri P, Grego S and Ceccanti B 1990 Ecological significance of the biological activity in soil. *In* Soil Biochemistry, Volume 6. Eds. Jean-Marc Bollag and G Stotzky. pp 293-355. Marcel Dekker, Inc., New York.

Noe J P and Barker K R 1985 Relation of within-field spatial variation of plant-parasitic nematode population densities and edaphic factors. Phytopathology 75, 247-252.

Noe J P and Campbell C L 1985 Spatial pattern analysis of plant-parasitic nematodes. J. Nematol. 17, 86-93.

North Carolina Agricultural Statistics Division 1990 North Carolina Agricultural Statistics: 1990. North Carolina Department of Agriculture, Raleigh. 76 p.

North Carolina Agricultural Statistics Division 1991 North Carolina Agricultural Statistics: 1991. North Carolina Department of Agriculture, Raleigh. 76 p.

Oliver M A 1987 Geostatistics and its application to soil science. Soil Use Manage. 3, 8-20.

Parmelee R W and Alston D 1986 Nematode trophic structure in conventional and no-tillage agroecosystems. J. Nematol. 18, 403-407.

Pielou P C 1977 Mathematical Ecology. John Wiley & Sons, New York. 385 p.

Platt H M, Shaw K M and Lambshead P J D 1984 Nematode species abundance patterns and their use in the detection of environmental perturbations. Hydrobiologia 118, 59-66.

Prot J C and Ferris H 1992 Sampling approaches for extensive surveys in nematology. Suppl. J. Nematol. 24, 757-764.

Rawlings J O 1988 Applied Regression Analysis: a Research Tool. Wadsworth & Brooks/Cole Advanced Books & Software, Pacific Grove, California. 553 p.

Saly A and Ragala P 1984 Free-living nematodes-bioindicators of the effects of chemization on the soil fauna. UVTIZ-Ochr. Rostl. 20, 15-21.

Samoiloff M R 1987 Nematodes as indicators of toxic environmental contaminants. *In* Vistas on Nematology. Eds. J A Veech and D W Dickson. pp 433-439. Society of Nematologists, Lakeland, Florida.

SAS Institute, Inc 1989 SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. SAS Institute Inc, Cary, NC. 1686 p.

SAS Institute, Inc 1992 SAS Technical Report P-229, SAS/STAT Software: Changes and Enhancements, Release 6.07. SAS Institute Inc, Cary, NC. 620 p.

Schmitt D P, Barker K R, Noe J P and Koenning S R 1990 Repeated sampling to determine the precision of estimating nematode population densities. J. Nematol. 22, 552-559.

Seastedt T R, James S W and Todd T C 1988 Interactions among soil invertebrates, microbes and plant growth in the tallgrass prairie. Agric. Ecosyst. Environ. 24, 219-228.

Sohlenius B 1982 Short-term influence of clear-cutting on abundance of soil-microfauna (Nematoda, Rotatoria and Tardigrada) in a Swedish pine forest soil. Appl. Ecol. 19, 349-359.

Sohlenius B, Bostrom S and Sandor A 1988 Carbon and nitrogen budgets of nematodes in arable soil. Bio. Fertil. Soils 6, 1-8.

Stinner B R and Crossley D A Jr 1982 Nematodes in no-tillage agroecosystems. *In* Nematodes in Soil Ecosystems. Ed. D W Freckman. pp 14-28. University of Texas Press, Austin.

Thorne G 1961 Principles of Nematology. McGraw-Hill Inc, New York. 553 p.

Twinn D C 1974 Nematodes. *In* Biology of Plant Litter Decomposition. Eds. C H Dickinson and G J F Pugh. pp 421-465. Academic Press, London.

U.S. Department of Commerce, Bureau of the Census 1987 1987 Census of Agriculture – County Data, North Carolina. U.S. Government Printing Office, Washington, DC.

Wasilewska L 1979 The structure and function of soil nematode communities in natural ecosystems and agrocenoses. Pol. Ecol. Stud. 5, 97-145.

Wasilewska L 1989 Impact of human activities on nematodes. *In* Ecology of Arable Land. Eds. M Charholm and L Bergstrom. pp 123-132. Kluwer Academic Publishers, Dordrecht, Holland.

Yeates G W, Bongers T, de Goede R G M, Freckman D W and Georgieva S S 1993 Feeding habits in soil nematode families and genera – An outline for soil ecologists. J. Nematol. 25, 315-331.

Yeates G W and Coleman D C 1982 Nematodes in decomposition. *In* Nematodes in Soil Ecosystems. Ed. D W Freckman. pp 55-80. University of Texas Press, Austin.