Mathematical Techniques to Study the Spatial Pattern of *Meloidogynejavanica* in relation to soil fungi and soil characteristics in a tomato-grown field

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Abstract

Mathematical techniques are widely applied in landscape ecology to quantify the spatial patterns exhibited within species and its associated root fungi. Several analyses have been developed and modified to improve the ability to detect and characterize the patterns by the growth of species and population in various scale. Analysis of spatial pattern for disease and pathogen are of interest for the understanding and management plan to control the increase or spread within plant populations due to the numerous shared channels. Mathematical approaches encounter and give quantitative information of infections caused by many soil borne plant pathogens which are generally found in clusters of patches. Reliable approach for the use of spatial data can be established either by the research objective or by the measurement types and sampling designs procedures. We applied several mathematical tools to quantify the quantitative nature of Meloidogyne javanica and its associated soil fungi and soil characteristics in the development of nematode populations in the tomato grown field.

Keywords: Mathematical techniques, Nematodes populations, spatial pattern analysis, Canonical correspondence analysis.

Introduction

Plant parasitic nematodes are well known parasites worldwide and utilize all parts of the host, and affect virtually every crop within their domain. Root-knot nematode species (*Meloidogyne*), are the most important plant-parasitic nematodes, infecting a wide range of cultivated plants, and are responsible for billions of dollar crop losses annually[1,2]. Since nematodes (saprotrophs or parasites) are more abundant in the rhizosphere, it is not surprising that their natural enemies will also be more numerous in such a habitat. The fungal antagonists of nematodes consist of a wide array of organisms, which include the nematode-trapping or predaceous fungi, endoparasitic fungi, parasites of nematode eggs and fungi, which produce metabolites toxic to nematodes[3]. In view of the long co-evolution of nematodes and fungi, which obviously occurred in the close confines of the soil habitat, it is a great variety of interrelationships have developed between two groups.

There are two important factors whereby environmental forces impact nematode community structure i.e., the community composition of nematode[45], and land management practices[6], mineral fertilizers[7], and toxic substances such as pesticides[8], affect nematode community composition. Most of these factors also influence the survival and proliferation of other micro-inhabitants including fungi in the rhizosphere.

Plant-parasitic nematodes generally have aggregated spatial distribution in an area, such as an agricultural field, with frequency distributions generally described by negative binomial function[9,10]. Taylor's Power Law[11], which empirically expresses the relation between variance and mean, has also been used to measure the distribution and to develop sampling strategies for nematodes[12,13]. While the soil sampling strategy often required is a systematic sampling over large areas, the studies usually report average values of nematode densities for the entire area field[14]. In the absence of information on spatial variation, site-specific management strategies cannot be formulated. Vast expanses involved in obtaining and analyzing soil/nematode samples makes it imperative that techniques of assessing spatial variability of root-knot nematode populations be highly efficient. Increased efficiency can be attained through robust sampling designs or by relating root-knot nematode populations to readily measured properties, such as soil pH, soil texture and organic matter that are expected to exhibit some degree of spatial pattern in the field[15].

Mathematical techniques enable us to assess the quantitative relationship of population dynamics of pathogens and has key role for the development of simulation models. It is also used for the design of experimental research[16,17].

Mathematical approach of canonical correspondence analysis (CCA) is a relatively recent technique that exposes the joint structure of populations and the associated environmental data[18]. In terms of a linear model, CCA is based on a weighted multivariate regression of transformed species data on the covariable environmental data set. Our central goal is to determine the causes by which environmental factors within

human management practice change the community composition of nematode. In this regard, published data only reported control plot or laboratory experimentation. Assessing the quality of soil at various scales depends on several factors and community composition[19].

Present study applied to check the non-randomness and analyze the spatial pattern of root-knot disease incited by *Meloidogyne javanica* (the root-knot nematode) in tomato grown field, nematode populations (in soil and roots), as well as colonies of the colonization incidence of three soilborne root-infecting fungi, *Fusarium solani*, *Paecilomyces lilacinus* and *Aspergillus* spp. to observe the relationship among nematode population with fungus in the study area.

