



## Nematodes as bioindicators of the state of disturbance of edaphic ecosystems irrigated with natural and polluted water

Nematodos como bioindicadores del estado de perturbación de ecosistemas edáficos irrigados con agua natural y contaminada

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### ABSTRACT

Proper soil assessment requires an understanding of the response of edaphic food webs. Therefore, in this study, the disturbance status of soils irrigated with natural and contaminated water was determined using edaphic nematofauna food webs. Preliminary sampling and analysis were performed to determine the number of subsamples for each type of soil (pasture, alfalfa crop, fig and willow) irrigated with natural and contaminated water, with the number of subsamples determined another sampling was performed, the samples were labeled and transported to the laboratory; with the data obtained, the NINJA program was used to calculate the food web percentages and food web indices. In soil ecosystems irrigated with natural water there was a high percentage of omnivorous and predatory nematodes; and in soil ecosystems irrigated with contaminated water there was a high percentage of bacteriovores and fungivores. The trophic network indices indicated that the edaphic ecosystems irrigated with natural water had little anthropic influence and therefore a greater number of healthy soils, and in the edaphic ecosystems irrigated with contaminated water there was a greater anthropic influence and therefore a greater number of disturbed soils.

**Keywords:** anthropic influence; soil quality; trophic index; trophic network.

### RESUMEN

La evaluación correcta del suelo requiere una comprensión de la respuesta de las redes tróficas edáficas. Por ello, en este estudio se determinó el estado de perturbación de suelos irrigados con agua natural y contaminada, mediante las redes tróficas de la nematofauna edáfica. Se realizó un muestreo y análisis preliminar para determinar el número de submuestras para cada tipo de suelo (pastizal, cultivo de alfalfa, higo y sauce) irrigados con agua natural y contaminada, con el número de submuestras determinadas se realizó otro muestreo, las muestras fueron rotuladas y transportadas al laboratorio; con los datos obtenidos se utilizó el programa NINJA para calcular los porcentajes de red trófica y los índices de red trófica. En los ecosistemas edáficos irrigados con agua natural hubo alto porcentaje de nematodos omnívoros y depredadores; y en los ecosistemas edáficos irrigados con agua contaminada presentan alto porcentaje de bacterióvoros y fungívoros. Los índices de la red trófica indicaron que los ecosistemas edáficos irrigados con agua natural presentan poca influencia antrópica por tanto existe mayor cantidad de suelos saludables; y en los ecosistemas edáficos irrigados con agua contaminada existe mayor influencia antrópica y, por tanto, mayor cantidad de suelos perturbados.

**Palabras clave:** Influencia antrópica, calidad del suelo, índice trófico; red trófica.

## 1. Introduction

Soil irrigation can become a threat to human consumption (Jiang et al., 2015), due to water pollution generated by anthropic activities (Fierro et al., 2019). Although irrigation is a valuable tool to increase the productivity of agricultural systems (Ezenne et al., 2019), little is known about the threat they pose to edaphic ecosystems (Kaya et al., 2015); here is where nematodes are presented as valuable tools to assess soil quality (Rosli et al., 2018).

At present, nematodes are considered one of the best indicators of soil quality (Fraschetti et al., 2016), since these organisms play an important role as nutrient cycling agents and soil fertility regulators (Procter, 1990), they are cosmopolitan (Grzelak et al., 2018), and occupy various positions in food webs (McGraw & Schlossberg, 2017); furthermore, each type of nematode changes predictably to the state of disturbance of its habitat (Rosli et al., 2018); therefore, there are indexes based on them to determine the quality of soils (Sánchez-Moreno & Talavera, 2013).

In this study, the state of disturbance of soils was determined, classified according to their use capacity, based on the edaphic nematofauna, in soils irrigated with natural and polluted water through the trophic percentage distribution and trophic network indices.

## 2. Materials and methods

### Study area

The edaphic ecosystem irrigated with natural water corresponds to the Añashuayco valley located at 16°25'12.32" south latitude and 71°40'09.91" west longitude, at an elevation of 1990 msnm; in the Uchumayo district of the Arequipa department, Peru. In the lower part of the Añashuayco ravine, there are springs from the water table and seepage from irrigation from surrounding areas, which allows the entire valley of Añashuayco to be irrigated (Trujillo, 2007). In this area, 4 types of soil were sampled, classified according to their capacity for use: pasture, clean cultivation (alfalfa cultivation), permanent cultivation (fig cultivation) and forest (willow).

