

Spatial Distribution of Nematodes at Organic and Conventional Crop Fields in Cape Coast, Ghana

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Abstract

Globally, plant-parasitic nematodes cause large reductions in crop yields and quality. The conditions prevalent in organic crop production fields can favour or inhibit nematode build-up. An overview of the spatial distribution of nematodes can help the design of targeted, site-specific management strategies. This paper assessed and compared the spatial distribution of nematode population in an organic crop field and a conventional crop field using Inverse Distance Weighted (IDW) and ordinary Kriging spatial interpolation techniques. The results show that nematode population is higher on the organic field compared to the conventional crop field. Spatial distribution of nematode population showed a north-south gradient in the organic field but small patches of large population in the conventional field. The two interpolation methods did not show substantial differences in mapping the spatial distribution of the nematode population. It is concluded that nematode control strategies employed on the organic field might be less effective than expected. Both inverse distance weighted and ordinary kriging can be used to map the spatial distribution of nematodes under similar conditions and in a non-complex terrain.

Introduction

Nematodes are roundworms that normally live in the thin films of water surrounding soil particles because water aids their mobility (Freckman, 2000). They complete their life cycle fully or almost fully in the soil. Nematodes are either beneficial (because they feed on other pathogens and help nutrient cycling in the soil) or parasitic because they feed on plant roots and thereby hamper nutrients and water uptake (Yeates, 2003). Globally, plant-parasitic nematodes cause huge yield and quality penalties (Adam *et al.*, 2013), with annual cost exceeding US\$ 100 billion (Bird and Kaloshian, 2003). Large infestations of nematodes are normally controlled with chemicals which have the potential to pollute soils, by

growing resistant varieties or rotating to a more tolerant crop (Mario *et al.*, 2011; Wyse-Pester *et al.*, 2002). As a result, nematode control in an organic crop field, especially for vegetables, can be very challenging compared to conventionally managed crop field (Oka *et al.*, 2007; Berkelmans *et al.*, 2003).

Controlling plant-parasitic nematodes, especially the root knot (*Meloidogyne* spp.), in an ecologically friendly manner has been a major challenge at the Teaching and Research Farm at the University of Cape Coast. A map of the spatial distribution of nematode populations can greatly aid management decisions regarding nematode control on the research farm (Wyse-Pester *et al.*, 2002).

Two factors key to producing such maps are sampling density and the appropriateness of the interpolation method. The sampling density will affect the cost of mapping and the ability to capture the spatial variability of the target attribute in the field. The interpolation method used will affect the accuracy of the predictions at unsampled locations. The spatial dependence (autocorrelation) in the distribution of the target attribute greatly influences the accuracy of the predictions at unsampled locations (Burrough, 1991).

Geostatistics can be used to analyse and quantify spatial autocorrelation by distance and direction (Evans *et al.*, 1999). Geostatistical analysis in previous studies has shown that field-scale autocorrelation in nematode populations can vary from less than 1 m to 160 m, or even up to over 640 m in large agricultural fields (Wyse-Pester *et al.*, 2002). The purpose of the current study was to assess the nematode population at an organic and conventionally managed vegetable crop fields and map the spatial distribution of nematode populations using two interpolation techniques.

Method

Study site

Soil samples were collected from an organic vegetable field and a conventional arable crop field at the Technology Village in the School of Agriculture Teaching and Research Farm, University of Cape Coast, Ghana. The organic field is used to produce vegetables, mainly sweet pepper, carrot, cucumber and lettuce while the conventional arable field is mainly cultivated with maize. The organic field is

hand-watered while the non-organic field is entirely rain-fed. The main nematode control measures in the organic field included, the use of neem extract, marigold (*Tagetes* spp.) and compost. No target nematode control measure had been used in the conventionally managed field. The study sites are in the coastal savannah agro-ecological zone of Ghana, with a major rainy season (April-July) and a minor rainy season (September–November) and a dry season (December–March). The mean annual rainfall is 920 mm and mean annual temperature is approximately 24 °C. Temperatures are uniformly high throughout the year. Soils at the site are classified as Haplic Acrisols (Asamoah, 1973).