Material and Methods

A field plot located at Gharo (approximately 50 km from Karachi city) was selected for sampling. The field chosen for the study is a cultivated field where generally tomato (*Lycopersicon esculentum* Mill.) and guar (*Cyamopsis tetragonoloba* L.) are grown in rotation for the last 10 years. For the purpose of sampling, a 14.4 × 14.4 meter square grid matrix was designed in the field. The grid matrix was divided into hundred and forty-four 1.2 m × 1.2m contiguous grids (quadrats). Each grid was sampled for *Meloidogyne javanica* (Treub.) Chitwood and fungi occurs in the soil and the rhizosphere. A number of microfungi were recorded but the analysis was restricted to only the abundant ones in the soil or nematode eggs; these included *Fusarium solani*, *Paecilomyces lilacinus*, *and Aspergillus* spp. Each grid unit arranged three tomato plants in two rows each row separated 60 cm apart and plant-to-plants distances were kept approximately 40 cm. A steel corer of 13 mm diam. × 189 cm deep is used to collect the six replicate cores near to the roots of selected field plot. The replicate soil samples from each grid were pooled to obtain composite samples. We used different mathematical techniques to analyze our data obtained from the field plot. Data matrix is further divided in four classes for the simplicity to compare the results. In First class (C1), we have taken 1x1 matrix grid (1 block), i.e., 12 rows and 12 columns, total 144 grids. In second class (C2), designed by dividing the whole field plot in terms of 2 x 2 matrix grid (4 blocks), i.e., each matrix consists 6 rows and 6 columns, total 36 grids. Third class (C3), designed by dividing whole field plot in terms of 3x3 matrix grid (3 blocks), i.e., 4 rows and 4 columns, total 16 grids. Fourth class (C4), designed by dividing whole field plot into 4 x 4 matrix grid (4 blocks), i.e., 3 rows and 3 columns, total 9 grids (Table-1).

Mathematical Techniques: Aggregation indices: To analyze the spatial pattern of nematode population, rhizosphere and egg parasitic fungi and the associated soil characteristics, an index of pattern detection, which is not affected by the changes in density caused by random thinning, is required[20]. Therefore, aggregation indices including Lloyd's[21] index of patchiness (*c*) and Morisita[22] index (I_δ) were employed because they are

insensitive to changes in density. First mean crowding m* was calculated as:

$$m^* = \frac{1}{N} \sum_{i=1}^{Q} X_i (X_i - 1)$$ (1)

X_i represents nematodes soil population (or colonization frequency by the fungi) in the *i*th quadrate, whereas, number of quadrats is denoted by Q, with $N = \sum X_i$. While $c = \frac{m}{\lambda}$, such that λ is almost equals to average density in each quadrat. Indices for Lloyd's and Morisita's were determined by the formula:

$$I_d = \frac{Q \sum X_i (X_i - 1)}{N(N-1)}$$ (2)

Morisita's index 1indicate the random distribution whereas, greater than 1 indicate for aggregated pattern of root-knot nematode soil population. The variance/mean ratio[23] for each variable (dependent and independent) was applied to measure the non-randomness. Indices for Lloyd's and Morisita's were also calculated in different blocks such as 1 × 1, 2 × 2, 3 × 3 and 4 × 4 simultaneously.

Spatial Autocorrelation: We applied spatial autocorrelation method by using Moran's[24] *I* statistics for continuous variables as opposed to binary variables. As this technique is closely associated to the spatial autocorrelation coefficient. Therefore, a strong positive spatial autocorrelations may determine if Moran's *I* gives a relatively larger value. We can depict spatial information by using Moran's *I* test for content of the field sampling by utilizing information on the location of each sample point[25]. Thus, spatial autocorrelation was employed to determine autocorrelation of the nematode, soil-borne fungi and some soil factors among the quadrates. The statistic was computed as follows:

$$I = \frac{\left(\frac{1}{W}\right) \sum_{i=1}^{Q} \sum_{j=1}^{Q} [W_{ij}(x_i - x_m)(x_j - x_m)]}{\left[\frac{1}{Q} \sum_{i=1}^{Q} (x_i - x_m)^2\right]}$$ (3)

