Rhizosphere 38 (2026) 101319

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Soil nematode communities and food web structure in Portuguese rice agroecosystems

Leidy Rusinque [a,b,c], Carla Maleita [a,b,*], Sofia R. Costa [d], Ana Rita Varela [a,e], Maria L. Inácio [a,c]

[a] Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Oeiras, 2780-159, Portugal
[b] CERES - Chemical Engineering and Renewable Resources for Sustainability, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima Pólo I —Pinhal de Marrocos, Coimbra, 3030-790, Portugal
[c] GREEN-IT Bioresources for Sustainability, ITQB NOVA, Av. da República, Oeiras, 2780-157, Portugal
[d] CBMA—Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, Braga, 4710-057, Portugal
[e] MED Mediterranean Institute for Agriculture, Environment and Development, CHANGE Global Change and Sustainability Institute, Institute for Advanced Studies and Research, University of Évora, Portugal

ARTICLE INFO

Keywords:
Nematodes
Oryza sativa
Rhizosphere
Root pathogens
Rice agroecosystems

ABSTRACT

Nematodes are essential contributors to soil food web, playing a crucial role in maintaining ecosystem functions and providing vital services. This study assessed the nematode diversity and food web structure in flooded rice fields in the main rice producing regions in Portugal. Soil samples were collected from rice fields along the basin of rivers Tagus, Mondego, and Sado in Portugal. Nematodes were extracted and identified to genus level to perform community analyses. Plant-parasitic nematodes (PPN) were identified to species level using 28S rDNA, 18S rDNA and COI markers. The study found 18 nematode genera associated to rice, including bacterivores, fungivores, omnivores, predators and PPN. Based on prominence value, the genus *Rhabditis* was found to be the most frequent followed by *Panagrolaimus* and *Helicotylenchus*. Nematode community composition differed significantly among regions, with small temporal shifts. Diversity patterns were also region specific; the Mondego basin showed the highest diversity index, while richness and evenness across basins remain stable. Functional diagnostics indicated enrichment-dominated food webs across regions, with Tagus and Mondego showing higher food-web structure than Sado. Molecular analysis identified eight PPN species, among which *H. dihystera* and *Pratylenchus crenatus* were widespread across the three regions, while *Aphelenchoides parietinus, Meloidogyne incognita* and *Paratrichodorus minor* were confined to Mondego, *Merlinius joctus* to Tagus and *Xiphinema citricolum* to Sado. *Meloidogyne graminicola*, the most serious pest of rice worldwide, was not found in Portuguese rice fields. These results provide the first baseline for nematode diversity and food-web condition in Portuguese rice agroecosystems and represent one of the first ecological characterisations of nematode communities in Europe, offering vital information for monitoring and management programmes.

1. Introduction

Nematode assemblages are widely used in soil ecological studies because changes in community composition and trophic structure reflect soil food web organisation and ecosystem functioning (Bongers and Ferris, 1999; Ferris et al., 2001). Soil biota contribute to nutrient cycling, organic matter decomposition, and the regulation of plant pests and pathogens, thereby supporting essential ecosystem services (Bardgett and van der Putten, 2014; Orgiazzi et al., 2016). On this basis, nematode ecological indices and metabolic footprints are often being applied in soil monitoring and biomonitoring programmes (Ferris, 2010; Sánchez-Moreno and Ferris, 2018).

In Europe, attention has been given to the development of soil health monitoring frameworks to evaluate soil biodiversity. Recent initiatives associated with the European Union Soil Strategy aim to integrate biological indicators into large scale assessments of soil condition across different land uses and climatic regions (European Commission, 2021; Guerra et al., 2021). Within this context and despite the limited availability of comprehensive datasets, the nematode community analysis is recognised as a valuable tool for assessing soil biodiversity and ecosystem functions in agricultural systems (Furmanczyk et al., 2025).

Rice agroecosystems represent unique environments where periodic

* Corresponding author. Instituto Nacional de Investigação Agrária e Veterinária (INIAV, I.P.), Oeiras, 2780-159, Portugal.
E-mail address: carla.maleita@uc.pt (C. Maleita).

https://doi.org/10.1016/j.rhisph.2026.101319
Received 11 March 2026; Received in revised form 17 March 2026; Accepted 25 March 2026
Available online 1 April 2026
2452-2198/© 2026 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

flooding and agricultural management practices influence soil biological communities (Ferris et al., 2001; Okada et al., 2011). Studies conducted in rice growing regions worldwide have shown that nematode assemblages respond to environmental conditions and cropping systems, making them useful indicators of soil ecological processes in paddy systems (Ferris et al., 2001; Neher, 2010; Korobushkin et al., 2019; Mokuah et al., 2023). However, compared with major rice producing regions in Asia, research on nematode community composition and trophic structure in the Mediterranean rice agroecosystems is still limited, leaving a significant gap in the current knowledge (Korobushkin et al., 2019; Du Preez et al., 2022).

In Portugal, rice is cultivated primarily in Mondego, Tagus, and Sado Valleys, in lowland areas and coastal wetlands, with a particular role in the preservation of biodiversity and soil conservation and significant socioeconomic importance (Gonçalves et al., 2022). However, while the diversity of aboveground organisms is well-documented, the overall diversity and ecological functions of soil biota, particularly nematofauna, remain largely unexplored. Hence, the establishment of baseline data on nematode diversity and trophic structure across rice growing areas is essential to support soil biodiversity assessments and future biomonitoring efforts.

Therefore, this study aimed to assess nematode diversity, soil food web indices, and metabolic footprints in irrigated rice fields in Portugal, to characterise nematode community structure and evaluate their potential as indicator of soil ecological condition, and plant-parasitic nematode (PPN) occurrence.

2. Materials and methods

2.1. Area of study and sampling

A survey in the main rice production areas (basins of rivers Tagus, Mondego, and Sado) was carried out from 2021 to 2023, collecting a total of 90 samples (Fig. 1). Sampling was conducted between July and September, corresponding to the reproductive to early ripening stages of the rice crop. All the sampled fields had rice as monoculture with comparable conventional management. Soil, root, and leaves samples were collected by the National Institute of Agrarian and Veterinarian Research (INIAV, I.P) and by inspectors of the National Plant Protection Organization (DGAV-Portugal) and Regional Directorate of Agriculture and Fisheries from the Central region (DRAPC-Portugal). Each soil sample, with an approximate volume of 1 dm^3, consisted of a composite of 15 subsamples collected at roughly equal intervals from the root-colonised soil at about 15-20 cm depth. Samples were individually coded and stored in polyethylene bags.

The regions surveyed in this study are represented by a Mediterranean climate, Csb and Csa of Köppen classification, characterised with mild and temperate summers, with almost no rainfall, while winters are rainy with mild temperatures (IPMA - Instituto Português do Mar e da Atmosfera, 2025). Soils are mainly alluvial with high agricultural quality, some of which are poorly drained, with waterlogging and salinization risks (Gonçalves et al., 2020).

Sample collection and species detection maps were made using the ArcMap 10.8 software (ESRI, United States), CAOP2022_PORTUGAL and CAOPP2022_DISTRITOS shapefiles (DGT, 2022).

2.2. Nematode extraction

Nematodes were extracted for 24 h from soil, leaves and roots using the modified Baermann's Funnel (tray method) from a 100 mL soil subsample or 100 g leaves/roots according to the standard protocol PM 7/119 (1) (EPPO - European Plant Protection Organisation, 2013). The obtained nematode suspension was concentrated by sieving through a 20 μm sieve. The suspensions were observed using a stereomicroscope (Nikon SMZ1500, Tokyo, Japan). In samples with a high number of nematodes an aliquot containing at least 100 nematodes was used for counting, in samples with fewer nematodes than 100 all specimens were counted. Direct examination of roots under a stereomicroscope was also performed to look for root-knot nematodes (RKN).