Soil Sampling

Soil samples for nematode counts and soil property determinations were collected from the fields at depths of 0–20 cm. The samples were collected in a zigzag pattern, starting from a corner at one end of the field to the opposite corner at the other end of the field. Soil cores were collected at regular intervals of 5 m between sampling points. Sampling from both fields was done in March 2016, just before the beginning of the major season. In all, 30 samples were collected at the organic field and 34 samples from the conventional crop field. The geographic coordinates of each sample location was collected using a Garmin Etrex Handheld Global Positioning System. For the organic field, an area of 50 m × 25 m was marked out for the sampling while an area of 50 m × 30 m was used at the conventional crop field.

Soil Properties

Dry bulk density of the soil was determined by the corer method after oven-drying the soil at 105 °C for 48 hours. Soil water content was determined gravimetrically. Soil pH was determined in 1:2.5 soil: water ratio using a pH meter. Nitrogen content was determined by the Micro-Kjedahl method (Faithfull, 2002) while organic carbon was determined by the Walkley Black method (Nelson and Sommers, 1996). Phosphorus was determined using the Bray-No.1 method while potassium was determined by ammonium acetate extraction and flame photometry (Faithfull, 2002). Soil texture was determined using the Bouyocous Hydrometer method (Klute, 1986).

Nematode Count

Nematode extraction was done using the method described by Whitehead and Hemming (1965). The soil samples were sieved and 100 g of sieved soil was weighed from each of the soil samples. The weighed samples were thinly spread over a single-ply tissue placed in a sieving basket, which in turn was placed in a shallow tray. Using a wash bottle, distilled water was introduced into the tray around the sieving basket to moisten (but not saturate) the soil samples. The setup was allowed to stand for three days to allow the nematodes move into the water in the tray. Within this period, it was ensured that the samples remained moist. After the third day, the sieving baskets, together with the soil samples, were gently removed from the trays as all living nematodes were assumed to have moved into the water. The suspension in the trays was transferred into

separate beakers, labelled and allowed to stand for 24 hours for nematode settling and decantation. After 24 hours, the top (about 75%) of the suspension in each of the beakers was siphoned off to concentrate nematodes and obtain a workable sample. The nematodes were relaxed in a water bath at 60 °C for three minutes and 25 cm³ double strength formalin acetic acid was added to fix the nematodes. Nematodes were counted under a compound microscope from 20 ml aliquots of the residual suspensions transferred into petri dishes in three replicates. The total nematode count for each sample location was the average of the counts in the three petri dishes.

Spatial Interpolation

The geographic coordinates of the sample locations and nematode count data were formatted in Microsoft Excel and imported as event theme in ArcGIS 10.1 (ESRI™, Redlands, California). Spatial distribution of the nematode counts was mapped using Inverse Distance Weighted (IDW) and Ordinary Kriging in the Geostatistical Analyst extension. The performance of each interpolation method was assessed using the cross-validation.

The IDW is deterministic and uses nearest neighbours to predict the values of unsampled locations as expressed in equation 1 (Yao *et al.*, 2013):

$$Z'(x_0) = \sum_{i=1}^n W_i Z(x_i) \quad (1)$$

where $Z'(x_0)$ is the nematode count at an unsampled location, W_i is the weight of each observed nematode count, and n is the number of close neighbouring points of observed nematode counts used in the

estimation of $Z'(x_0)$. The weights are proportional to the inverse of the squared distance between $Z'(x_0)$ and the neighbouring observed points, and they add up to 1 as in equation 2:

$$W_i = \frac{(1/d_i^2)}{\sum_{i=1}^n 1/d_i^2} \quad (2)$$

where d_i is the distance between the observed and estimated point. In the IDW, a maximum of 10 nearest neighbours were used since the land surface is nearly flat and the sampling was uniform in distance. A distance power of two was used. Kriging is a linear unbiased method in which attributes values at unsampled locations are predicted based on the hypothesis that the variogram depends on the distance between samples and not on the sample location. Kriging helps understand the spatial structure and autocorrelation in the data. In ordinary Kriging, the sample variogram is estimated and used to predict the value of the variable of interest at an unsampled location (Yao *et al.*, 2013):

$$(h) = \frac{1}{zn} \sum_{i=1}^n [Z(x_i) - Z(x_i + h)]^2 \quad (3)$$

where x_i and x_i+h are sampled locations separated by a distance h (lag) and $Z(x_i)$ and $Z(x_i+h)$ are the observed values of nematode counts at the corresponding locations. Based on the sample variogram, an appropriate variogram model is fitted and assessed using cross-validation parameters. Spatial autocorrelation was explored using the Global Moran's I statistic (Chen, 2013) in the Spatial Analyst toolbox (ESRI™). In computing the Global Moran's I, the Euclidean distance method and the inverse distance conceptualization were used (Chen, 2013).