The edaphic ecosystem irrigated with polluted water corresponds to the new town of Molino Chuquicaña located at 16°27'18.51" south latitude and 71°34'57.27" west longitude, at an elevation of 2144 msnm; in the Tiabaya district of the Arequipa department, Peru. This area is irrigated by the polluted waters of the Chili River. since Along this river up to the study area there are

many points of discharge of wastewater of different origin (Yupanqui & Bernabé, 2018). In this area, 4 types of soil classified according to their use capacity were also sampled: pasture, clean cultivation (alfalfa cultivation), permanent cultivation (fig cultivation) and forest (willow).

### Field work

The sampling was carried out in one hectare for each type of soil. In grassland and alfalfa soils, it was sampled in zigzag (Piedra, 2015); in star-shaped fig cultivation and willow (Coyne et al., 2007); the samples were extracted with a 4 cm diameter cannula bit at a depth of 25 cm (Piedra, 2015). The preliminary sampling consisted of a sample composed of 10 subsamples for each type of soil (Coyne et al., 2007); after obtaining the number of subsamples, another sampling was carried out for each type of soil, with 3 repetitions; that were placed and labeled in black polyethylene bags and transported to the nematology laboratory of the Faculty of Biological Sciences of the National University of San Agustín de Arequipa, Peru; in the period from September 2014 to April 2015.

### Laboratory work

To obtain representativeness of mobile nematodes, the modified Whitehead tray method was used (Hernández-Ochandia et al., 2016), in each type of soil, a tray system was used which consisted of a 150 cc container in which a 250 µm mesh strainer was placed, covered internally by a 70 mm diameter Whatman brand circular filter paper; then using a spatula a 100 cc container was filled with soil sample and poured onto the filter paper, then the tray system was filled with distilled water until the water covered the soil sample; thus it was left for 48 hours; After 48 hours, the soil sample was removed from the system and the remaining water with the nematodes was passed through a Yichang brand sieve with a 50 µm mesh size, said sample was dropped into a Petri dish of 55 mm in diameter and 14.2 mm in height; and with the help of an Olympus stereoscope of 0.8 to 4.5 X magnification, the nematodes were identified at the taxonomic genus level. The identification of the specimens was carried out based on the taxonomic keys (Guerrero, 2017).

To obtain representativeness of inactive forms and slow nematodes, the modified centrifugation-flotation method was used (Hernández-Ochandia et al., 2016), placing 100 cc of soil sample in a 2 liter container and it was completed with distilled water up to 500 ml, the soil clumps were crumbled

for the separation of the nematodes from the soil particles, it was left to rest for 10 seconds so that the particles larger soil sediment, the suspension was passed through the 500 um and 50 um sieves, the remainder of which was deposited in a 10 cc centrifuge tube with 1 ml of 34.5% sucrose; which was centrifuged at 2000 rpm for 1 minute; This solution passed through a 50 um sieve and was immediately rinsed with distilled water to remove the sugar and prevent the nematodes from plasmolyzing, the nematodes that remained on the sieve were collected with a wash-bottle to deposit them in a Petri dish and it was finally observed, counted and recognized at the genus level on the stereoscope.