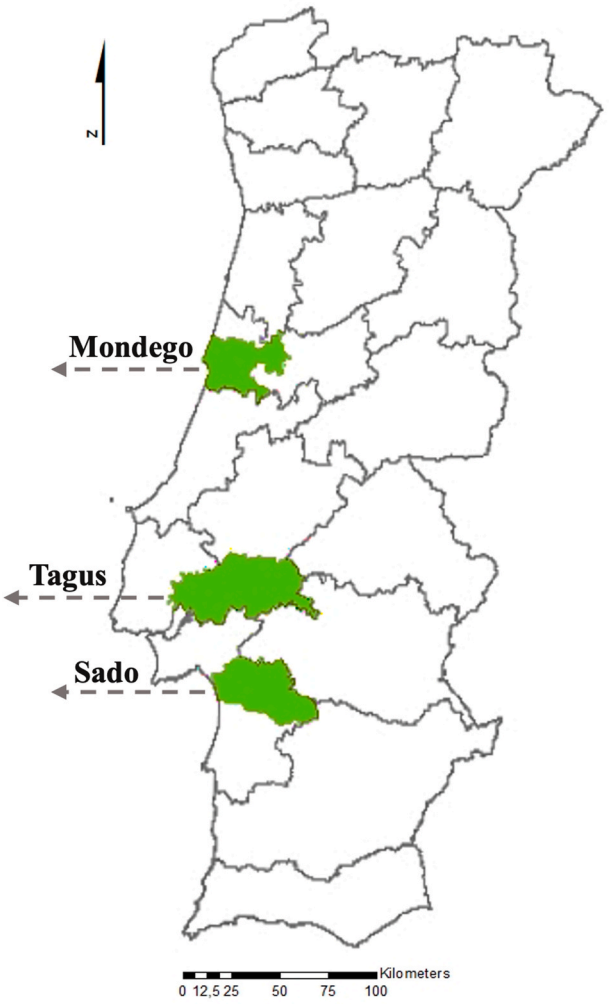


Fig. 1. Location of the main rice producing regions in Portugal, surveyed between 2021 and 2023. Sampling was conducted in the Mondego, Tagus, and Sado river basins (highlighted in green).

2.3. Morphological characterisation and trophic analysis

Nematodes were identified using a brightfield light microscope (Olympus BX-51, Hamburg, Germany) and classified in trophic groups through their mouth and upper digestive tract anatomy (Bongers and Bongers, 1998; Yeates et al., 1993). Identification to genus level was performed using simplified keys (Goodey, 1963; Hunt, 1993; Jairajpuri and Ahmad, 1992; Mai and Mullin, 1996; Siddiqi, 2000). Individuals belonging to the genus *Aphelenchus* were considered fungivores in this survey though some of them may also feed on roots, moss, and algae (Yeates et al., 1993; Siddiqi, 2000; Sohlenius et al., 1977). Specimens belonging to the genus *Aphelenchoides* were considered fungivores by Yeates et al. (1993), however, in this survey they were considered PPN due to the importance that some of them have as pathogens in rice.

Nematodes were grouped into five trophic groups based on their feeding habits: bacterivores (BF), fungivores (FF), PPN, omnivores (OM), and predators (PD) according to Yeates et al. (1993).

To assess the frequency and dominance of nematode genera in rice agroecosystems absolute frequency, absolute density, prominence value and relative prominence values (Norton, 1978) were calculated.

1) Absolute frequency $= \frac{\text{Number of samples containing genus/species}}{\text{Total number of samples collected}} X100$
2) Absolute density = Number of individuals of species per unit volume of soil
3) Prominence value (pv) = Absolute density $\sqrt{\text{Absolute}}$ frequency
4) Relative prominence value $= \frac{\text{Prominence value}}{\text{Total prominence value}}$ X 100

To characterise nematode diversity, a set of taxonomic indices that included Shannon's diversity, Margalef's richness and Pielou's evenness were estimated (Magurran, 2021).

2.4. Functional diversity and soil food web

The genera identified were categorised into life strategy groups based on colonizer/persister values (c-p 1 to c-p 5 and p-p 2 to p-p 5), using the NINJA software (Sieriebriennikov et al., 2014). From these data, functional diversity indices were calculated, including the maturity index of free-living nematodes belonging to colonizer-persister group 1–5 (MI), maturity index excluding c-p 1 nematodes (MI 2–5) (Bongers, 1990), basal index (BI), channel index (CI), enrichment index (EI), structure index (SI) (Ferris et al., 2001) and metabolic footprints (Ferris, 2010) using the VEGAN package in R (Oksanen et al., 2019). The MI, MI 2–5, BI and CI provide information on the level of disturbance in the ecosystem and the condition of the soil food web, EI indicates the availability of food and nutrient enrichment, SI infers the food web structure and its complexity, and the metabolic footprints indicate the ecosystem services provided by the soil food web (Du Preez et al., 2022; Sánchez-Moreno and Ferris, 2018).

2.5. Molecular characterisation

Various genomic regions were used to provide the molecular identification of the PPN species detected in the surveyed rice fields. Total DNA was extracted from a single second-stage juvenile (J2) using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Polymerase Chain Reactions (PCR) were performed using primers D2A/D3B (Nunn, 1992) to amplify the D2–D3 expansion segments of the 28S rDNA; 18S-CL-F3/1912R (Carta and Li, 2018; Holterman et al., 2006) to amplify the 18S rDNA region; and JB3/JB5 (Hu et al., 2002) to amplify the mitochondrial cytochrome oxidase subunit I (COI). PCR reactions were performed in a 25 μL final volume mixture containing 12.5 μL Supreme NZYTaq II Green Master Mix (NZYTech, Lisbon Portugal), 2 μL of isolated DNA, and 0.4 μM of each primer in a Biometra TGradient thermocycler (Biometra, Göttingen, Germany). Thermal cycling conditions consisted of an initial step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 43 °C (COI), 54 °C (18S) or 51 °C (28S) for 30 s, and elongation at 72 °C for 1 min. The thermal cycle was concluded with a 5 min elongation step at 72 °C for all genomic regions. PCR products were resolved by electrophoresis at 5 V.cm−1 in agarose gel (1.5%) containing 0.5 μg/mL ethidium bromide and 0.5x Tris-Borate-EDTA (TBE) running buffer. Amplifications were visualized using the Versa-Doc Imaging System (BioRad Laboratories, Hercules, California, USA). PCR products were purified using the DNA clean and concentrator kit (Zymo Research Corp, California, USA), according to the manufacturer's instructions. Amplicons were sequenced in both directions at STABVida Sequencing Laboratory (Lisbon, Portugal) on a DNA analyser ABI PRISM 3730xl (Applied Biosystems). The newly obtained sequences were manually checked, edited, and assembled using MEGA 7 (Kumar et al., 2016). Sequence identity was carried out by comparison with references deposited in GenBank using BLAST. Sequences generated in this study were deposited in GenBank.

2.6. Data analysis

To assess differences in overall nematode community composition, a permutational multivariate analysis of variance (PERMANOVA) was performed using Bray-Curtis dissimilarities (9999 permutations), significance level of $\alpha = 0.05$. Homogeneity of multivariate dispersion was evaluated with betadisper and a permutation test (9999 permutations) using the VEGAN package in software R (Version 4.5). When Region was significant, pooled pairwise comparisons among regions were performed to estimate the average regional effect across years (permutation tests; Bonferroni adjustment) (Anderson, 2017; Oksanen et al., 2019).

Nematode abundance was analysed using a Generalised Linear Model with a negative binomial distribution and a log link (GLM-NB) to account for overdispersion (Ver Hoef and Boveng, 2007). Overdispersion was evaluated via Pearson residual tests for each genus. The response variable in each model was the abundance of individual nematode genus. Models were fitted using the MASS package in software R (Version 4.5) (Venables and Ripley, 2002). The models included Region, Year, and their interaction (Region x Year) as fixed factors but, due to the unbalanced distribution of samples among Region - Year combinations, interaction effects were interpreted with caution and considered exploratory.