There was no row standardization or weight matrix. The interpolated surfaces for the two sites were compared.

Results

Descriptive statistics of soil properties and nematodes

On the organic field, nitrogen content ranged from 0.09 to 1.43% and organic carbon ranged from 0.52 to 2.14% (Table 1). Phosphorus had a wide range over the study area (8.09–39.70 $\mu\text{g P soil}^{-1}$), with the highest standard deviation amongst the soil properties. The soil pH ranged from slightly acidic to neutral while the mean dry bulk density ranged from 1.19 to 1.52 g cm^{-3} . The water content also had a wide range with standard deviation greater than one. On the conventional field, the values of most of the soil properties were lower than those of the organic field except the dry bulk density (Table 2). The pH of the conventional field ranged from acidic to slightly acidic. The dry bulk density ranged from 1.32 to 1.73 g cm^{-3} . Again, phosphorus and water content showed the largest standard deviations.

Nematode counts ranged from 84 to 152 and 59 to 107 on the organic and conventional fields, respectively (Table 3). The mean nematode counts were 112 and 81, while the mode was 130 and 90 for the organic and conventional fields, respectively. Total nematode counts for the two fields were 2,120 and 1,622 for the organic and conventional fields respectively.

Spatial distribution of nematodes

On the organic field, the nematode population increases from south to north

TABLE 1
Soil properties at the organic field (n = 30)

<i>Soil property</i>	<i>Maximum</i>	<i>Minimum</i>	<i>Mean</i>	<i>Standard deviation</i>
Nitrogen (%)	1.43	0.09	0.17	0.10
Organic carbon (%)	2.14	0.52	1.33	0.50
Phosphorus ($\mu\text{g P g soil}^{-1}$)	39.70	8.09	20.50	7.42
Potassium (Cmolc kg^{-1})	1.59	0.70	1.06	0.33
pH	7.61	6.78	7.13	0.29
Dry bulk density (g cm^{-3})	1.52	1.19	1.31	0.13
Water content (%)	9.15	3.01	5.82	2.11

TABLE 2
Soil properties at the conventional field (n = 35)

<i>Soil property</i>	<i>Maximum</i>	<i>Minimum</i>	<i>Mean</i>	<i>Standard deviation</i>
Nitrogen (%)	0.42	0.05	0.17	0.38
Organic carbon (%)	1.26	0.81	0.94	0.27
Phosphorus ($\mu\text{g P g soil}^{-1}$)	23.67	15.44	16.72	3.58
Potassium (Cmolc kg^{-1})	0.86	0.08	0.38	0.11
pH	6.78	4.82	5.24	0.15
Dry bulk density (g cm^{-3})	1.73	1.32	1.52	0.13
Water content (%)	7.04	2.71	4.93	1.49

TABLE 3
Nematode counts at the organic and conventional crop fields.

<i>Sample site</i>	<i>Nematode Numbers</i>						
	<i>Minimum</i>	<i>Maximum</i>	<i>Mean</i>	<i>Median</i>	<i>Mode</i>	<i>Standard deviation</i>	<i>Sum</i>
Organic field	84	152	112	108	130	18	2,120
Conventional field	59	107	81	82	90	10	1,622

(Fig. 1). The largest nematode count is concentrated in a small patch in the north-west. Both IDW and kriging show a similar pattern of spatial distribution even though there were slight differences in the gradations. While the IDW image shows large distances between breakpoints in the gradient from north to south, the Kriging image shows gradual and small

breakpoints. Overall, the two interpolation methods did not seem to differ substantially in the pattern of spatial distribution of the nematode counts.

On the conventional field, however, nematode count did not show any particular pattern of spatial distribution. Large nematode counts were concentrated in small patches in the south-east and