**Data analysis and processing**

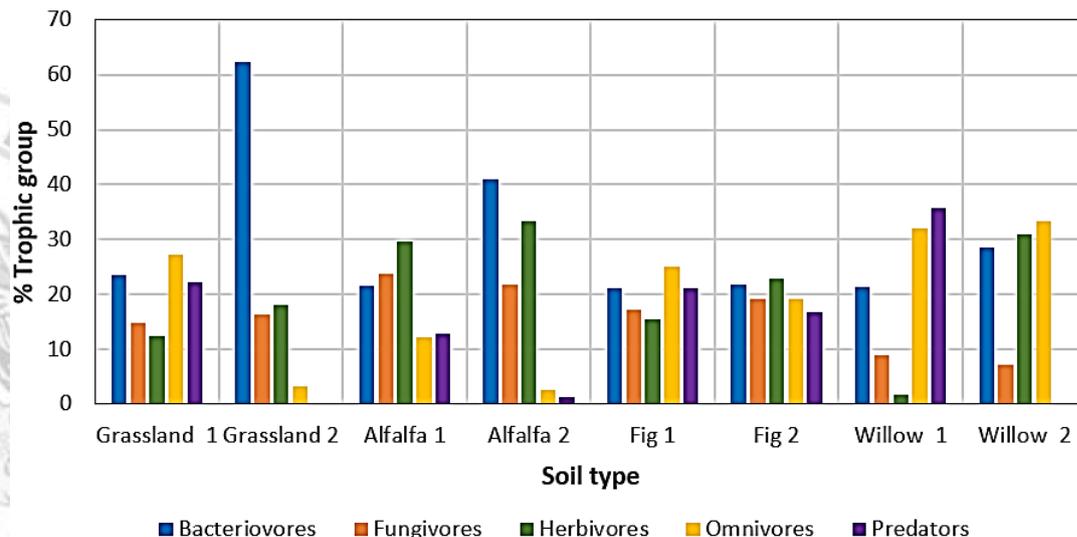
The NINJA (Nematode INDicator Joint Analysis) program was used, which is a free automated calculation software for monitoring the quality of the soil based on nematodes (Sieriebriennikov et al., 2014); with this program, the trophic web percentages and the trophic web indices were calculated.

**3. Results and discussion**

On soils irrigated with natural water; the grassland presented an equitable percentage distribution of the trophic groups of nematodes (Figure 1): 23.46% of bacteriovores (*Rhabditis* and *Acrobeles*), 14.81% of fungivores (*Aphelenchus* and *Aphelenchoides*), 12.35% of herbivores (*Heterodera* and *Tylenchus*), 27.16% omnivores (*Dorylaimus*) and 22.22% predators (*Mononchus*); in the same way in fig cultivation: 21.15% of bacteriovores (*Rhabditis*), 17.31% of fungivores

(*Aphelenchus* and *Aphelenchoides*), 15,38% of herbivores (*Criconemoides*, *Hemicycliophora*, *Heterodera*, *Xiphinema*, *Paratricho-dorus* and *Tylenchus*), 25% of omnivores (*Dorylaimus*) and 21.15% predators (*Mononchus*). In alfalfa cultivation a slight predominance of herbivorous nematodes is observed with 29.73% (*Criconemoides*, *Hemicycliophora*, *Heterodera*, *Paratricho-dorus* and *Tylenchus*); However, the other trophic groups are also well represented with 21.62% of bacteriovores (*Rhabditis*), 23.65% of fungivores (*Aphelenchus* and *Aphelenchoides*), 12.16% of omnivores (*Dorylaimus*) and 12.84% of predators (*Mononchus*). Finally, in the willow soil there is a predominance of omnivorous nematodes with 32.14% (*Dorylaimus*) and predators with 35.71% (*Mononchus*).

In soils irrigated with polluted water; the pastureland has a predominance of bacteriovores (*Rhabditis*) with 62.3% and fungivores (*Aphelenchus* and *Aphelenchoides*) with 16.39%. In alfalfa cultivation, a predominance of bacteriovorant nematodes (*Rhabditis*) is observed with 41.03% and herbivores (*Criconemoides*, *Hemicycliophora*, *Heterodera*, *Xiphinema* and *Tylenchus*) with 33.3%. In the soil of the fig crop an equitable presence of the trophic groups of nematodes is observed with 21.69% of bacteriovores (*Rhabditis*), 19.28% of fungivores (*Aphelenchus* and *Aphelenchoides*), 22.89% of herbivores (*Criconemoides*, *Heterodera* and *Tylenchus*), 19.28% omnivores (*Dorylaimus*) and 16.87% predators (*Mononchus*). In the willow soil a predominance of omnivorous nematodes is observed (*Dorylaimus*) 30.95% and predators (*Mononchus*) 33.33%.



**Figure 1.** Percentage distribution of the dynamics of the trophic groups of nematodes in soils irrigated with natural (1) and polluted water (2).