To evaluate nematode diversity, indices were calculated for each individual sample using the VEGAN package in R (Version 4.5), this approach allowed for a more robust statistical comparison, as it accounted for within region variation (Neher and Darby, 2006; Oksanen et al., 2019). Modelling was carried out with Generalised Linear Models (GLMs), Shannon diversity and Margalef richness via Gamma (log), Pielou's evenness via beta (logit). Functional indices were analysed similarly: EI, SI, BI, CI with beta (logit) models, and MI, MI2-5 and metabolic footprints with Gamma (log) models. Main effects in Gamma GLMs, were analysed using Type-III tests with CAR package in R (Version 4.5), whereas Beta regression models were evaluated using likelihood-ratio (drop-term) tests. When main effects were significant, estimated marginal means (EMMs) were calculated and Tukey-adjusted pairwise comparisons performed using the EMMEANS package in R (Version 4.5) (Lenth, 2024). The Region × Year interaction was tested initially for diversity and functional indices but excluded from final models due to unbalanced sampling across Region - Year combinations, which led to non-estimable or unstable contrasts.

3. Results

3.1. Nematode communities in rice agroecosystems

Nematodes were recovered only from soil samples. A total of 18 different nematode genera representing different trophic groups were identified. Among trophic groups, PPN showed the highest genus richness (n = 12 genera) followed by bacterivores (n = 3 genera) and fungivores, omnivorous and predatory nematodes (n = 1 genera). Within bacterivores, the genus *Rhabditis* was particularly prominent, showing the highest frequency (76.7%) and density (21/100 mL). Other genera within this group, such as *Panagrolaimus* (53.3%) and *Eucephalobus* (10%) were also frequent but less abundant. Fungivores were represented by the genus *Aphelenchus* (26.7%), which displays high frequency but low abundance. Nematodes belonging to the omnivores group *Dorylaimus* (36.7%) and predators *Mononchus* (24.4%) were commonly found, though at low densities. Many genera of PPN were present in the soil samples, being the genus *Helicotylenchus* the most frequently found (46.7%) and more abundant within this group (11/100 mL), followed by *Aphelenchoides* (20%) and *Pratylenchus* (16.7%). Other genera were detected at low frequencies and densities. Based on relative frequency, absolute density and relative prominence value, the genus *Rhabditis* was found to be the most prominent followed by *Helicotylenchus*, and *Panagrolaimus* (Fig. 2; Table S1).

Table S2 contextualizes these patterns regionally by showing the distribution of mean abundances across regions. Free-living nematodes such as bacterivores and fungivores occurred at moderate levels and revealed little regional variation, whereas several PPN, including

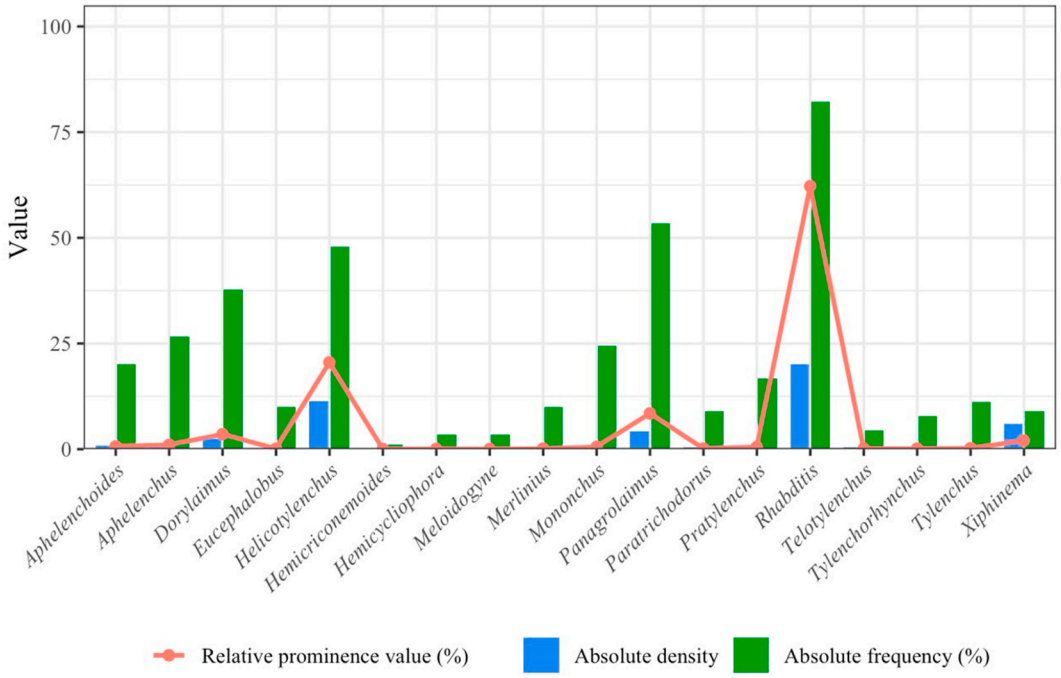


Fig. 2. Relative frequency (%), absolute density ((#/100 mL) and relative prominence value (%) of nematode genera found in Portuguese rice agroecosystems.

Helicotylenchus, *Aphelenchoides*, and *Xiphinema*, displayed differences in abundance among regions.

3.2. Community composition

The PERMANOVA based on Bray-Curtis distances showed significant differences in nematode community composition among regions ($F = 6.31$, $R^2 = 0.125$, $p = 0.001$), while the main effect year ($F = 1.34$, $R^2 = 0.026$, $p = 0.18$) and the Region × Year interaction ($F = 1.78$, $R^2 = 0.03$, $p = 0.053$) were not significant. Dispersions were homogeneous among regions (Region: $F = 1.84$, permutation $p = 0.163$) but differed among years (Year: $F = 10.51$, permutation $p < 0.001$). Given the non-significant Region × Year interaction, pooled permutation-based pairwise among regions was performed, indicating that all regions differed significantly (Mondego vs Sado, $p = 0.003$; Mondego vs Tagus, $p = 0.012$; Sado vs Tagus, $p = 0.003$; Bonferroni adjusted).

At genus level, negative binomial GLM indicated that spatial variation (Region) was significant for many genera including: *Aphelenchoides* (p = 1,008e-10), *Hemicriconemoides* (p = 2.025e-04), *Helicotylenchus* (p = 1.063e-05), *Hemicycliophora* (p = 9.628e-08), *Meloidogyne* (p = 1.795e-02), *Merlinius* (p = 4.281e-05), *Panagrolaimus* (p = 1.792e-02), *Paratrichodorus* (p = 5.043e-09), *Telotylenchus* (p = 5.680e-19), *Tylenchus* (p = 6.501e-04) and *Xiphinema* (p = 8.814e-245). Regarding temporal changes, while Year did not affect overall community composition (PERMANOVA), genus-wise GLM-NB indicated year-to-year changes in a subset of taxa *Hemicriconemoides* (p = 4.05e-03) and *Telotylenchus* (p = 4.7e-15), consistent with taxon-specific responses that do not shift the multivariate centroid. On the other hand, significant Region × Year interactions were found for *Dorylaimus* (p = 2.030e-03), *Panagrolaimus* (p = 1.792e-02), *Tylenchorhynchus* (p = 3.960e-03), and *Tylenchus* (p = 1.792e-02), suggesting that for these genera temporal dynamics changed among regions. The estimated marginal means (EMMs) supported the overall PERMANOVA and GLM-NB results, revealing strong regional differences in most of the genera identified, decline in abundance in two genera over the years and non-uniform changes in abundance for only four genera across regions.

3.3. Diversity indices

Due to the unbalanced Region x Year replication, the interaction for diversity indices was not possible to estimate with precision and so it was excluded. In terms of diversity, the Shannon index differed among regions ($\chi^2 = 6.43$, $p = 0.040$) (Fig. 3) but did not differ among years. Post-hoc Tukey comparisons showed significant differences between Mondego and Tagus ($p = 0.042$), whereas Sado did not differ from either basin. Margalef richness also showed a marginal regional effect ($\chi^2 = 6.20$, $p = 0.046$) but no significant differences among years, moreover, pairwise comparisons did not reveal significant differences after Tukey adjustment. Pielou evenness index had no effect of region or year.

