THE USE OF PERMUTATION TESTS IN CO-INERTIA ANALYSIS: APPLICATION TO THE STUDY OF NEMATODE-SOIL RELATIONSHIPS

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Abstract

The relationships between the nematode populations parasitizing two cultivars of yam (*Dioscorea cayenensis-rotundata* and *D. alata*) and several physico-chemical soil parameters in Martinique (FWI) were studied using the co-inertia analysis method. Permutation tests were then applied to assess the strength of the detected relationships. Results show that they are significant for both cultivars. For *D. cayenensis-rotundata*, the probability of making an error in rejecting H_0 (no relationship between soil parameters and nematode species composition) was found to be extremely low ($P < 10^{-5}$). For *D. alata* about 0.3% of random permutations gave a value of the total inertia criterion higher than the experimental one ($P \sim 0.3\ 10^{-2}$); the test is thus still significant, but the significance is much lower.

This difference between the cultivars is explained by the fact that the *D. cayenensis-rotundata* yam cultivar is mainly infested by *Pratylenchus coffeae*, an endemic nematode which occurence is linked to the soil characteristics. Indeed, *P. coffeae* is mostly found in soils with higher levels of organic matter, and its abundance is influenced by pH, and sodium rate.

The *D. alata* cultivar is infested by *P. coffeae* or *Scutellonema bradys*, an exotic nematode species. *S. bradys* and *P. coffeae* are extremely rarely found together in the same tuber. In this case, the relationship with the soil parameters is still significant because of the infestation by *P. coffeae*, and other species, but it is weakened by *S. bradys* infestation, which do not show relationships with the soil parameters, as it is exclusively introduced by the seed tuber.

1. Introduction

Nematodes have bio-morphological characteristics which make them different from other parasites : they are little mobile and they spend a part of their life history in the soil. For these organisms, as for other parasites, the host (the plant) is obviously a necessary condition determining their existence, but it is not a sufficient condition. For nematodes, the soil environment must allow the life cycle part belonging to it to take place. Subsequently, it is interesting to investigate the soil abiotic factors influencing the nematode development. Co-inertia analysis is a multivariate method well adapted to identify the species-environment relationships. But the statistical significance of the relationships unveilled by this technique must be assessed. Classical inference tests cannot be used here because the assumptions on which they are based (normality of the distributions and homocedasticity) are not met. Conversely, distribution-free methods like Monte-Carlo tests are very convenient, and their application to co-inertia analysis is very easy.

2 Material and methods

Observations were made in Martinique (French West Indies). Over a hundred yam fields were sampled approximately during the appearance of the neo-tubers. About half of them are cultivated with the cultivar *Dioscorea cayenensis-rotundata* and the second half with *Dioscorea alata*. In each field, about 1 dm^3 soil, 30 g roots, and 10-20 g of neo-tuber surface skin were collected on 3 to 5 plants along a transect. Each soil sample is subdivided in two subsamples, one for the pedology study, and one for nematode study.

Nine nematode species were observed, among which three were found both in soil and plant tissues (roots or tuber) : *Scutellonema bradys* (in the soil : Scus; in the roots : Scur), *Pratylenchus coffeae* (in the soil : Pras; in the roots : Prar), *Meloidogyne spp.* (in the soil : Mels; in the roots : Melr), *Rotylenchulus reniformis* (Roty), *Helicotylenchus erythrinae* (Heli), *Criconemella spp.* (Cric), *Hemicriconemoïdes cocophilus* (Hemi), *Xiphinema setariae* (Xiph), *Aorolaimus luci* (Aoro). The analyses performed on the soil samples are : carbon (C), nitrogen (N), organic matter (M.O), pH (water : pHO and KCl : pHK), soil granulometry (clay : Arg; fine silt : LiF; coarse silt : LiG; fine sand : SaF; coarse sand : SaG), total posphorus (P2O), exchange capacity (CEC), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), saturation coefficient (V%), wilting points (2.5 : pF2; 3.0 : pF3; 4.2 : pF4).