On soils irrigated with natural water; the grassland is in quadrant C (Figure 2), which is characterized by having a structured trophic network, therefore a structured soil with a low level of enrichment. The cultivation of alfalfa, fig cultivation and willow soil are found in quadrant B, which is characterized by having a mature trophic network, therefore a mature soil with good structure and enrichment. In soils irrigated with polluted water, the pasture and the alfalfa crop are in quadrant A, which is characterized by having a disturbed trophic network therefore a disturbed soil, that is, they are soils with poor structure, but with a good level of enrichment, on the other hand the cultivation of fig and willow soil are found in quadrant B, characteristic of mature soils. In the grassland irrigated with natural water there is an equitable percentage of trophic groups and it is found in quadrant C, indicative of a healthy soil, these results agree with the studies reported by Sechi et al. (2018), who points out that this is produced by a sustainable management of the soil, evidenced by an optimal state of the soil structure.

On the other hand, the pasture irrigated with polluted water is found in quadrant A and a predominance of bacterivores and fungivores is observed; ground disturbance indicators (Bal et al., 2017).

The alfalfa crop irrigated with natural water is found in quadrant B and a slight predominance of herbivorous nematodes is observed; however, the other trophic groups are also well represented; this can be attributed to the fact that this soil is not used only for the production of alfalfa, since it is usually rotated for the production of other plant species, a situation that favors a healthy soil (McGraw & Schlossber, 2017). Unlike the alfalfa crop irrigated with polluted water that is found in quadrant A and a predominance of bacterivores and herbivores is observed; which is a sign of stress in the soil, this result agrees with what was reported by Simmons et al. (2008); besides, Gu et al. (2018) indicates that alfalfa crops are highly affected due to the fact that they are constantly tilled and undergoing severe artificial fertilization processes.