3.4. Functional diversity

The nematode classification into different life strategy groups revealed the presence of higher PPN classes (p-p 4 and p-p 5), represented by the genera *Paratrichodorus* and *Xiphinema*, being of particular interest the genus *Xiphinema* (p-p 5), which was found exclusively and abundantly in the Sado Basin. Otherwise, assemblages were dominated by c-p 1-2, enrichment opportunists with short life cycles and a preference for disturbed environments. This group includes bacterivores and fungivores, being the genera *Rhabditis* and *Panagrolaimus* the most found. Nematodes from higher c-p classes, such as *Dorylaimus* and *Mononchus* (c-p 4), typically associated with stable, mature ecosystems, were also detected across all regions. PPN were predominantly assigned to p-p class 3, with *Helicotylenchus, Pratylenchus*, and *Tylenchorhynchus* occurring in all three basins.

The generalised linear models (GLMs) revealed significant regional differences, mainly in the soil food-web index MI2-5 ($X^{2=}$ 6.31, p = 0,042), whereas no differences were detected among years (2021–2023). Therefore, a summary by region is presented in Table 1. The MI was very low (1.30 and 1.50) and did not differ significantly among regions, a pattern consistent with enrichment/disturbance dominated by opportunistic taxa. In contrast, MI2-5 displayed significant differences between Sado and Tagus (p = 0.037). Higher MI2-5 values in Tagus and Mondego basins (>3) imply a greater contribution

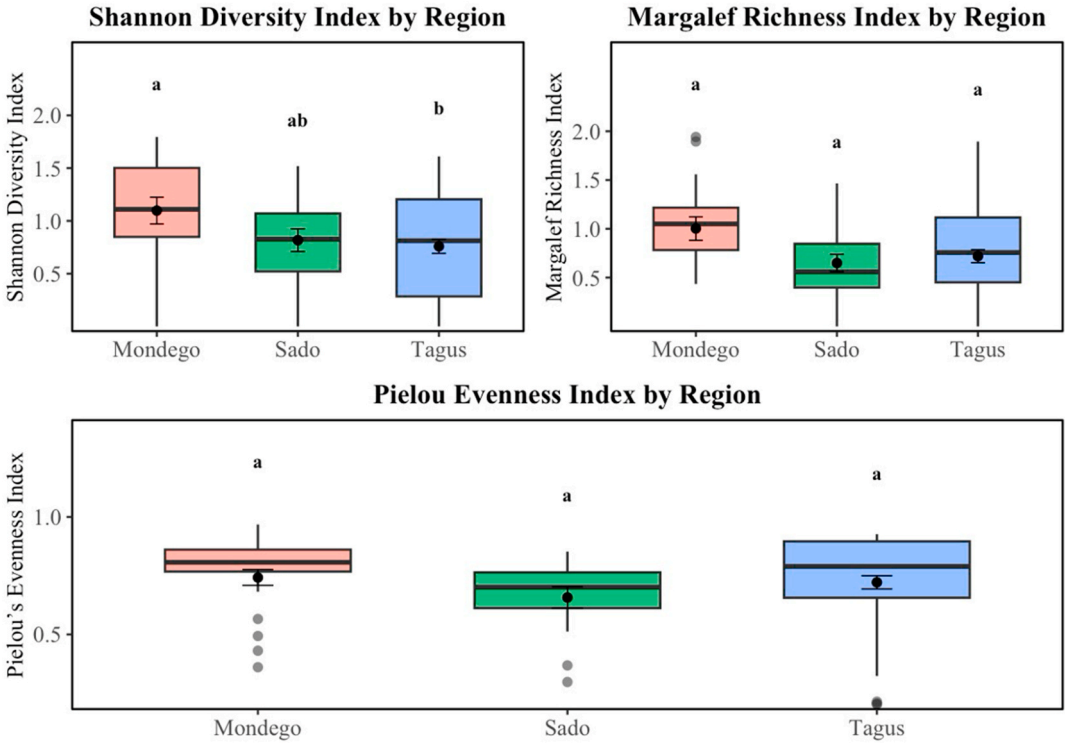


Fig. 3. Boxplots of Shannon diversity index, Margalef richness index, and Pielou's evenness index for nematode communities across the Mondego, Sado, and Tagus River Basins. Different letters indicate statistically significant differences among regions ($p < 0.05$). The horizontal line within each box represents the median of the index values. The box limits denote the interquartile range (IQR) from the 25th to the 75th percentile indicating the central 50% of the data. The "whiskers" extend to the most extreme values within 1.5 × IQR from the quartiles, they help indicate the spread and variability of the data. Dots outside the whiskers represent outliers, indicating individual values that deviate substantially from the rest of the data.

Table 1
Soil food web indices and metabolic footprints (mean ± SD)/region.

Index	Mondego	Tagus	Sado	*p*-value
Maturity Index	1.37 ± 0.41	1.51 ± 0.62	1.32 ± 0.38	0.199
Maturity Index 2-5	3.51 ± 0.76[a]	3.42 ± 0.82[a]	2.64 ± 0.83[b]	0.037*
Basal Index	2.69 ± 9.49	4.46 ± 12.13	5.86 ± 11.91	0.637
Channel Index	4.61 ± 18.89	8.51 ± 27.57	9.18 ± 23.00	0.881
Enrichment Index	97.08 ± 9.58	94.64 ± 13.85	93.84 ± 11.94	0.661
Structure Index	81.01 ± 36.74	75.99 ± 39.93	38.20 ± 47.49	0.148
Enrichment footprint	52.69 ± 60.57	46.35 ± 83.95	18.92 ± 15.91	0.085
Structure footprint	16.00 ± 30.11	18.69 ± 55.14	5.49 ± 12.66	0.847

*Significant differences at $p \leq 0.05$, different letters indicate significant differences between regions (Tukey-adjusted pairwise comparisons).

and persistence of higher c-p 2–5 taxa and a more developed food web, while in Sado, the low MI2–5 suggests that additional stressors beyond simple nutrient enrichment are affecting food web development. Indicators of ecosystem function, such BI and CI, were variable in all three regions but below 10%, and EI was above 90% across the three basins, showing a pattern consistent with bacterial dominated decomposition. SI and EFP were higher in Mondego and Tagus basins than in Sado, but the differences were not statistically significant. SFP were lowest in Sado, matching its lower SI.

Nematode communities in all regions are strongly enriched, with Tagus supporting more higher c–p taxa and Sado displaying the lowest food web structure. When genera were grouped by feeding habits, regional patterns were found (Fig. S1). The Sado basin was dominated by PPN, suggesting disturbance prone environments where nutrient inputs or stressors favour these nematodes. This pattern aligns with the low MI2–5 and SI observed, indicating a simplified, less structured food web with limited higher trophic activity and weaker top-down control. The Tagus basin was dominated by bacterivores, consistent with high enrichment and active bacterial decomposition pathways, supported by the high EI and enrichment footprint values. This indicates that bacterial decomposition dominates organic matter processing, a typical response to elevated productivity or organic inputs. In contrast, the Mondego basin showed a mixed containing both bacterivores and PPN, suggesting an intermediate system, where energy channels from both bacteria and plants contribute to nematode communities. This trend is supported with the relatively high MI2–5 and EI values, implying a moderately enriched system where both bottom-up resource availability and food-web structure are sustained.

To further evaluate the nematode community, a food web analysis was conducted using EI against SI (Fig. 4). This approach provided a complementary view of food-web status, highlighting the balance between enrichment responses and the degree of trophic complexity across regions.