X_m represent the mean, W_{ij} is the weight assigned to the join between quadrats *i* and *j*, and *W* is the sum of all weights. There is no trend detected if Moran's *I* statistic is approximately equal to zero in the spatial pattern. Spatial autocorrelation is tested against the null hypothesis that sample *I* do not differ from the expected value:

$$E(I) = \frac{-1}{Q-1}$$ (4)

Randomization of null hypothesis is used to check the significance of Moran's *I* whereas, a wide range of pattern

detection indices techniques were used in research because they measure slightly different aspects of pattern[23].

Canonical Correspondence Analysis: The canonical correspondence analysis (CCA)[26] is employed here to expose the correlation structure between the species data set (nematode and fungi) and the environmental data set (i.e., soil variables). CCA has been widely used in community ecology to unravel the interrelationships between species populations and environmental characteristics. In CCA, nematode and fungal species and associated environmental factors are arranged in multidimensional space, with the restriction that the ordination axes must be linear combinations of the specified environmental variables. CANOCO, version 4.5[27] was used to accomplish canonical correspondence analysis. Data on the abundance of fungal species were log-transformed to dampen the influence of dominant species.

Results and Discussion

Field plot design for analysis are given in Table-I. Results of Lloyd's index (m[*]), Morisita's index (I_δ), variance mean ratio and Moran's index for autocorrelation for number of root-knot nematode eggs root and juvenile population density in soil, egg parasitism by *F. solani*, *P. lilacinus* and *Aspergillus* spp are presented in Table-II. Generally, mean crowding (the clumping together of individuals within a quadrat) for nematode eggs per root system increased with the increase in block size. By contrast, mean crowding of nematode juveniles, egg parasitism by fungi and rhizosphere population of fungi decreased if we increased the size of blocks (quadrats). In most cases, variances of Morisita's index were found relatively low as compare to the variance of Lloyd's index. The variance/mean ratios were much higher than unity in every instance, implying aggregation of root-knot nematodes and associated soil-borne fungi. The variances of Lloyd's index for different organisms, in general, increased with increasing of block size. Morisita's index for *F. solani* and *Aspergillus* spp. generally increased with increasing block size but for other organisms it varied. Moran's *I* statistic for spatial autocorrelation was found significantly greater than the expected value *E(I)* for the nematode population in soil (p<0.05), and rhizosphere colonization by *F. solani* (p<0.001) and *P.lilacinus* (p<0.01) and egg-parasitism by fungi (p<0.001). In comparison of the results of Lloyd's and Morisita's index, the characteristics of mean crowding values found to be independent of block size (quadrat). In this regard, if the value of Morisita's index varies with the increase or decrease of block size may reflect the scale and pattern intensity. However, index value larger than 1 (expected value for random distribution) in Morisita's index showed significantly at various block sizes with the exception of block size 4 × 4 for *P. lilacinus* (p<.001).

The frequency of eggs produced by *M. javanica* was significantly positively correlated at small block sizes i.e., 1 × 1 (p<0.001) and 2 × 2 (p<0.01). The rhizosphere colonization

frequency of the fungi (*F. solani*, *P.lilacinus* and *Aspergillus* spp.) showed negative correlation with number of eggs produced by *M. javanica* intensity at all block sizes. However, total numbers of eggs were found correlated with parasitism by fungi only at smaller block sizes (1 x 1 and 2 x 2).

A perusal of CCA ordination discloses that the quadrats (grid units) are distributed in almost the entire ordination plane but they exhibit clustering at low values of the first CCA axis and low to middle values of the second canonical axis (figure-1). These quadrats generally belong to the lower left side of the sampled grid. The total variation of species composition data set was 0.193, and sum of all canonical eigenvalues (total explained variation) was 0.174 (table-3). The Monte Carlo permutation test (499 permutations) showed that a significant relationship between quadrat to quadrat variation in fungal and nematode populations and the environmental (soil) variables (F=1.815, P=0.0220). The first two CCA axes were also significant based on the Monte Carlo permutation test with 499 permutations. For the first canonical axis the eigenvalues was 0.097 corresponding to second axis was 0.042 (p<0.01) (table-4).