2.1 Co-inertia analysis

Co-inertia analysis (Chessel & Mercier, 1993; Dolédec & Chessel 1994) is a multivariate analysis technique that belongs to the "data coupling" approach, enabling two data tables to be analysed simultaneously. Numerous methods have been suggested to analyse such data sets (e.g., Canonical Correspondence Analysis, ter Braak, 1986; or the PLS regression, Höskuldsson, 1988), but one of the simplest from the theoretical point of view is the co-inertia analysis. In the fields of agronomy and ecology, the two tables often correspond to a table of environmental data and a floro-faunistic table (Cadet et al., 1994). The geometrical interpretation of co-inertia analysis is simple. Classical methods like principal components analysis (PCA), correspondence analysis (CA) or multiple correspondence analysis (MCA) aim at summarizing a table by searching orthogonal axes on which the projection of the sampling points (rows of the table) have the highest possible variance. This characteristic ensures that the associated graphs (factor planes) will best represent the initial results. To extract information common to both tables canonical analysis (Gittins, 1985) searches successive pairs t_i and u_i with a maximum correlation. By using the covariance instead of the correlation, the co-inertia analysis maximizes the product of the correlation by the projected variances on axes t_i and u_i. This ensures that the co-inertia axes will both have a good correlation between one other and correspond to a significant part of the variance in each of the two tables: $\mathrm{cov}(t_i, u_i) = \mathrm{cor}(t_i, u_i)\sqrt{\mathrm{var}(t_i)\mathrm{var}(u_i)}$

2.2 Permutation tests

The principle of the Monte-Carlo tests used in co-inertia analysis is based on the permutation of the rows of each tables. If a relationship exists between the rows of the tables, then the permutation will cancel this relationship. The criterion computed for each permutation is the total inertia (i.e., the sum of all the eigenvalues of the co-inertia analysis, that is the sum of the covariances between successive pairs of axes). The distribution of the criterion is plotted and the observed value (without permutation) is compared to this distribution. The significance of the test is given directly by the frequency of the permutations leading to a value of the criterion higher than the observed one.