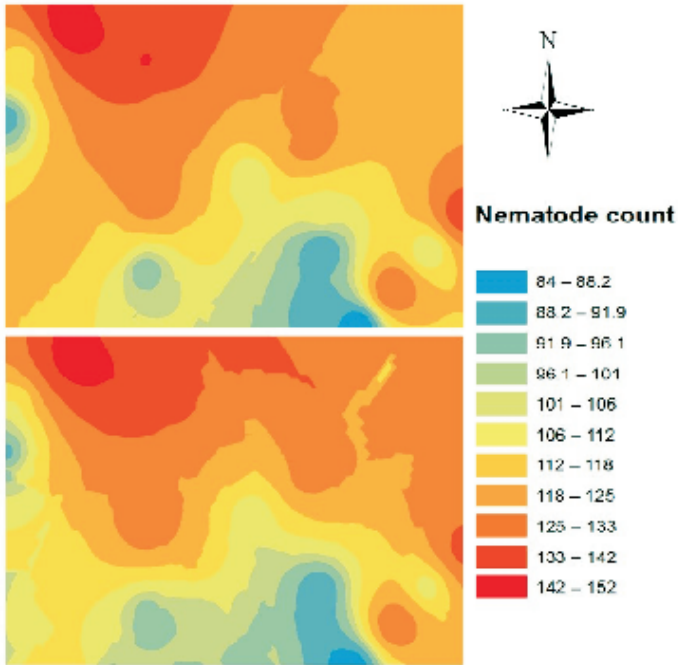


Fig. 1: Spatial distribution of nematodes on the organic field using IDW (top) and ordinary Kriging (bottom).

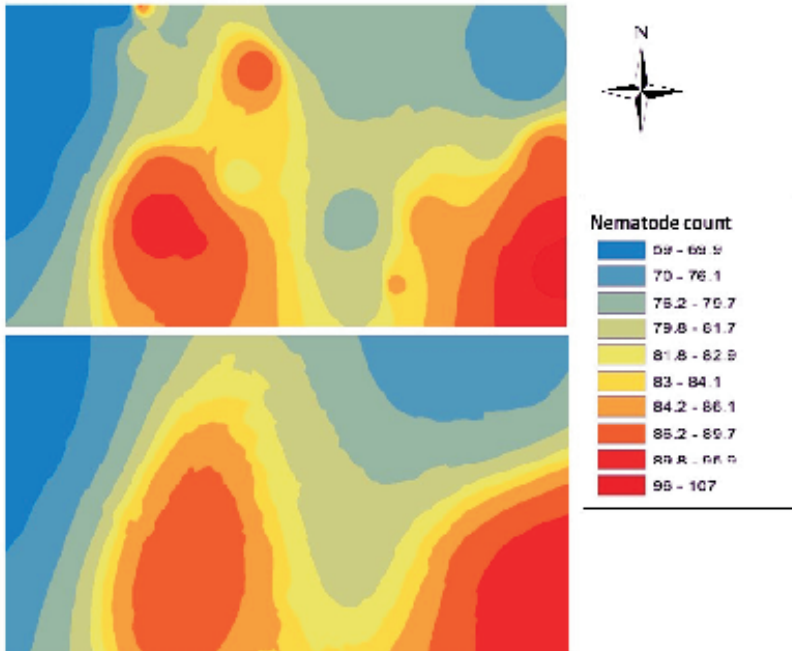


Fig. 2: Spatial distribution of nematodes on the conventional field using IDW (top) and ordinary Kriging (bottom).

south-central parts of the field (Fig. 2). In contrast to the IDW image which clearly shows patches of high population concentration of nematodes, the kriging image smooths out these small patches and rather shows two large patches of high population concentration.

Discussion

The nematode counts found on both sites are lower than those reported in other studies in Ghana and elsewhere. Okae-Anti *et al.* (2013) reported much higher populations of nematodes in a Haplic Lixisol cultivated to plantain in the Forest

Savannah Transitional Zone of Ghana, with the *Meloidogyne* spp. being dominant. Total nematode counts ranged from 326 (control plot) to 5602 per 100 g soil, with a mean of 623; the *Meloidogyne* spp. accounted for approximately 48%. Ohemeng (1978) found a total of 539 counts of nematodes per 100 g of soil (consisting of 13 genera) in 10 farms in the coastal savanna zone of Ghana.