The food web analysis shows that most samples from all three regions are represented in quadrants A and B, indicating nutrient-enriched, maturing and regulated communities. This suggests that nematode communities have large numbers of enrichment opportunists but still contain higher c–p taxa that contribute to food-web complexity. Samples from the Tagus region display the greatest spread, with some extending toward lower SI or EI values, pointing to greater heterogeneity in functional community structure. Mondego samples group more tightly in quadrant B, whereas several Sado samples fall in quadrant A, indicating nutrient-enrichment, disturbed communities and conducive to pests and diseases (Ferris et al., 2001).

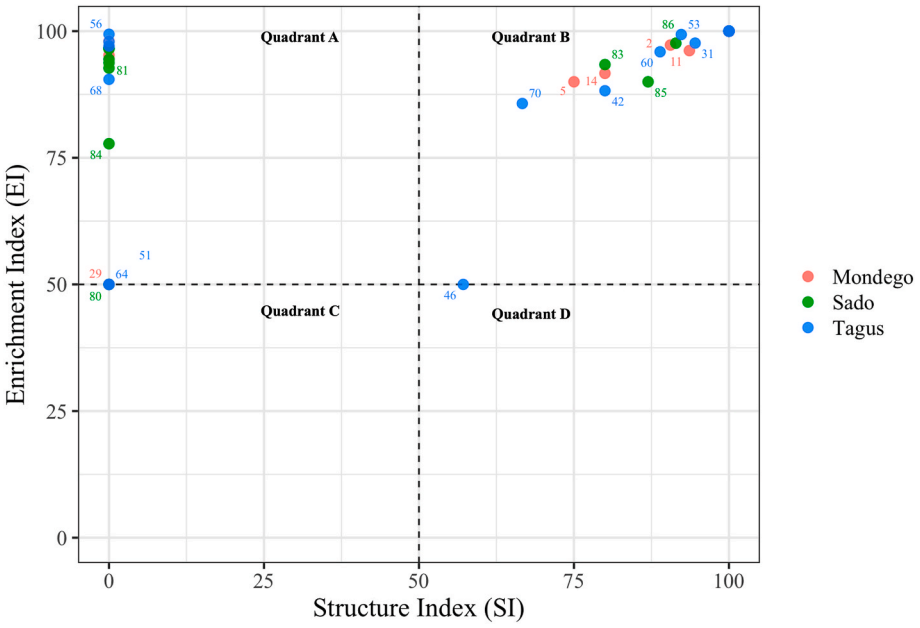


Fig. 4. Food web analysis showing the relationship between Enrichment Index (EI) and Structure Index (SI). Each point represents a sample, overlapping points appear as a single dot. Dashed lines indicate quadrants used to characterise food web condition. **Quadrant A – Disturbed**: N-enriched, low C:N, bacterial decomposition channel (high disturbance); **Quadrant B – Maturing**: N-enriched, low C:N, balanced decomposition channels (low to moderate disturbance); **Quadrant C – Structured**: moderate enrichment, moderate C:N, fungal decomposition channel (undisturbed); **Quadrant D – Degraded:** nutrient depleted, high C:N, fungal decomposition channel (Stressed).

3.5. *Molecular identification of PPN species*

Sequence identity was consistently high (>98%), supporting reliable identification of eight PPN species in Portuguese rice fields (Table S3). These include *Aphelenchoides parietinus*, *Helicotylenchus dihystera*, *Hemicriconemoides ortonwilliamsi*, *Meloidogyne incognita*, *Merlinius joctus*,

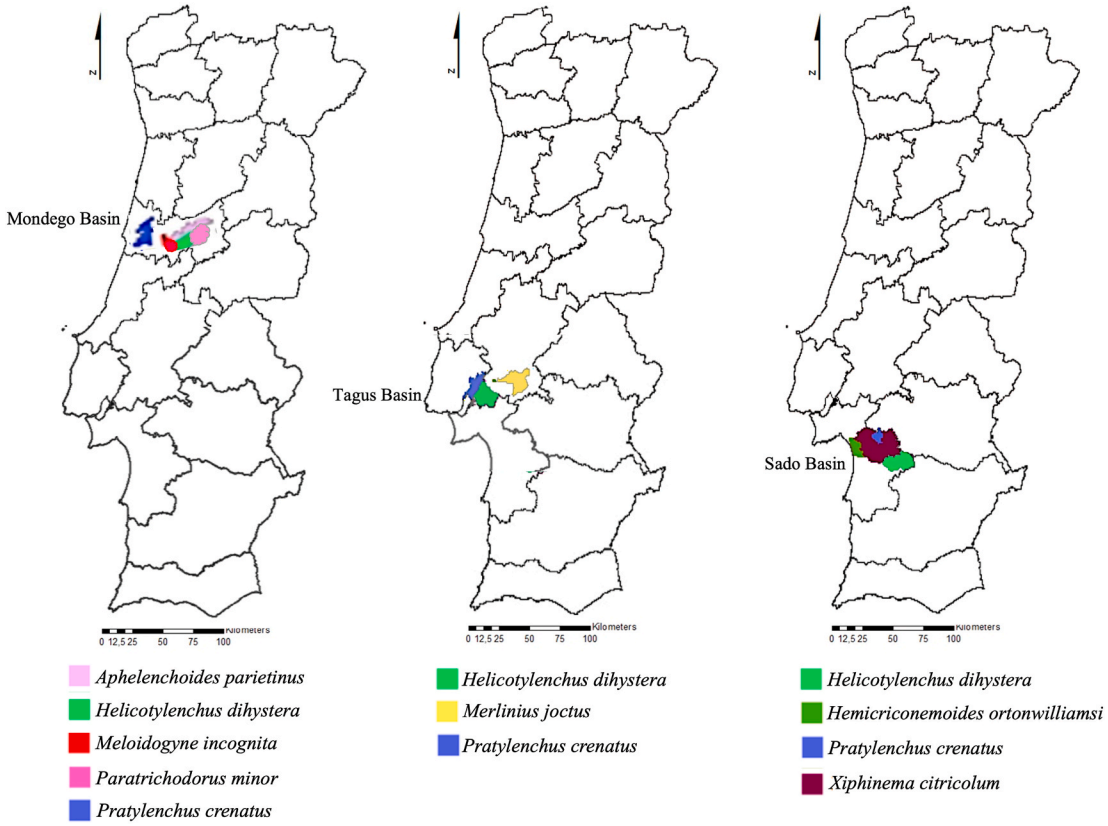


Fig. 5. Distribution maps of plant-parasitic nematode species identified in the three rice-producing regions of Portugal. From left to right the panels show the Mondego, Tagus, and Sado regions.

Paratrichodorus minor, *Pratylenchus crenatus*, and *Xiphinema citricolum*. Fig. 5 summarizes where each species was detected within Portugal's three main rice-producing regions.

The distribution maps clearly show regional patterns, some species, such as *H. dihystera* and *P. crenatus*, were widely distributed across regions, whereas others showed a more restricted occurrence, such is the case of *A. parietinus*, *M. incognita* and *P. minor* present only in the Mondego region, *M. joctus* in the Tagus region and *X. citricolum* in the Sado region. Despite the limited number of species identified molecularly, these results support the community level patterns described above, where Sado exhibited stronger dominance by PPN and reduced structural complexity, while Tagus supported a more heterogeneous assemblage. *Meloidogyne graminicola*, considered the most damaging rice nematode worldwide, was not detected in any of the surveyed fields.

