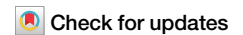


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Divergent alpha and beta diversity trends of soil nematode fauna along gradients of environmental change in the Carpathian Ecoregion



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There is a significant lack of research on how climate change influences long-term temporal trends in the biodiversity of soil organisms. Nematodes may be specifically adequate to test soil biodiversity changes, because they account for ~80% of all Metazoans and play key roles in the functioning of terrestrial ecosystems. Here, we report on the first synthesis study focused on temporal trends of nematode fauna over a period of 14 years (1986–1999) across the Carpathian Ecoregion. We provide new evidence that wetter conditions associated to global change contributes to driving nematode diversity at genus/family level. We observed opposite trends in soil nematode alpha diversity (increase) and beta diversity (decrease) consistent across ecosystem types and soil horizons, providing strong evidence for the influence of climate change on soil biodiversity at large spatial scales. An increase in the community functional uniformity along with a decline in beta diversity indicated more homogenous soil conditions over time. The Soil Stability Index (metric devised to assess soil homeostasis based on the functional composition of nematode communities) increased over time, indicating a decline of soil disturbances and more complex soil food webs. Our results highlight the importance of nematodes as powerful indicators of soil biodiversity trends affected by multiple facets of environmental change in long-term soil monitoring.

As a growing human footprint is reshaping the Earth's ecosystems¹, addressing temporal changes in biodiversity emerges as a key challenge^{2,3}. Moreover, biodiversity trends differ among groups of organisms, metrics, and scales^{4,5}. Traditionally, studies have focused on alpha and gamma diversity, but elucidating temporal changes in community composition is needed to fully explain and predict anthropogenic biodiversity changes^{5–7}. For example, temporal variations in compositional reorganization and biotic homogenization or differentiation of communities may be more sensitive indicators of biodiversity change^{8–13}. Despite some recent advances, research efforts on the temporal trends and underlying drivers of soil communities are scarce¹⁴. However, knowledge on biodiversity change comes from time-series abundance datasets for thousands of species over large geographic and temporal scales¹⁵. While many studies have focused on

plants and vertebrates, soil fauna, such as nematodes, have been neglected in most taxonomic databases, conservation actions, and policies^{16,17}. Given that soil biodiversity accounts for ~59% of all species on Earth^{17,18} and may show dissimilar distribution patterns than better-studied aboveground taxa^{19,20}, we urgently need more information on how different facets of soil biodiversity change over time and what are the underlying drivers of change.

Land-use change and climate change are key determinants of temporal changes in biodiversity^{2,3,21,22}, and these factors may even exert synergistic effects when acting simultaneously^{23–27}. In particular, soil biodiversity may be threatened by intensive land-use measures such as inorganic fertilization and tillage, pollution with pesticides and heavy metals, as well as altered climatic conditions and extreme weather events such as warming and droughts²⁸. The co-occurrence of different stressors can have exceptionally

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detrimental effects on soil biodiversity and functioning^{29,30}. However, soil biodiversity responses to global change drivers may also be context dependent, varying with the ecosystem type and soil depth. For example, effects of climate change and extreme weather events on alpha diversity may differ between forests and grasslands^{31–33}, with little being known about temporal trends of soil biodiversity across ecosystem types. Moreover, soil biodiversity is known to strongly decline with soil depth^{32,34}, while climate change effects on soil communities may be more pronounced in the organic as compared to more buffered, deeper soil layers^{35,36}, more difficult to sample and therefore less investigated.

Nematodes are the most abundant soil animals, with estimates ranging between 80 and 90% of all individual animals on Earth and a total number of nematode species of 1–10 million^{37,38}. Soil nematodes are represented in most trophic levels of the soil food web and are driving fundamental ecological processes such as root herbivory and litter decomposition processes³⁹. This makes them useful indicators for assessing environmental disturbances and soil health^{40–42}. While the global distribution and main drivers of nematode communities were reported recently³⁷, long-term temporal trends of soil nematode diversity remain unexplored. Global change effects on nematode fauna may be ecosystem specific⁴³, and therefore understanding drivers of nematode community composition across ecosystems is essential in forecasting long-term climate change-related effects on ecosystem functions and services.

Here, we address this research gap by exploring: (i) temporal changes in soil nematode communities considering multiple biodiversity facets, i.e., different diversity metrics that contain complementary information on how biodiversity has changed across scales, reflecting taxonomic distinctiveness and functional variation in community composition across different ecosystem types and soil horizons; and (ii) the effects of climate change-related variables on the temporal changes in soil nematode communities. We used empirical data from an extensive survey carried out between 1986 and 1999 on nematode fauna of organic and mineral soil horizons in forests and grasslands distributed in the Carpathian Ecoregion of Eastern Europe, with such highly biodiverse mountain ecosystems being particularly sensitive to climate change^{44–46}. As this survey spans over 14 years, the investigation meets the recommendations for temporal studies on soil biodiversity, which are supposed to exceed 10 years⁴⁷.