The global Moran’s Indices and related z-scores indicated that the pattern in the spatial distribution of nematodes on both fields was not significantly different than random (illustrated with Fig. 3 for the

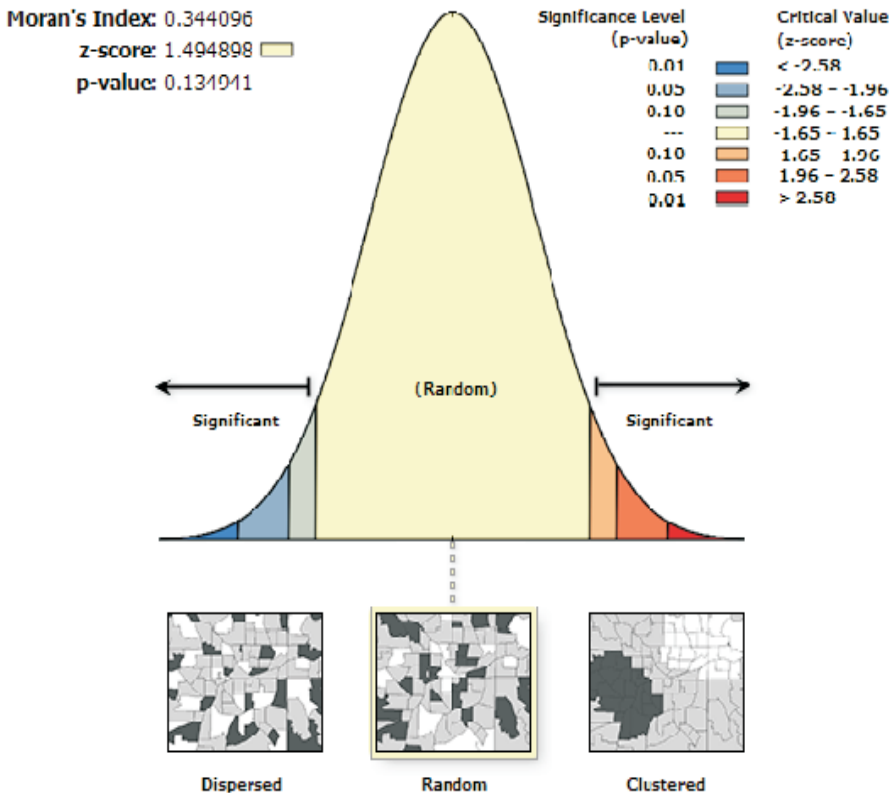


Fig. 3. Global Moran’s I of nematode distribution on the organic field

organic field). Thus, the spatial distribution of nematodes did not show significant spatial autocorrelation.

Between 2600 and 2300 nematode counts per 100 g soil has been reported for organic farms in Cairo (Adam *et al.*, 2013). In Ghana, economically important nematodes (in terms of population, distribution, and economic damage to crops) include the *Meloidogyne* spp., *Pratylenchus* spp., *Paratylenchus* spp. and *Helicotylenchus* spp (Okae-Anti *et al.*, 2013; Addoh, 1971; Ohemeng, 1978). The *Meloidogyne* spp. is the most destructive and prevalent, having over 150 host plants associated with it (Addoh, 1971). The *Meloidogyne* spp. are the commonest on the School of Agriculture Teaching and Research Farm, University of Cape Coast.

In the current study, nematode occurrence was 100% (found in all samples) for both fields. The slightly lower nematode counts found in the conventionally managed field might be due to the presence of nut-grass (*Cyperus rotundus*, during off-season) which is known to be resistant to nematodes (Ohemeng, 1978) and *Pennisetum purpureum* (elephant grass) which is reported to help control phyto-nematodes (Matsumoto *et al.*, 2002). Berkelmans *et al.* (2003) found higher population densities of plant-parasitic root lesion nematodes in organic fields than in conventional fields. Problems with nematode build up in organic farms can be challenging during the transition from conventional to organic farming (van Bruggena and Termorshuizen, 2003). These problems of nematode build up can also be realized within five to ten years

after conversion to organic farming (Hallman *et al.*, 2007).

While conditions prevalent in organic crop production sites might favour nematode build up, use of organic materials such as compost might contribute antagonistic substances to reduce the number of nematodes (Hallmann *et al.*, 2007). In the current study, organic crop production on the organic site started in 2009. Prior to this, the field was rarely used for crop production. The conventional field, on the other hand, has been subjected to cultivation for a long time for both research and non-research purposes. In the organic field, agronomic management practices that might control nematode population and activities include the application of compost, poultry manure, and extracts from neem leaf (*Azadirachta indica*), pawpaw leaf (*Karika papaya*) and the bark of mahogany (*Kaya senegalensis*). These extracts are believed to have antagonistic or nematicidal properties. In addition, marigold (*Tagetes* spp.) intercropping has been tried as a nematode control measure recently. Root exudate from marigold is reported to suppress several genera of plant parasitic nematodes (Wang *et al.*, 2007). It is possible that these have helped control adverse feeding activities of the nematodes on the crops grown on the organic field but have been less effective in lowering the nematode population below that of the conventional field. Adam *et al.* (2013) reported that the composition of plant-parasitic nematode populations and occurrence in organic farms were similar to those on conventionally managed crop fields under similar environmental