4. Discussion

Environmental variability in flooded rice fields drives contrasting patterns in soil community composition (Bai et al., 2017). In this study, eighteen nematode genera were recorded across bacterivores, fungivores, omnivores, predators and PPN, with bacterivores, especially the genus *Rhabditis,* being the most frequent in Portuguese paddy fields. In rice production, this pattern is typical due to the frequent inputs (mineral N–P fertiliser, low C:N organic amendments) and their water regime (flooded and drained periods), which favours fast bacterial pathways and early successional opportunistic taxa (Ferris, 2001). This bacterivore prominence has also been reported in rice systems across Africa and Asia, indicating that the water level management drives this dominance more than local conditions (Liu et al., 2008; Mokuah et al., 2023; Okada et al., 2011). PPN *Helicotylenchus, Pratylenchus, Tylenchorhynchus* were abundant across the three basins under study, reflecting the host driven selection pressure imposed by continuous rice monoculture. Similar dominance has been documented in other rice systems: *Pratylenchus, Tylenchorhynchus,* and *Helicotylenchus* were among the most prevalent PPN in Chinese rice agroecosystems (Mo et al., 2021), while Eche et al. (2021) also reported their presence in Nigerian rice fields, highlighting the role of PPN as key components of rice nematofauna across different geographic regions. In this study, the genus *Tylenchus* was classified as a PPN despite of some species being considered facultative fungivores, this was based on its morphology, feeding structure, and ecological role. Moreover, several *Tylenchus* species are known to parasitize cereals and grasses, including rice, and are commonly grouped with PPN in similar agroecosystems (Mo et al., 2021; Okada et al., 2011; Siddiqi, 2000; Yeates et al., 1993).

Region was the primary driver of nematode assemblage, with communities separating clearly among Mondego, Tagus and Sado. Although not addressed in this study, the pronounced regional structuring has been associated with differences in water scheduling (flooding, mid-season drainage, reflooding), residue and fertiliser handling, soil texture and, in some cases, salinity. These drivers alter oxygen supply and the delivery of fresh carbon and nitrogen into the system, thereby shifting the balance among trophic groups (Du Preez et al., 2022; Korobushkin et al., 2019; Lv et al., 2014; Wu et al., 2008). GLM-NB at genus level reinforced this interpretation by showing spatial filtering across functional guilds. Rapid colonisers like *Panagrolaimus* are also prevalent in Portuguese rice fields, suggesting there are frequent inputs of fresh, easily decomposed organic matter as mentioned in previous studies (Ferris, 2001; Ferris et al., 2004; Ferris and Bongers, 2006). As reported in recent surveys on rice fields in Thailand and Kenya, PPN composition had regional patterns (Nimnoi et al., 2024; Ndirangu et al., 2024). For instance, *Paratrichodorus* and *Xiphinema* displayed strong spatial differences, in other studies, these genera have been linked often to coarser or better drained microsites and along field margins, whereas *Hemicriconemoides* and *Hemicycliophora* (ectoparasites with protective cuticles) tend to persist where disturbance is recurrent, but soils are not persistently reduced (Ndirangu et al., 2024). High prevalence of genus *Helicotylenchus* has also been described from Kenya and the Philippines (Coyne and Plowright, 2000; Mokuah et al., 2023). Moreover, Coyne et al. (2001) reported there is a positive correlation between flooded rice and the genus *Helicotylenchus*. Notably, the genus *Xiphinema* was detected at high abundance only in the Sado basin; however, model diagnostics indicated poor fit due to extreme sparsity/zero inflation, so differences for *Xiphinema* should be interpreted with caution.

Although all three river basins share similar rice-cropping practices involving continuous flooding and regular fertiliser inputs, differences in nematode diversity and food-web structure reflect contrasts in hydrology, soil properties, and management intensity. In the Mondego basin, rice cultivation is concentrated in the Lower Mondego Valley, within the Baixo Mondego Irrigation Scheme, a gravity-fed network with limited automation and collective management (Cunha et al., 2021; Gonçalves et al., 2022). This traditional water regime produces small temporal and spatial variation in flooding depth and drainage, generating hydrological heterogeneity that favours the coexistence of nematodes with different life-strategy groups. Consequently, both opportunistic (c-p 1/2) and more persistent taxa (c-p 3/4) coexist, maintaining moderate food-web structure. In contrast, the Tagus basin, Portugal's largest and most intensively managed rice-growing area relies on fully regulated irrigation and higher agrochemical use, which homogenise soil conditions and increase chemical stress (Almeida et al., 2018; Calha et al., 2023). These practices reduce habitat heterogeneity and the abundance of sensitive taxa, leading to lower Shannon diversity and dominance of opportunistic nematodes. Nevertheless, the relatively high MI2-5 values in Tagus suggest that field margins and drainage canals still support higher c-p taxa where organic residues and variable moisture maintain microhabitat diversity. The Sado basin, with intermediate rice-field density but fine-textured, poorly drained floodplain soils and a strong estuarine influence, exhibited the lowest MI2-5 and SI values, indicating a simplified and stress-prone food web. Long-term chemical inputs and periodic salinity intrusions from the estuary intensify anaerobic and osmotic stress, favouring tolerant and opportunistic taxa while excluding higher c-p groups (Biguino et al., 2024). The strong PPN pressure observed in Sado likely results from the combined effects of monoculture, reduced biological regulation, and environmental stress. Flooded and saline soils suppress nematode predators and microbial antagonists, allowing root-feeding PPN to persist and reproduce, while pesticide residues further weaken top-down control (Coyne and Plowright, 2000; Mokuah et al., 2023; Sánchez-Moreno and Ferris, 2018).

The EI - SI analysis supports these interpretations as samples mainly fall within Quadrants A and B, representing nutrient-enriched environments where bacterial-feeding, fast-reproducing nematodes (c-p 1/2) dominate, as expected in flooded rice soils that receive continuous nutrient inputs (Ferris et al., 2001, 2004). This pattern agrees with the consistently high EI (>90%) across all regions, confirming that rapid organic matter turnover through bacterial pathways is a common feature of Portuguese rice systems. However, regional contrasts in SI and MI2-5 revealed differences in the degree of food-web maturity. Sado samples, clustering mainly in Quadrant A, show high EI but low SI, characteristic of communities experiencing chronic disturbance and limited structural recovery. In contrast, Mondego and Tagus samples, grouped mainly in Quadrant B, displaying higher SI and MI2-5, consistent with more balanced systems where both enrichment and structure coexist. Similar EI–SI patterns, enriched yet structurally resilient nematode communities have been reported in Chinese and Japanese rice paddies and in Kenyan irrigated systems, where controlled flooding and moderate disturbance maintain functional stability (Liu et al., 2008; Mokuah et al., 2023; Okada et al., 2011).

Molecular identification confirmed the presence of eight PPN species in Portuguese rice fields. The distribution of these species was not uniform across regions, reinforcing the regional differences in nematode community structure described earlier. For instance, the wide distribution of *Helicotylenchus dihystera* and *Pratylenchus crenatus* is consistent

with their generalist feeding strategies and wide host ranges (Sikora et al., 2018). These restricted distributions emphasize the importance of local soil and management conditions in shaping PPN assemblages, a pattern that has also been reported in other rice systems (Coyne and Plowright, 2000; Mokuah et al., 2023; Okada et al., 2011).

Although *Aphelenchoides besseyi* has been recently reported in Portuguese rice fields (Inácio et al., 2025), it was not detected in our survey. This is consistent with its biology, since the species survives mainly in seeds and is rarely found in soil. In this study seed samples were not included because they represent a different epidemiological pathway linked to post-harvest survival and international trade.

Interestingly, *Meloidogyne graminicola*, considered the most damaging PPN of rice worldwide (Mantelin et al., 2017; Rusinque et al., 2021), was not detected in any of the surveyed fields. This absence diverges from reports from South America, Asia and Africa, where *M. graminicola* is prevalent and causes severe rice yield losses (De Waele and Elsen, 2007; Win et al., 2015). The apparent absence of *M. graminicola* in Portugal may be due to climatic limitations, differences in irrigation management, or absence of introduction pathways. However, the detection of *M. incognita* indicates that Portuguese rice fields remain vulnerable to significant PPN impacts.

5. Conclusion

This survey provides the first baseline of soil nematode communities associated to Portuguese rice agroecosystems. The communities across the studied basins were enrichment-dominated and region-structured, with higher trophic structure in Tagus and simplified food webs in Sado basin. Eight PPN species were found, however, the most damaging root nematode of rice, *M. graminicola* was not detected. These findings support regular nematode monitoring and targeted surveillance to maintain soil function and pest risks reduction.