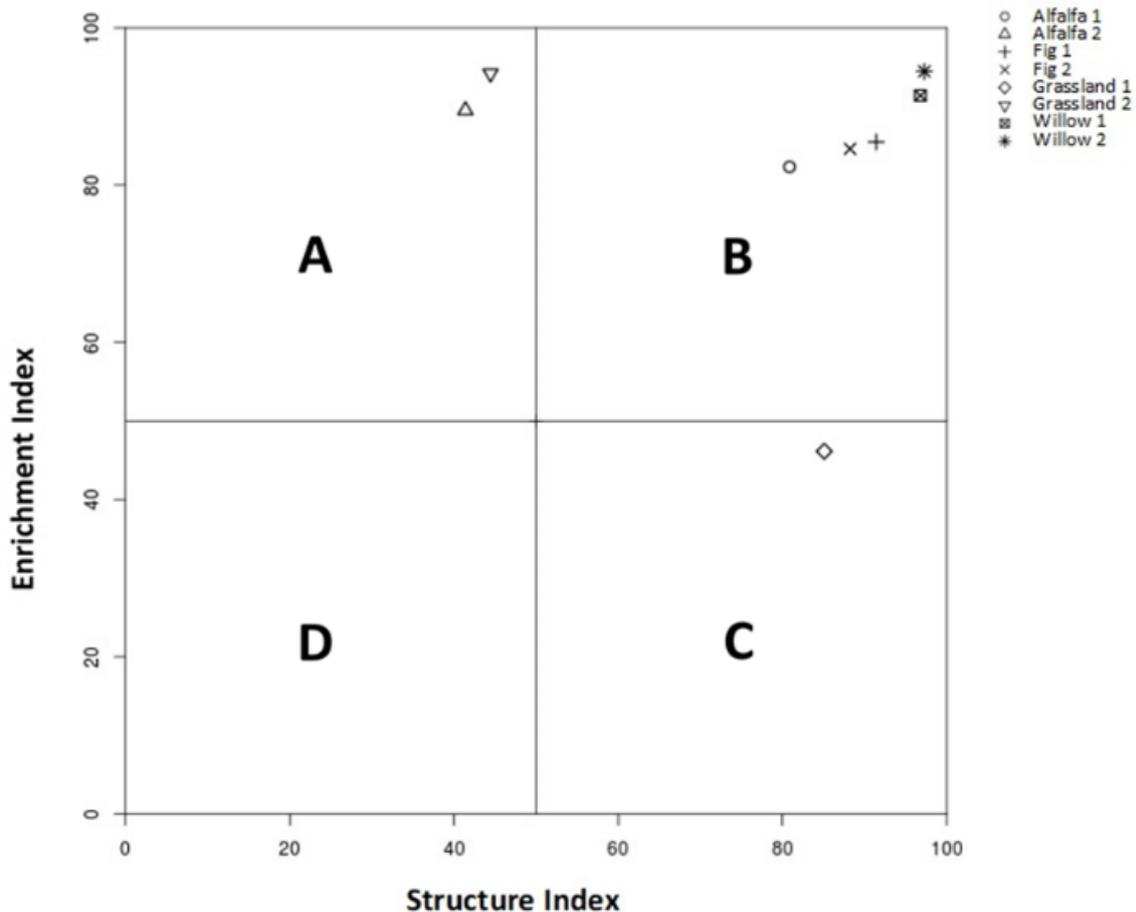


Figure 2. Diagnosis of the status of the trophic network of nematodes in soils irrigated with natural (1) and polluted water (2).

In the soil of the fig crop with both types of irrigation an equitable presence of trophic groups is observed and they are found in quadrant B, significant of a healthy soil, this agrees with Song et al. (2017) who point out that the homogeneous distribution of trophic groups occurs in soils with sustainable management. Further, Fernando et al. (2018) point out that in perennial crops, tillage is reduced and fertilization measured, which promotes favorable conditions for the soil and the crop, reflecting little stress on the soil.

In willow soils irrigated with natural and polluted water, they are found in quadrant B and a predominance of omnivores and predators is observed; indicative of a healthy soil, this result agrees with those reported by Landi et al. (2018) who point out that the decrease or absence of these trophic groups indicates deterioration in the health of the soil. Additionally, according to Steel & Ferris (2016) the presence and more particularly the abundance of predatory nematodes represents an undisturbed soil.

#### 4. Conclusions

The percentage distribution of the dynamics of the trophic groups and the indices of the trophic network of edaphic nematodes allowed us to infer that the edaphic ecosystems irrigated with natural water present little anthropic influence and therefore there is a greater quantity of healthy soils; On the other hand, in edaphic ecosystems irrigated with polluted water, there is a greater anthropic influence and, therefore, a greater number of disturbed soils.