conditions in Egypt. The genera and the occurrence of *Meloidogyne* spp. found in organic farms in Egypt (Adams *et al.*, 2013) were also similar to those in organic farms in Germany (Hallmann *et al.*, 2007). This suggests that, under similar environmental conditions, the occurrence of nematodes in organic farms might not differ substantially from that of conventionally managed crop fields. On the contrary, the current study shows that the total nematode count in the organic field is significantly larger than that of the conventional field regardless of the similar environmental conditions.

An understanding of site-specific distribution of nematodes is crucial for effective management of nematodes. Nematode population mapping of the entire teaching and research farm of the University of Cape Coast has yet to be done. In the current study, the nematode distribution shows a gradient at the organic site. The north-south gradient of nematode counts in the organic field follows the gentle elevation gradient in the field. Otherwise, it is not clear what is accounting for the observed gradient in nematode counts as the nematode distribution on both fields was found to have insignificant spatial autocorrelation. Similarly, the pockets of large nematode densities observed in the conventional fields coincide with small depressions in the field. This probably suggests that water aids the mobility and/or concentration of nematodes at points of low elevation or depressions in the fields (Chen *et al.*, 2012). This requires further investigations. Further, the high bulk density of the conventional field might explain the

slightly lower nematode counts than the organic field (Chen *et al.*, 2012).

In the current study, the two interpolation methods used did not show substantial difference in predicting the spatial distribution of the nematodes. It was expected that Kriging would be better than IDW due to the problems associated with distance-based interpolation methods (Yao *et al.*, 2013). However, the problems arising from distances between unsampled and sampled locations can be more prominent in a complex, uneven terrain or under random sampling conditions (Yao *et al.*, 2013). The similarity in the interpolation methods in the current study might be due to the relatively small area of the field and evenly distributed sample points. In environmental sciences, ordinary Kriging and IDW are the most commonly and widely used interpolation methods and have been found to perform comparably in non-complex terrains (Li & Heap, 2011). Hence, either IDW or ordinary kriging can be adopted to predict spatial distribution of nematodes under conditions similar to those in the current study.

Conclusions

Plant parasitic nematodes pose huge challenges to crop production globally. The count, occurrence and spatial distribution of soil nematodes in organic and conventional crop fields were assessed and compared. The total count of nematodes in the organic field was substantially higher than that of the conventional crop field. It is possible that nematode build-up in the organic field could be much larger than the observed if some control measures were not adopted.

The spatial distribution of nematodes showed a north-south gradation in the organic field but large concentrations in small patches in the southern part of the conventional crop fields. This spatial distribution information can be used to focus management efforts while undertaking further investigation to understand the ecological conditions accounting for the observed distribution. The study shows that both ordinary kriging and inverse distance weighted interpolation methods can be used to predict the spatial distribution of nematodes on relatively flat or gently sloping fields, with evenly spaced sampling.