Funding

This research was supported by the “Fundação para a Ciência e a Tecnologia” (FCT, Portugal) and the European Social Funds, through the “Programa Operational Regional Centro”, under the Ph.D. fellowship 2020.05541.BD and through the R&D Units GREEN-IT—Bioresources for Sustainability (UIDB/04551/2020) and Chemical Engineering and Renewable Resources for Sustainability (UID/00102/2025 - https://doi.org/10.54499/UID/00102/2025; UID/PRR/00102/2025 - https://doi.org/10.54499/UID/PRR/00102/2025). SRC acknowledges funding from CBMA (https://doi.org/10.54499/UIDB/04050/2020), supported by the “Contrato-Programa” UIDB/04050/2020 by national funds through the FCT I.P.

CRediT authorship contribution statement

Leidy Rusinque: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Carla Maleita:** Funding acquisition, Resources, Supervision, Writing – review & editing. **Sofia R. Costa:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Ana Rita Varela:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Maria L. Inácio:** Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the technicians of the Laboratory of Nematology INIAV—Nema-INIAV, Margarida Fontes, Nídia Laureano, Marina Cardoso e Lourdes Silva, and the Laboratory of Biochemistry and Molecular Genetics.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2026.101319.

Data availability

Data will be made available on request.

References

Almeida, C., Ramos, T.B., Segurado, P., Branco, P., Neves, R., Proença de Oliveira, R., 2018. Water quantity and quality under future climate and societal scenarios: a basin-wide approach applied to the Sorraia river, Portugal. Water 10 (9), 1186. https://doi.org/10.3390/w10091186.

Anderson, M.J., 2017. In: Balakrishnan, N., Colton, T., Everitt, B., Piegorsch, W., Ruggeri, F., Teugels, J.L. (Eds.), Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online, John Wiley & Sons, Ltd., Hoboken, NJ, pp. 1–15. https://doi.org/10.1002/9781118445112.stat07841.

Bai, R., Xi, D., He, J.Z., Hu, H.W., Fang, Y.T., Zhang, L.M., 2017. Microbial community and functional structure significantly varied among distinct types of paddy soils but responded differently along gradients of soil depth layers. Front. Microbiol. 8, 945. https://doi.org/10.3389/fmicb.2017.00945.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515 (7528), 505–511. https://doi.org/10.1038/nature13855.

Biguino, A., Antunes, J.C., Dias, J.M., Pires, A., Lillebo, A.I., 2024. Long-term trends (1986–2019) in the physicochemical properties of the Sado Estuary (Portugal): river flow, marine influence and meteorological conditions. Mar. Pollut. Bull. 207, 116806. https://xxxxx.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14–19. https://doi.org/10.1007/BF00324627.

Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. Appl. Soil Ecol. 10 (3), 239–251.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends Ecol. Evol. 14 (6), 224–228. https://doi.org/10.1016/S0169-5347(98)01583-3.

Calha, I., Oliveira, MdF., Reis, P., 2023. Weed management challenges in rice cultivation in the context of pesticide use reduction: a survey approach. Sustainability 15 (1), 244. https://doi.org/10.3390/su15010244.

Carta, L.K., Li, S., 2018. Improved 18S small subunit rDNA primers for problematic nematode amplification. J. Nematol. 50 (4), 533–542.

Coyne, D., Smith, M., Plowright, R., 2001. Plant parasitic nematode populations on upland and hydromorphic rice in Côte d'Ivoire: relationship with moisture availability and crop development on a valley slope. Agric. Ecosyst. Environ. 84, 31–43. https://doi.org/10.1016/S0167-8809(00)00193-6.

Coyne, D., Plowright, R.A., 2000. Nematode threats to intensifying smallholder upland rice production in the Guinea savannah of Côte d'Ivoire. Trop. Sci. 40, 67–74. https://hdl.handle.net/10568/114652.

Cunha, M., Marques, J., Azevedo, J., Castilho, A., 2021. Understanding the impact of a major hydro-agricultural project in low mondego area (Portugal). Land 10 (2), 114. https://doi.org/10.3390/land10020114.

De Waele, D., Elsen, A., 2007. Challenges in tropical plant nematology. Annu. Rev. Phytopathol. 45, 457–485. https://doi.org/10.1146/annurev.phyto.45.062806.094438.

DGT, 2022. Carta administrativa oficial de Portugal - CAOP2022 (Direção-Geral do Território). Available at: https://www.dgterritorio.gov.pt/cartografia/cartografia tematica/caop#tab-24b1t-1. June 2025.

Du Preez, G., Daneel, M., De Goede, R., Du Toit, M.J., Ferris, H., Fourie, H., et al., 2022. Nematode-based indices in soil ecology: application, utility, and future directions. Soil Biol. Biochem. 169, 108640. https://doi.org/10.1016/j.soilbio.2022.108640.

Eche, C.O., Oluwatayo, J.I., Okeme, A.E., 2021. Spatial distribution and community analysis of plant-parasitic nematodes associated with rice (*Oryza sativa* L.) in Benue state, Nigeria. Acta Entomol. Zool. 2 (2), 39–47. https://doi.org/10.33545/27080013.2021.v2.i2a.42.

EPPO - European Plant Protection Organisation, 2013. Standard protocol PM 7/119 (1). Nematode extraction. EPPO Bull. 43, 471–495.

European Commission, 2021. EU Soil Strategy for 2030: Reaping the Benefits of Healthy Soils for People, Food, Nature and Climate.

Ferris, H., 2001. Contribution of nematodes to the structure and function of the soil food web. J. Nematol. 33 (4), 193–199.

Ferris, H., 2010. Form and function: metabolic footprints of nematodes in the soil food web. Eur. J. Soil Biol. 46, 97–104. https://doi.org/10.1016/j.ejsobi.2010.01.003.

Ferris, H., Bongers, T., de Goede, R.G.M., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18 (1), 13–29. https://doi.org/10.1016/S0929-1393(01)00152-4.

Ferris, H., Venette, R.C., Lau, S.S., 2004. Dynamics of nematode communities in tomatoes grown in conventional and organic farming systems, and their impact on