Discussion: Our results showed lowest I_δ value or small plot size (4 x 4) and greater values in plot size (3 x 3). In this regard sampling was consider more reliable if I_δ value could be minimized[22], and selection of appropriate plot size for sampling could have significant bearing on sampling efficiency[17]. Lowest I_δ value in block size (4 x 4) elaborate that highest sampling precision can be achieved. The sampling intensity must be greater enough to measure the populations so that the management decisions can be made at an acceptable level of risk[14]. Not surprisingly, the frequency of eggs produced by *M. javanica* was positively correlated (p<0.001) with nematode densities in the soil, particularly at larger block sizes i.e., 1 × 1 and 2 × 2. However, such relationships were not recorded at smaller block sizes i.e., 3 × 3 and 4 × 4. The colonization frequency of the fungi (*F. solani*, *P.lilacinus* and *Aspergillus* spp.) indicates significantly positive correlation with egg masses intensity relatively (3 x 3) or (4 x 4) blocks reflecting their interaction at a small spatial scale.

Relationship among root-knot nematode and their associated root-infecting fungi is in the cultivation field is very familiar and often ignored to measure their interaction at different scales. After analyzing two data sets, the spatial pattern of nematode population has found to be direct effects of management practices. Ordination of sites and genera for each dataset were performed separately most of them influence in structuring nematode populations, i.e., soil moisture, and pH[28]. Environmental class variables (i.e., Soil moisture, Organic matter, pH, and calcium carbonate, Nitrate, Phosphorus and Potassium) were coded as nominal variables. Log transformation were applied to normalize the data of nematode abundance prior to application of CCA[29].

Table-1
Arrangements of field plot of the study area, data were arranged in four classes before the analyses.

Class 1 (C1)	Class 2 (C2)	Class 3 (C3)	Class 4 (C4)
1 x 1	2 x 2	3 x 3	4 x 4
144 grids	36 grids	16 grids	9 grids
12R x 12C	6R x 6C	4R x 4C	3R x 3C

Table-2
Jackknife estimates of Lloyd's index, Morisita's index and Moran's index, variance-mean ratio (V:M) with their autocorrelation for nematode and fungi in soil population

Variables	Blocks	Mean	V:M	Lloyd's index		Morisita's index		Moran's index	
				value	variance	value	variance	I	P(I = E(I))
No. of eggs	1 x 1	74311	30527	104857	3.2	1.410	0.0030	0.005	n.s.
	2 x 2	-	43874	119349	36.2	1..594	0.030	-	-
	3 x 3	-	30581	145894	58.3	1..275	0.015	-	-
	4 x 4	-	29610	166702	111.9	1.234	0.016	-	-
Nematode soil population	1 x 1	4313	2266	6563.2	5570	1.521	2.97	0.014	<0.05
	2 x 2	-	1846	6154.3	2871	1.411	7.54	-	-
	3 x 3	-	2030	5444.0	1096	1.549	3.28	-	-
	4 x 4	-	1415	5117.0	6660	1.325	1.97	-	-
Fusarium solani	1 x 1	27.6	95.02	122.64	299.7	4.38	0.357	0.051	<0.001
	2 x 2	-	74.28	97.32	425.1	4.52	0.683	-	-
	3 x 3	-	72.67	109.39	3118	6.80	5.157	-	-
	4 x 4	-	29.38	50.43	772.3	6.10	4.105	-	-
Paecilomyces lilacinus	1 x 1	4.08	8.76	11.91	1.73	2.908	0.037	0.031	<0.01
	2 x 2	-	7.09	9.84	11.5	3.102	0.465	-	-
	3 x 3	-	1.383	2..25	1.85	1.50	0.550	-	-
	4 x 4	-	0.252	0.607	0.09	0.483	0.043	-	-
Aspergillus niger	1 x 1	27.6	95.02	122.64	299	4.388	0.357	0.007	n.s.
	2 x 2	-	74.28	97.32	425	4.523	0.683	-	-
	3 x 3	-	17.45	72.67	109	3118	6.80	-	-
	4 x 4	-	29.38	50.43	772	6.10	4.105	-	-
Egg parasitism	1 x 1	10.9	4.68	14.618	0.91	1.334	2.17	0.039	<0.001
	2 x 2	-	3.20	11.668	1.53	1.225	3.54	-	-
	3 x 3	-	2.04	8.3210	1.55	1.133	7.12	-	-
	4 x 4	-	1.39	6.8360	1.29	1.0534	4.08	-	-