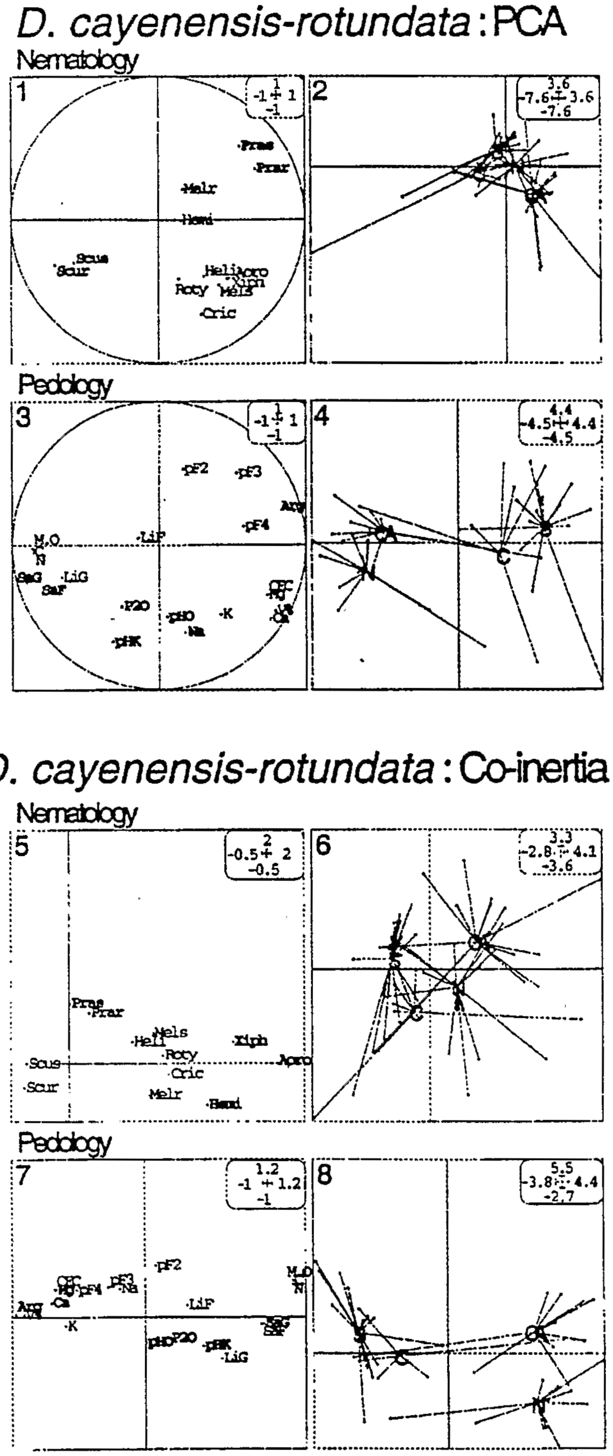
3. Results

3.1 D. cayenensis-rotundata : PCA

The first factor map of the PCA (Fig. 1.1) on the nematological data shows that *Scutellonema* is placed in the left part of the map, while all other species appear in the right part. The second axis opposes *Pratylenchus* to most other ectoparasite species.

Almost all samples are placed in the center or right part of the map (Fig. 1.2) because they are mainly infested by *Pratylenchus*, particularly in the north (N) and south (S). Those of center (C) can be infested occasionally by *Scutellonema*, and the atlantic center ones (CA) have less *Pratylenchus* and large ectoparasites populations.

Figure 1: First factor maps of the PCA (top) and Co-inertia analysis (bottom) for *D. cayenensis-rotundata* data sets. 1.1 and 1.2: species map and fields maps of the PCA on *D. cayenensis-rotundata* abundance. 1.3 and 1.4: pedological variables and fields maps of the PCA on soil physico-chemical parameters. 1.5 and 1.6: species map and fields maps of the Co-inertia analysis on *D. cayenensis-rotundata* abundance. 1.7 and 1.8: pedological variables and fields maps of the Co-inertia analysis on soil physico-chemical parameters.



The first factor map of the PCA (Fig. 1.3) on the pedological data shows that the first axis corresponds to a granulometry and organic matter gradient. The second axis is mostly linked to pH, sodium and phosphorus rates.

The samples taken in the south (S) and center (C) of the island are in the right part of the map because they come from soils with a high clay content (Fig. 1.4). Conversely, the samples from atlantic center (CA) and north (N) are in the right part because the soil is more sandy and richer in organic matter.

3.2 D. cayenensis-rotundata : Co-inertia

The factor map of the co-inertia analysis (Fig. 1.5) is not very different from that of the PCA. The main difference is the fact that *Aorolaimus*, *Xiphinema* and *Hemicriconemoïdes* have moved to the right, due to their association with sandy and organic matter rich soils. For the second axis, the most important species is *Pratylenchus*. Its presence in the soil is linked to a relatively higher exchange capacity (high rate of sodium, magnesium and calcium) and lower pH (Fig. 1.6).

The co-inertia analysis reverses the first axis, which restores the granulometry clay-sand gradient, but it also takes into account the chemical factors : calcium, magnesium, pH and phosphorus (Fig. 1.7). It increases the correlation between organic matter and the second axis. The north and atlantic center samples present a high level of organic matter and sand, while the south and center samples have a high level of clay content (Fig. 1.8).

3.3 *D. cayenensis-rotundata* : Permutation tests

Among the 100 000 permutations, none gave a total inertia greater than the one obtained on the original (not permuted) data set (Fig. 2). The distribution of the values of the criterion is very acute and the observed value is very far from the right tail of the distribution.

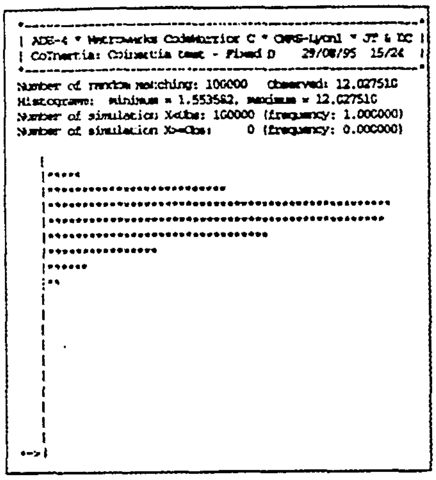
3.4 *D. alata* : PCA

The F1×F2 map (Fig. 3.1) shows that *Scutellonema* is opposed to *Meloidogyne* and most ectoparasites on the first axis. On the second axis, *Scutellonema* is opposed to *Pratylenchus* and *Aorolaimus*.