soil fertility. Appl. Soil Ecol. 26 (2), 143–158. https://doi.org/10.1016/j.apsoil.2003.10.009.
Ferris, H., Bongers, T., 2006. Nematode indicators of organic enrichment. J. Nematol. 38 (1), 3–12.
Furmanczyk, E.M., Kozacki, D., Ourry, M., Bickel, S., Olimi, E., Masquelier, S., et al., 2025. An analysis of soil nematode communities across diverse horticultural cropping systems. Soil Syst. 9 (3), 77. https://doi.org/10.3390/soilsystems9030077.
Gonçalves, J.M., Ferreira, S., Nunes, M., Eugénio, R., Amador, P., Filipe, O., et al., 2020. Developing irrigation management at district scale based on water monitoring: study on Lis Valley, Portugal. AgriEngineering 2 (1), 78–95. https://doi.org/10.3390/agriengineering2010006.
Gonçalves, J.M., Nunes, M., Ferreira, S., Jordão, A., Paixão, J., Eugénio, R., et al., 2022. Alternate wetting and drying in the center of Portugal: effects on water and rice productivity and contribution to development. Sensors 22 (10), 3632. https://doi.org/10.3390/s22103632.
Goodey, T., 1963. Soil and Freshwater Nematodes. Rewritten by J. B. Goodey, second ed. London: John Wiley & Sons, New York, p. 544.
Guerra, C.A., Bardgett, R.D., Caon, L., Crowther, T.W., Delgado-Baquerizo, M., Montanarella, L., et al., 2021. Tracking, targeting, and conserving soil biodiversity. Science 371 (6526), 239–241.
Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J., Helder, J., 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Mol. Biol. Evol. 23 (9), 1792–1800. https://doi.org/10.1093/molbev/msl044.
Hu, M., Höglund, J., Chilton, N.B., Zhu, X., Gasser, R.B., 2002. Mutation scanning analysis of mitochondria cytochrome c oxidase subunit 1 reveals limited gene flow among bovine lungworm subpopulations in Sweden. Electrophoresis 23, 3357–3363. https://xxx.
Hunt, D.J., 1993. Aphelenchida, Longidoridae and Trichodoridae. In: CABI. Practical Keys to Genera in Three Agriculturally Important Groups (Includes virus-vector Genera) xxxxx.
Inácio, M.L.S., Rusinque, L.C.M., Nóbrega, F., Varela, R., 2025. Detection of *Aphelenchoides besseyi* in major rice-growing regions of Portugal: tracing a possible introduction pathway through seed trade. Plant Dis. https://doi.org/10.1094/PDIS-07-25-1513-SC. Advance online publication.
IPMA - Instituto Português do Mar e da Atmosfera, 2025. Clima De Portugal Continental, Classificação De Köppen (Área Educativa). Available at: ipma.pt, Área educativa, Tempo e Clima (Accessed 25 September 2025).
Jairajpuri, M.S., Ahmad, W., 1992. Dorylaimida: free-living, predaceous and plant-parasitic nematodes. Fundam. Appl. Nematol. 3, 286–287, 1993.
Korobushkin, D.I., Butenko, K.O., Gongalsky, K.B., Saifutdinov, R.A., Zaitsev, A.S., 2019. Soil nematode communities in temperate rice-growing systems. Eur. J. Soil Biol. 93, 103099. https://doi.org/10.1016/j.ejsobi.2019.103099.
Kumar, S., Glen, S., Koichiro, T., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33 (7), 1870–1874. https://doi.org/10.1093/molbev/msw054.
Lenth, R.V., 2024. EMMEANS: estimated marginal means, aka least-squares means. https://doi.org/10.32614/CRAN.package.emmeans.
Liu, M., Chen, X., Qin, J., Wang, D., 2008. A sequential extraction procedure reveals that water management affects soil nematode communities in paddy fields. Appl. Soil Ecol. 40 (2), 250–259. https://doi.org/10.1016/j.apsoil.2008.05.001.
Lv, Y., Gu, X., Zhang, W.D., 2014. The responses of soil nematode assemblages to disturbance in liaohe estuary wetlands. Eur. J. Soil Biol. 61, 6–11. https://doi.org/10.1016/j.ejsobi.2013.12.003.
Magurran, A.E., 2021. Measuring biological diversity. Curr. Biol. 31 (19), R1174–R1177. https://doi.org/10.1016/j.cub.2021.07.049.
Mai, W.F., Mullin, P.G., 1996. Plant-Parasitic Nematodes: a Pictorial Key to Genera, fifth ed. Cornell University Press, Ithaca, NY.
Mantelin, S., Bellafiore, S., Kyndt, T., 2017. *Meloidogyne graminicola*: a major threat to rice agriculture. Mol. Plant Pathol. 18 (1), 3–15. https://doi.org/10.1111/mpp.12394.
Mo, Y.X., Chen, W.J., Ye, W., Yu, H., 2021. Systematic investigation of plant-parasitic nematodes in rice fields: occurrence, diversity, and community structure. Front. Microbiol. 12, 799270.
Mokuah, D., Karuri, H., Nyaga, J.M., 2023. Food web structure of nematode communities in irrigated rice fields. Heliyon 9 (2), e13183. https://doi.org/10.1016/j.heliyon.2023.e13183.
Ndirangu, K., Pili, N.N., Chelal, J., Biwot, J., Kandie, F., Razieh, Y., et al., 2024. Occurrence of plant-parasitic nematodes on rice (*Oryza sativa*) in Kenya with a focus on *Hirschmanniella oryzae*. Nematology 26 (10), 1179–1193. https://doi.org/10.1163/15685411-bja10364.
Neher, D.A., 2010. Ecology of plant and free-living nematodes in natural and agricultural soil. Annu. Rev. Phytopathol. 48, 371–394. https://doi.org/10.1146/annurev-phyto-073009-114439.
Neher, D.A., Darby, B.J., 2006. Computation and application of nematode community indices: general guidelines. In: Abebe, E., Traunspurger, W., Andrássy, I. (Eds.), Freshwater Nematodes: Ecology and Taxonomy. CABI, Wallingford, UK, pp. 211–222. https://doi.org/10.1079/9780851990095.0211.
Nimnoi, P., Pirankham, P., Srimuang, K., Ruanpanun, P., 2024. Insights into soil nematode diversity and bacterial community of Thai jasmine rice rhizosphere from different paddy fields in Thailand. PeerJ 12, e17289. https://doi.org/10.7717/peerj.17289.
Norton, D.C., 1978. Ecology of plant-parasitic Nematodes. John Wiley & Sons, New York, USA, p. 268.
Nunn, G.B., 1992. Nematode Molecular Evolution. University of Nottingham, Nottingham, UK. PhD dissertation.
Okada, H., Niwa, S., Takemoto, S., Komatsuzaki, M., Hiroki, M., 2011. How different or similar are nematode communities between a paddy and an upland rice fields across a flooding–drainage cycle? Soil Biol. Biochem. 43, 2142–2151. https://doi.org/10.1016/j.soilbio.2011.06.018.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2019. Vegan: community ecology package. https://CRAN.R-project.org/package=vegan.
Orgiazzi, A., Singh, B., Wall, D., Barrios, E., Kandeler, E., Moreira, F., et al., 2016. Global Soil Biodiversity Atlas. Publications Office of the European Union, Luxembourg.
Rusinque, L., Maleita, C., Abrantes, I., Palomares-Rius, J.E., Inácio, M.L., 2021. *Meloidogyne graminicola* - a threat to rice production: review update on distribution, biology, identification, and management. Biology 10 (11), 1163. https://doi.org/10.3390/biology10111163.
Siddiqi, M.R., 2000. Tylenchida: parasites of plants and insects. In: CABI. Authoritative Keys for Tylenchid (plant-parasitic) Genera, second ed.
Sieriebriennikov, B., Ferris, H., de Goede, R.G.M., 2014. NINJA: an automated calculation system for nematode-based biological monitoring. Eur. J. Soil Biol. 61, 90–93. https://doi.org/10.1016/j.ejsobi.2014.02.004.
Sikora, R.A., Coyne, D., Hallmann, J., Timper, P., 2018. Reflections and challenges: nematology in subtropical and tropical agriculture. In: Sikora, R.A., Coyne, D., Hallmann, J., Timper, P. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI, Wallingford UK, pp. 1–19.
Sánchez-Moreno, S., Ferris, H., 2018. Nematode ecology and soil health. In: Sikora, R.A., Coyne, D., Hallmann, J., Timper, P. (Eds.), Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CABI, Wallingford UK, pp. 62–86.
Sohlenius, B., Persson, H., Magnusson, C., 1977. Distribution of roots and nematodes in a young Scots pine stand in central Sweden. Ecol. Bull. 25, 340–347.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, fourth ed. Springer.
Ver Hoef, J.M., Boveng, P.L., 2007. Quasi-Poisson vs. negative binomial regression: how should we model overdispersed count data? Ecology 88, 2766–2772. https://doi.org/10.1890/07-0043.1DigitalObjectIdentifier.
Win, P.P., De Waele, D., Kyi, P.P., Maung, Z.T.Z., Myint, Y.Y., 2015. Comparison of the damage potential and yield loss of the rice root-knot nematode *Meloidogyne graminicola* on lowland and upland rice varieties from Myanmar. Russ. J. Nematol. 23 (1), 53–72.
Wu, H.Y., Li, X.X., Shi, L.B., Wang, Z.H., Ma, F.Y., 2008. Distribution of nematodes in wetland soils with different distance from the Bohai sea. Plant Soil Environ. 54, 359–366. https://doi.org/10.17221/417-PSE.
Yeates, G.W., Bongers, T., De Goede, R., Freckman, D.W., Georgieva, S., 1993. Feeding habits in soil nematode families and genera an outline for soil ecologists. J. Nematol. 25, 315–331.