Responses of nematode populations to indirect effects were estimated using partial CCA[26] ordinations. CCA biplots indicate relative importance of a vector is its length; the angle indicates correlation with other vectors and CCA axes and triplot from a CCA of the root-knot nematode and soil fungi of tomato egg plants are shown in figure-2.

Environmental variables are represented by Eigenvalues for CCA axes indicate the importance of the axes in explaining relationships in the genera-environment data matrices. The total variation of species composition dataset was 0.193, and sum of all canonical Eigenvalues (total explained variation) was 0.174 (i.e., 17.4% of variation in species distribution was explained by the seven (environmental) variables.

However, results of canonical correlations and corresponding Eigenvalues shows the cumulative percentage of variance of species-environmental relations for first axis was 56.2% and for the first two axes nearly 80.1% are indicating high degree of environmental variables control of nematode and fungi populations. Significance of canonical correlation was verified by Monte Carlo test and showed that a significant relationship between quadrat to quadrat variation in fungal and nematode populations and the environmental (soil) variable at $P=0.022$. The first two CCA axes were also found to be significant, the first canonical axis the Eigenvalues was 0.097 corresponding to second axis was 0.042 ($p < 0.01$). The F-ratio for the first canonical axis (Eigenvalues=0.097) was 1.024 ($p=0.004$) while the F-ratio for all canonical axes (all Eigenvalues, trace=0.174) was 1.815 ($p=0.022$).

Conclusion

Plant parasitic nematodes (*Meloidogyne javanica*) largely damaged vegetation of cultivation field used in traditional method to design the plot. However, our mathematical technique to design the field in terms of matrix model in various block sizes (i.e., 1 x 1, 2 x 2, 3 x 3 and 4 x 4) before the vegetation of crops give more reliable results in the prevention of root-knot nematode disease. Moreover, our study also suggested that in small block sizes, disease frequency of root-knot nematode in tomato plants found significantly low, at the same time nematode were controlled by their root infected fungi (*Paecilomyces lilacinus*) as a biocontrol in small blocks.

Table-3
Results of canonical correspondence analysis. Eigenvalues, species-environmental correlations, cumulative percentage variance of species data and cumulative percentage variance of species-environment relations

Axes	1	2	3	4	Total inertia
Eigenvalues	0.097	0.042	0.018	0.016	0.193
Species-environmental correlation	1.000	0.999	0.988	0.997	
Cumulative percentage variance of species data	50.6	72.1	81.3	89.8	
Cumulative percentage variance of species-environmental relation	56.2	80.1	90.3	99.7	
Sum of all Eigenvalues	0.193				
Sum of all canonical eigenvalues	0.174				

Table-4
Monte Carlo permutation test, test for significance of first canonical axis