No structure appears according to the geographical position of the field (Fig. 3.2) : points in the fourth quadrant have higher *Scutellonema* infestation rates than those in the second one, but they may come from any part of the island.

Figure 2: Results of the permutation test of the co-inertia analysis for *D. cayenensis-rotundata* data sets (see text).

D. cayenensis-rotundata : Permutation tests



The first axis of the soil variables map (Fig. 3.3) also corresponds to a granulometry gradient, and the second one to a gradient linked to the rate of exchangeable bases, potassium and organic matter.

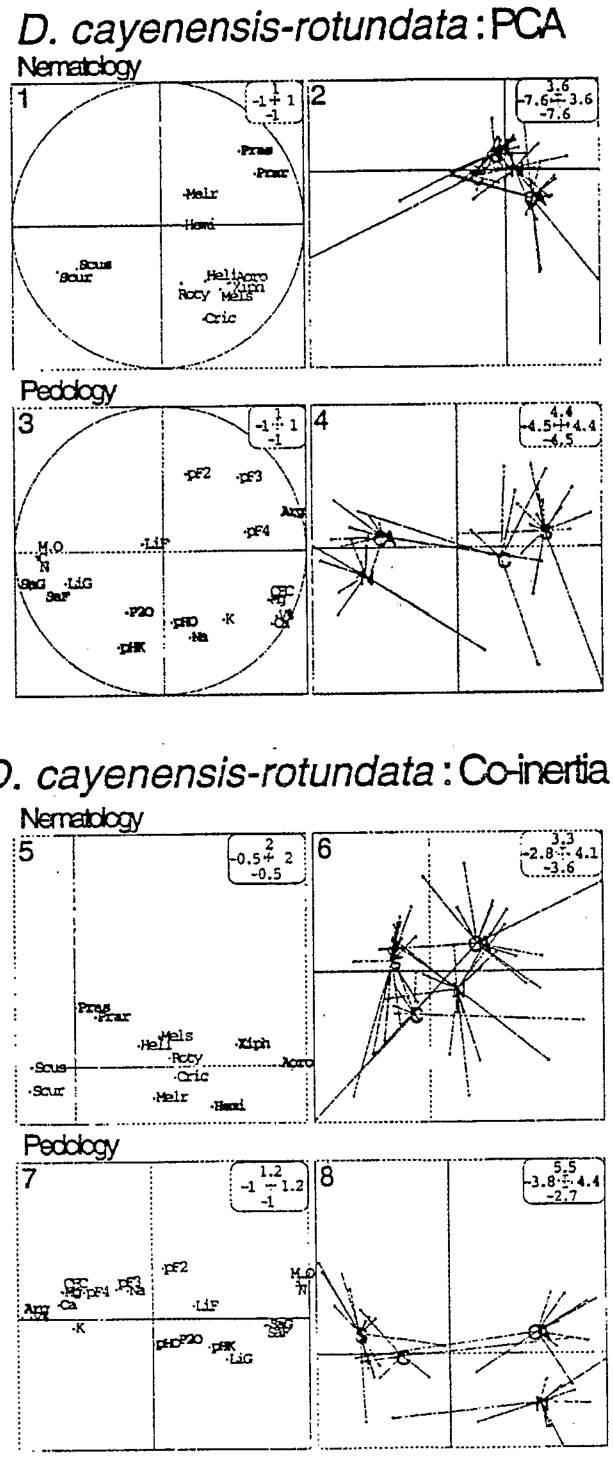
On the first factor map (Fig. 3.4), the samples are ordered from left to right according to their north-south position : the more sandy soils are situated in the north of the island.