References

- Asamoah G. K.** (1973). Soils of the proposed farm site at University of Cape Coast. Soil Research Institute (CSIR) Technical Report No. 88.
- Adam M., Heuer H., Ramadan E. M., Hussein M. A. and Hallmann J.** (2013). Occurrence of plant-parasitic nematodes in organic farming in Egypt. *International Journal of Nematology* **23**: 82–90.
- Addoh P. G.** (1971). The distribution and economic importance of plant parasitic nematodes in Ghana. *Ghana Journal of Agricultural Science* **4**, 21–32.
- Berkelmans R., Ferris H., Tenuta M. and van Bruggen A. H. C.** (2003). Effects of long-term crop management on nematode trophic levels other than plant feeders disappear after 1 year of disruptive soil management. *Applied Soil Ecology* **23**: 223–235.
- Bird D. M. and Kaloshian I.** (2003). Are roots special? Nematodes have their say. *Physiological and Molecular Plant Pathology* **62**(2): 115–123.
- Burrough P. A.** (1991). Sampling designs for quantifying map unit composition. In SSSA Special Publication no. 28. Spatial variabilities of soils and landforms. (M. J. Mausbach and L. P. Wilding, eds.), pp. 89–125. Madison, WI: Soil Science Society of America.
- Chen Y.** (2013). New approaches for calculating Moran's Index of spatial autocorrelation. *PLoS ONE* **8**(7): e68336. oi:10.1371/journal.pone.0068336.
- Chen S. Y., Sheaffer C. C., Wyse D. L., Nickel P. and Kandel H.** (2012). Plant-parasitic nematode communities and their associations with soil factors in organically farmed fields in Minnesota. *Journal of Nematology* **44**(4): 361–369.
- Evans K., Webster R., Barker A., Halford P., Russell M., Stafford J. and Griffin S.** (1999). Mapping infestations of potato cyst nematodes and the potential for patch treatment with nematicides. In *Proceedings of the Second European Conference on Precision Agriculture*, Odense, Denmark, July 11–15, 1999, (J. V. Stafforred.) 505–515.
- Faithfull N. T.** (2002). *Methods in agricultural chemical analysis: a practical handbook*. CABI Publishing, Wallingford, UK.
- Freckman D. W.** (2000). Bacterivorous nematodes and organic matter decomposition. *Agriculture, Ecosystems and Environment* **24**: 195–217.
- Hallman J., Frankenberg A., Paffrath A. and Schmidt H. S.** (2007). Occurrence and importance of plant-parasitic nematodes in organic farming in Germany. *Nematology* **9**: 869–879.
- Klute A.** (1986) (Ed.). *Methods of soil analysis. Part 1. Physical and mineralogical methods*, 2nd edn. Agronomy monograph No. 9, American Society of Agronomy/Soil Science Society of America, Madison, Wisconsin.
- Li J. and Heap A. D.** (2011). A review of comparative studies of spatial interpolation methods in environmental sciences: Performance and impact factors. *Ecological Informatics* **6**: 228–241.
- Mario F. P., Erwin A. E. and Manuel C. P.** (2011). Soil properties influencing phytoparasitic nematode population on Chilean vineyards. *Chilean Journal of Agricultural Research* **71**(2): 240–248.
- Matsumoto M. N., Goulart A. M. C., Homechin M., Sakamoto M. M. Y. and Santiago D. C.** (2002). Mulching with *Pennisetum purpureum* and other cultural practices for management of *Meloidogyne javanica* on tomato under

- greenhouse conditions. *Nematologia Brasileira* **26**(1): 101–104.
- Nelson D. W. and Sommers L. E.** (1996). Total carbon, organic carbon and organic matter. In *Methods of soil analysis*. Part 3, chemical methods. (D. L. Sparks ed.). Soil Science Society of America Book Series No.5, pp. 961–1010.
- Ohemeng O. B.** (1978). Plant parasitic nematodes associated with maize in Ghana. 1. Population and host range studies of *Pratylenchus brachyurus*. *Ghana Journal of Agricultural Science* **11**: 81–88.
- Oka Y., Shapira N. and Fine P.** (2007). Control of root-knot nematodes in organic farming systems by organic amendments and soil solarisation. *Crop Protection* **26**(10): 1556–1565.
- Okae-Anti D., deGraft-Estison V., Adu-Bitherman P. and Idriss A. R. M.** (2013). Effect of inorganic fertilizers and palm bunch ash on nematode population dynamics and plantain yield in a haplic lixisol. *International Journal of Agricultural Policy and Research* **1**(8): 205–209.
- Van Bruggena A. H. C. and Termorshuizen A. J.** (2003). Integrated approaches to root disease management in organic farming systems. *Australian Plant Pathology* **32**(2): 141–156.
- Wang K. H., Hooks C. R. and Ploeg A.** (2007). Protecting crops from nematode pests: using marigold as an alternative to chemical nematicides. *Plant Disease* **35**: 1–6.
- Whitehead A. G. and Hemming J. R.** (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* **55**: 25–38.
- Wyse-Pester D. Y., Wiles L. J. and Westra P.* (2002). The potential for mapping nematode distributions for site-specific management. *Journal of Nematology* **34**: 80–87.
- Yao X., Fu B., Lu Y., Sun F., Wang S. and Min L.** (2013). Comparison of four spatial interpolation methods for estimating soil moisture in a complex terrain catchment. *PLoS ONE* **8**(1), e54660. Doi: 10.1371/journal.pone.0054660.
- Yeates G. W.** (2003). Nematodes as soil indicators: functional and biodiversity aspects. *Biological Fertility of Soils* **37**: 199–210.