#### References

- Bal, H. K., Acosta, N., Cheng, Z., Grewal, P. S., & Hoy, C. W. (2017). Effect of habitat and soil management on dispersal and distribution patterns of entomopathogenic nematodes. *Applied Soil Ecology*, 121, 48-59.
- Coyne, D. L., Nicol, J. M., & Claudius-Cole, B. (2007). *Practical plant nematology: a field and laboratory guide*. SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin.
- Ezenne, G. I., Jupp, L., Mantel, S. K., & Tanner, J. L. (2019). Current and potential capabilities of UAS for crop water productivity in precision agriculture. *Agricultural Water Management*, 218, 158-164.
- Fernando, A. L., Costa, J., Barbosa, B., Monti, A., & Rettenmaier, N. (2018). Environmental impact assessment of perennial crops cultivation on marginal soils in the Mediterranean Region. *Biomass and Bioenergy*, 111, 174-186.
- Fierro, P., Valdovinos, C., Arismendi, I., Díaz, G., Ruiz de Gamboa, M., & Arriagada, L. (2019). Assessment of anthropogenic threats to Chilean Mediterranean freshwater ecosystems: Literature review and expert opinions. *Environmental Impact Assessment Review*, 77, 114-121.
- Fraschetti, S., Guamieri, G., Gambi, C., Bevilacqua, S., Terlizzi, A., & Danovaro, R. (2016). Impact of offshore gas platforms on the structural and functional biodiversity of nematodes. *Marine Environmental Research*, 115, 56-64.
- Grzelak, K., Tamborski, J., Kotwicki, L., & Bokuniewicz, H. (2018). Ecostructuring of marine nematode communities by submarine groundwater discharge. *Marine Environmental Research*, 136, 106-119.
- Gu, Y. -J., Han, C. -L., Fan, J. -W., Shi, X. -P., Kong, M., Siddique, K.H.M., et al. (2018). Alfalfa forage yield, soil water and P availability in response to plastic film mulch and P fertilization in a semiarid environment. *Field Crops Research*, 215, 94-103.
- Guerrero, R. (2017). *Manual de nematodos fitoparásitos identificados de especies cuarentanarias*. Agrocalidad. Ecuador: Quito 136: 1-42.
- Hernández-Ochandia, D., Rodríguez-Hernández, M. G., Miranda-Cabrera, I., & Holgado R. (2016). Métodos para la extracción de nematodos presentes en suelos del agrupamiento Ferralítico en Cuba. *Revista Protección Vegetal*, 31(3), 228-232.
- Jiang, Y., Sun, B., Li, H., Liu, M., Chen, L., & Zhou, S. (2015). Aggregate-related changes in network patterns of nematodes and ammonia oxidizers in an acidic soil. *Soil Biology and Biochemistry*, 88, 101-109.
- Kaya, Ç. I., Yazar, A., & Sezen, S. M. (2015). SALTMED Model Performance on Simulation of Soil Moisture and Crop Yield for Quinoa Irrigated Using Different Irrigation Systems, Irrigation Strategies and Water Qualities in Turkey. *Agriculture and Agricultural Science Procedia*, 4, 108-118.
- Landi, S., Papini, R., d'Errico, G., Brandi, G., Rocchini, A., Roversi, P.F., Bazzoffi, P., & Mocali, S. (2018). Effect of different set-aside management systems on soil nematode community and soil fertility in North, Central and South Italy. *Agriculture, Ecosystems and Environment*, 261, 251-260.
- McGraw, B. A., & Schlossberg, M. J. (2017). Fine-scale spatial analysis of soil moisture and entomopathogenic nematode distribution following release in wetting agent-treated turf. *Applied Soil Ecology*, 114, 52-61.
- Piedra, R. (2015). *Guía de muestreo de nematodos fitoparásitos en cultivos agrícolas*. Instituto Nacional de Innovación y Transparencia en Tecnología Agropecuaria.
- Procter, D. L. C. (1990). Global overview of the functional roles of soil-living nematodes in terrestrial communities and ecosystem. *Journal of Nematology*, 22(1), 1-7.
- Rosli, N., Leduc, D., Rowden, A. A., Probert, P. K., & Clark, M. R. (2018). Regional and sediment depth differences in nematode community structure greater than between habitats on the New Zealand margin: Implications for vulnerability to anthropogenic disturbance. *Progress in Oceanography*, 160, 26-52.
- Sánchez-Moreno, S., & Talavera, M. (2013). Los nematodos como indicadores ambientales en agroecosistemas. *Ecología y Medio Ambiente*, 22, 50-55.
- Sechi, V., De Goede, R. G. M., Rutgers, M., Brussaard, L., & Mulder, C. (2018). Functional diversity in nematode communities across terrestrial ecosystems. *Basic and Applied Ecology*, 30, 76-86.
- Sieriebriennikov, B., Ferris, H., & de Goede, R. G. M. (2014). NINJA: An automated calculation system for nematode-based biological monitoring. *European Journal of Soil Biology*, 61, 90-93.
- Simmons, B. L., Niles, R. K., & Wall, D. H. (2008). Distribution and abundance of alfalfa-field nematodes at various spatial scales. *Applied Soil Ecology*, 38, 211-222.
- Song, D., Pan, K., Tariq, A., Sun, F., Li, Z., Sun, X., Zhang, L. et al. (2017). Large-scale patterns of distribution and diversity of terrestrial nematodes. *Applied Soil Ecology*, 114, 161-169.
- Steel, H., & Ferris, H. (2016). Soil nematode assemblages indicate the potential for biological regulation of pest species. *Acta Oecologica*, 73, 87-96.
- Trujillo, C. C. (2007). Impacto ambiental en el geosistema de las canteras de sillar de Añashuayco - Arequipa. *Espacio y Desarrollo*, 216, 207-216.
- Yupanqui, M., & Bernabé, J. C. (2018). Grado de contaminación del río Chili por oligoelementos metálicos y su efecto en el cultivo de *Illium cepa* L. (cebolla) en el subsector de riego - Tiabaya. *Campus*, 23, 43-58.