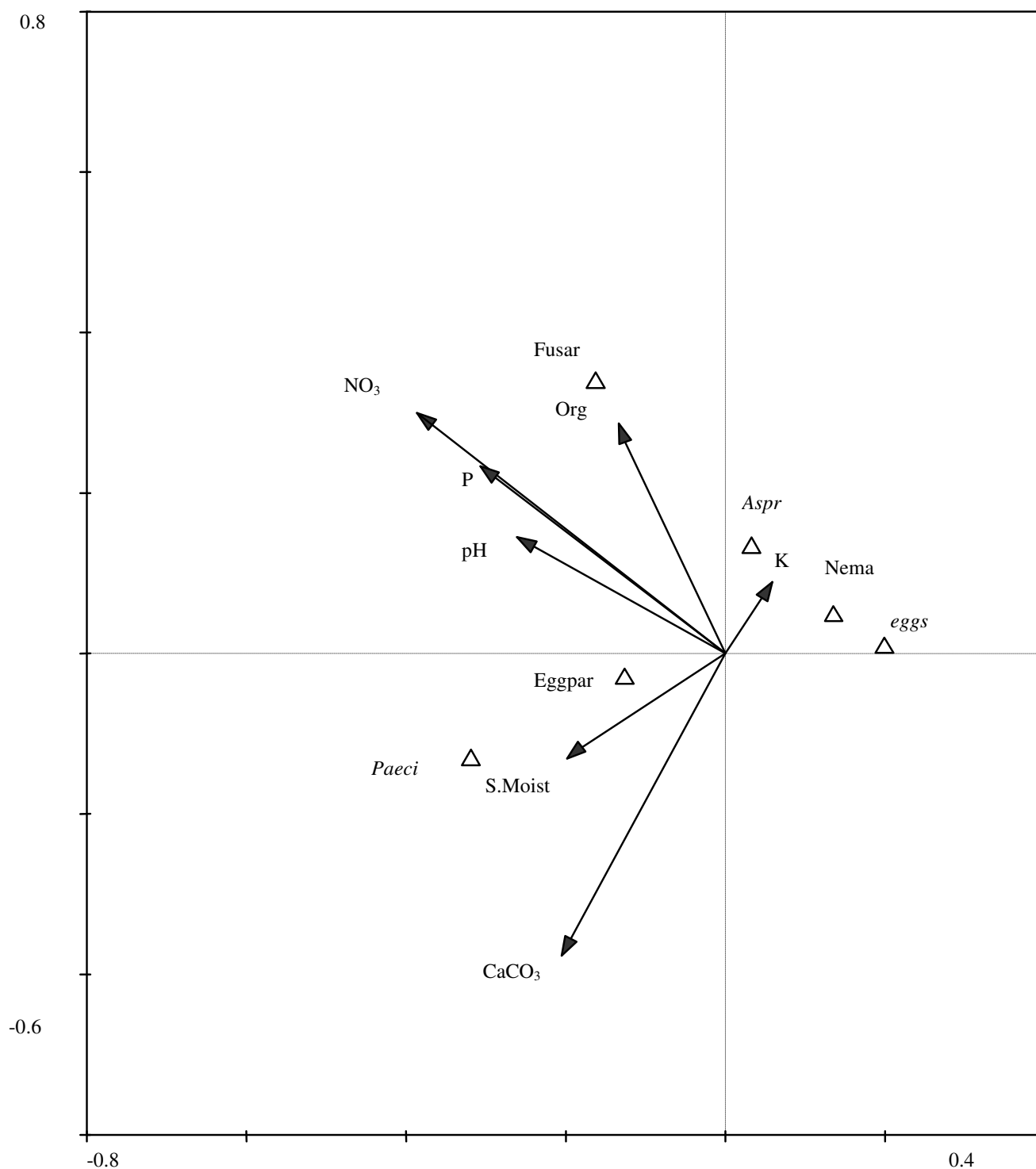| **Eigenvalues**	**0.097**
F-ratio	1.024
P-value	0.004
Trace	0.174
F-ratio	1.815
P-value	0.022