3.5 *D. alata* : Co-inertia

The factor map of the co-inertia analysis on nematological data is different (Fig. 3.5) : all the nematode species have new positions. The importance of *Scutellonema* collapses as it moves near the origin, and the importance of *Pratylenchus* (in roots) increases largely as this species is dependent on soil factors (soil with organic matter). The abundance of *Pratylenchus* in the soil is linked to lower pH, lower calcium and magnesium rate and higher sodium rate). *Aorolaimus* is also found in sandy soils, but with a high level of organic matter and phophorus. Thus, no relationship is found between *Scutellonema* and the soil characteristics (Fig. 3.6).

The factor map of pedological data in co-inertia analysis (Fig. 3.7) reflects the same granulometry gradient. The wilting points (pF) have a lower importance and move near the origin. The organic matter migrates to the positive values of the second axis to underline the importance of the relationship with *Aorolaimus*. Since the co-inertia analysis keeps the granulometry gradient on the first axis, the factor maps of fields (Fig. 3.7 and 3.8) are very close to those obtained with PCA.

Figure 3: First factor maps of the PCA (top) and Co-inertia analysis (bottom) for *D. alata* data sets. 1.1 and 1.2: species map and fields maps of the PCA on *D. alata* abundance. 1.3 and 1.4: pedological variables and fields maps of the PCA on soil physico-chemical parameters. 1.5 and 1.6: species map and fields maps of the Co-inertia analysis on *D. alata* abundance. 1.7 and 1.8: pedological variables and fields maps of the Co-inertia analysis on soil physico-chemical parameters.

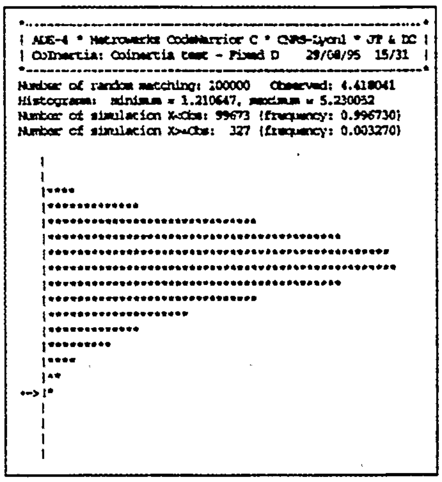


3.6 *D. alata* : Permutation tests

Among the 100 000 permutations, 327 gave a total inertia greater than the one obtained on the original (not permuted) data set (Fig.4). The distribution of the values of the criterion is wider and the observed value is within the right end of the distribution tail.

Figure 4: Results of the permutation test of the co-inertia analysis for *D. alata* data sets (see text).

D. alata: Permutation tests



4. Discussion and conclusion

The results of the co-inertia analysis show that some relationships exist between the nematode populations infesting the yam fields and the physico-chemical parameters of the soil in these fields. These relationships mainly concern the granulometry and the organic matter content, as well as chemical compounds such as sodium, calcium and phosphorus. The nematode species showing a response to these parameters are *Pratylenchus coffeae*, *Aorolaimus luci*, *Xiphinema setariae* and *Hemicriconemoïdes cocophilus*.

However, the strength of these relationships must be assessed in order to compare them between the two yam cultivars. The permutation tests allowed us to show that they are stronger for *D. cayenensis-rotundata* than for *D. alata*. The sum of the covariances between nematode species and soil parameters is equal to 12.03 for *D. cayenensis-rotundata* and to 4.42 for *D. alata*. With 100 000 permutations, none gave a sum of the covariances higher than the one obtained for *D. cayenensis-rotundata*, while 327 were higher for *D. alata*.

A biological hypothesis can explain this mathematical result. The two major nematode species, *P. coffeae* and *S. bradys* have different behaviours : *S. bradys* is an exotic species that does not exist in the fields in Martinique, and that is exclusively introduced with the seed tubers when planting. It is therefore normal that for *D. alata*, highly infested by this species, the fauna-soil relationship be weaker. But it nevertheless exists because of the other nematode species, some of which are strongly dependent on the soil physico-chemical characteristics.

On the other hand, *D. cayenensis-rotundata* is infested mostly by endemic species, particularly *P. coffeae*, which are highly dependent on soil parameters.

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