Figure-1
CCA-ordination diagram of the sampling plots with biological (Δ) and environmental variables (arrows); first axis is horizontal, second axis vertical. The biological variables including root-knot nematode and fungi. The fungi are: A. spp. = Aspergillus spp, Paeci = Paecilomyces lilacinus, Fusar = Fusarium solani. And nema = nematode soil population, eggs = number of eggs, eggpar = egg parasitism
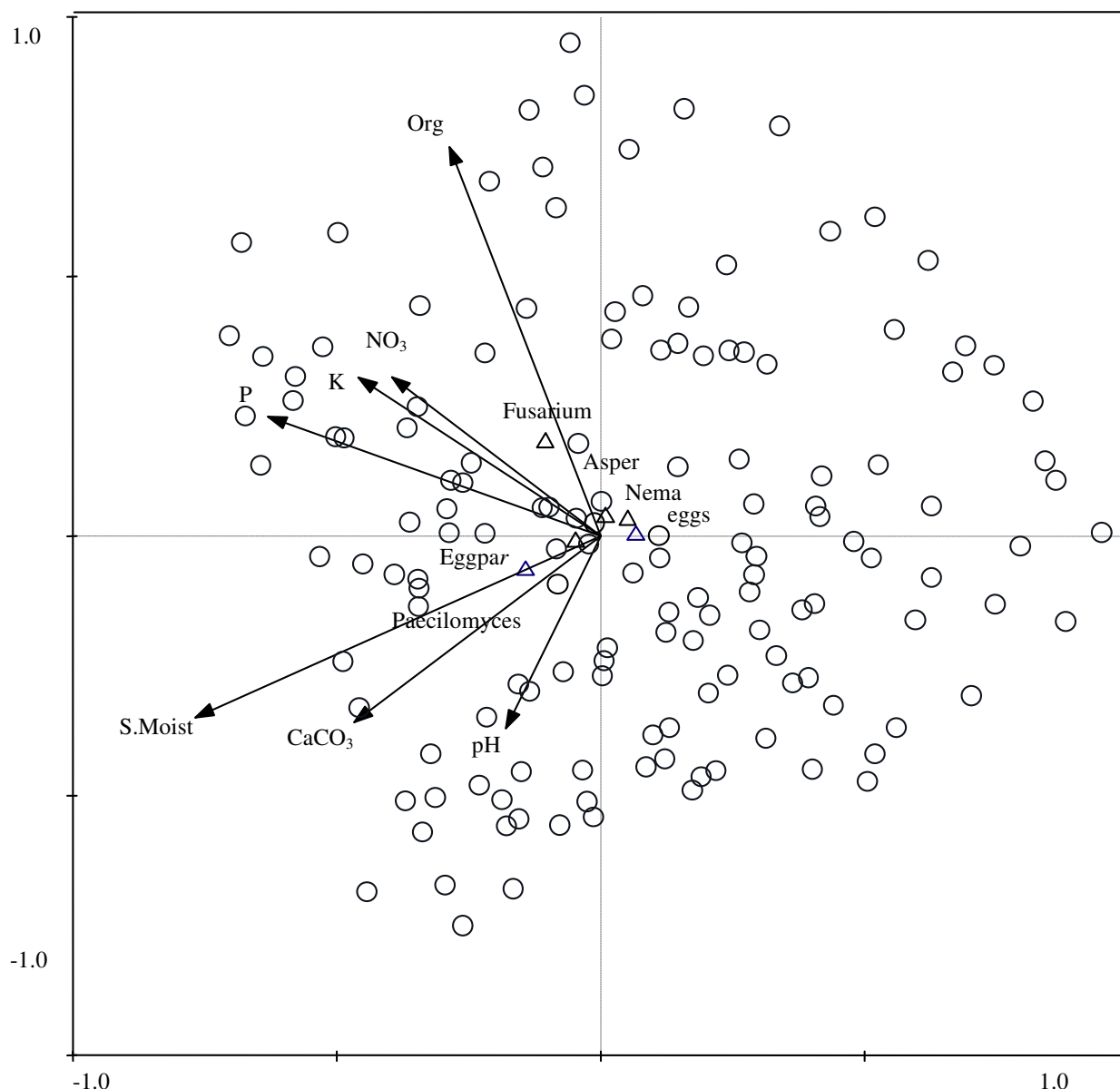
Figure-2
Triplot from a CCA of the root-knot nematode and soil fungi of tomato egg plants. Environmental variables are represented by arrows, samples (quadrats) by small open circles, and species by triangle (Δ). The species are listed by the initial letters of the genus and the specific epithet, they include: A. fumig. = Aspergillus spp., Paeci = Paecilomyces lilacinus, Fusar = Fusarium solani. And nema = nematode soil population, eggs = number of eggs, eggpar = egg parasitism

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