We monitored temporal changes in nematode diversity across different spatial scales by exploring alpha and beta diversity²⁵. At the local scale, we quantified the taxa richness of each sample to assess temporal changes in alpha diversity. We further used temporal beta diversity to compare the composition of local nematode communities considering the variance of community data among samples within a single site, i.e., local scale beta diversity^{5,48}. Additionally, we assessed the temporal variation in the functional composition of nematode communities by considering different life-history strategies (i.e., feeding habits and if they are rather efficient colonizers or persister (colonizers of new resources in dynamic ecosystems and persists in undisturbed habitats., cp-scale)), allowing for a detailed understanding of the dynamics of community structure over time⁴⁹. On the basis of this data, we employed nematode-based indices to explore temporal changes in soil health status (i.e., Soil Stability Index) (SSI, see “Methods”) and its response to climate change. Given the historical water stress in the Carpathian Ecoregion before the early 1980s, with extensive drainage and acid rain events causing soil degradation^{50,51}, we included the annual variation of drought in our study.

We hypothesized that: (1) nematode taxonomic diversity at genus/family level changes considering multiple spatial scales significantly over time and that these changes are influenced by temporal variation in climatic variables. More specifically, we expected to observe an increase in nematode taxa richness coupled with increasing dissimilarity between community assemblages over time due to increasing soil moisture levels and increasingly beneficial environmental conditions in degraded soils in the late 70 s and early 80 s. Similarly, (2) the nematode-based Soil Stability Index (SSI) (i.e., indicating soil health status) was expected to increase over time due to increasingly beneficial climatic conditions. Moreover, we hypothesized (3) the impact of temporal changes in precipitation to induce different temporal

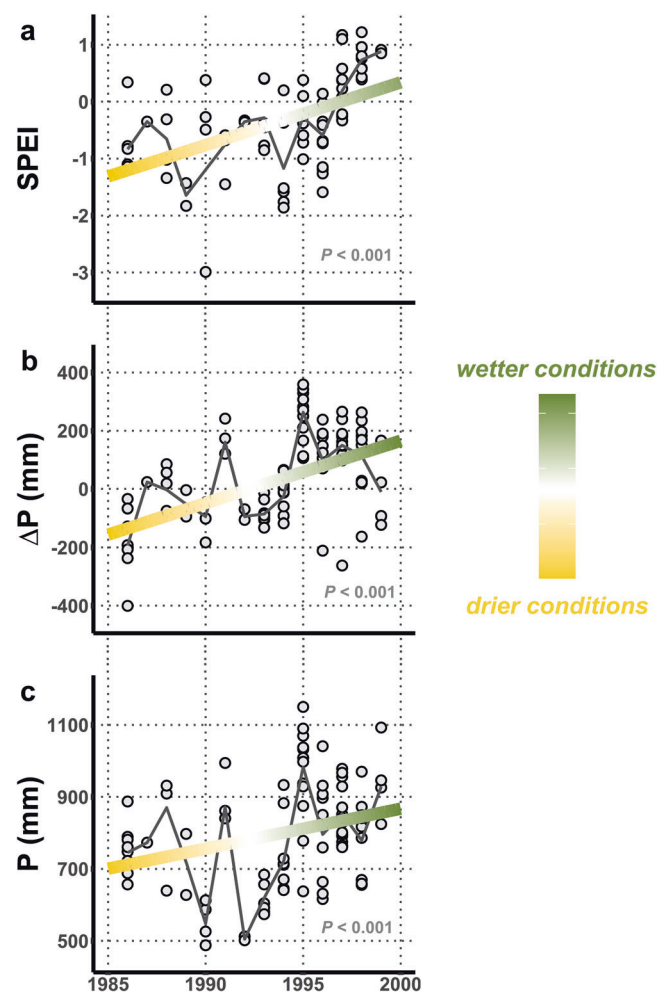


Fig. 1 | Temporal trends in precipitation-related variables. Time data analysis of the environmental water conditions is examined using the indices of **a** Standardized Precipitation-Evapotranspiration Index (SPEI), **b** the inter-annual variability of precipitation (ΔP) and **c** the annual mean precipitation for the sampling year (P). Black lines indicate the mean of the climate variables in each sampling year. All fitted trends are fitted by a linear trend based on the best GLS model according to Akaike information criterion (AIC).

changes in nematode taxa richness and beta diversity across ecosystem types (forests and grasslands) and soil horizons, because grasslands generally have lower levels of resilience than forest ecosystems, and mineral soil horizons are assumed to be better buffered against environmental fluctuations than organic horizon. Finally, we expected (4) changes in the functional composition of the nematode communities to occur over time, with an increasing proportion of specialist taxa with more conservative life strategies (more persisters than colonizers), and a higher proportion of predators and omnivores that indicate higher food web complexity and stability.

Results

Temporal climate changes

Temporal change models, conducted at the regional site level, demonstrated evidence of a climate change scenario (~14 years; 1986–2000) in the Romanian Carpathians and the Transylvanian Plateau (Fig. 1). Annual mean temperature (T) and precipitation (P), inter-annual variability of temperature (ΔT) and precipitation (ΔP), the standardized precipitation-evapotranspiration index (SPEI), and the number of severe cold days (NSCD) were retained for the forward selection procedure, after accounting for correlated variables driving spatial-temporal patterns of nematode communities. As expected, the observed climate change referred mainly to temporal changes in precipitation-related variables (Fig. 1). Although no

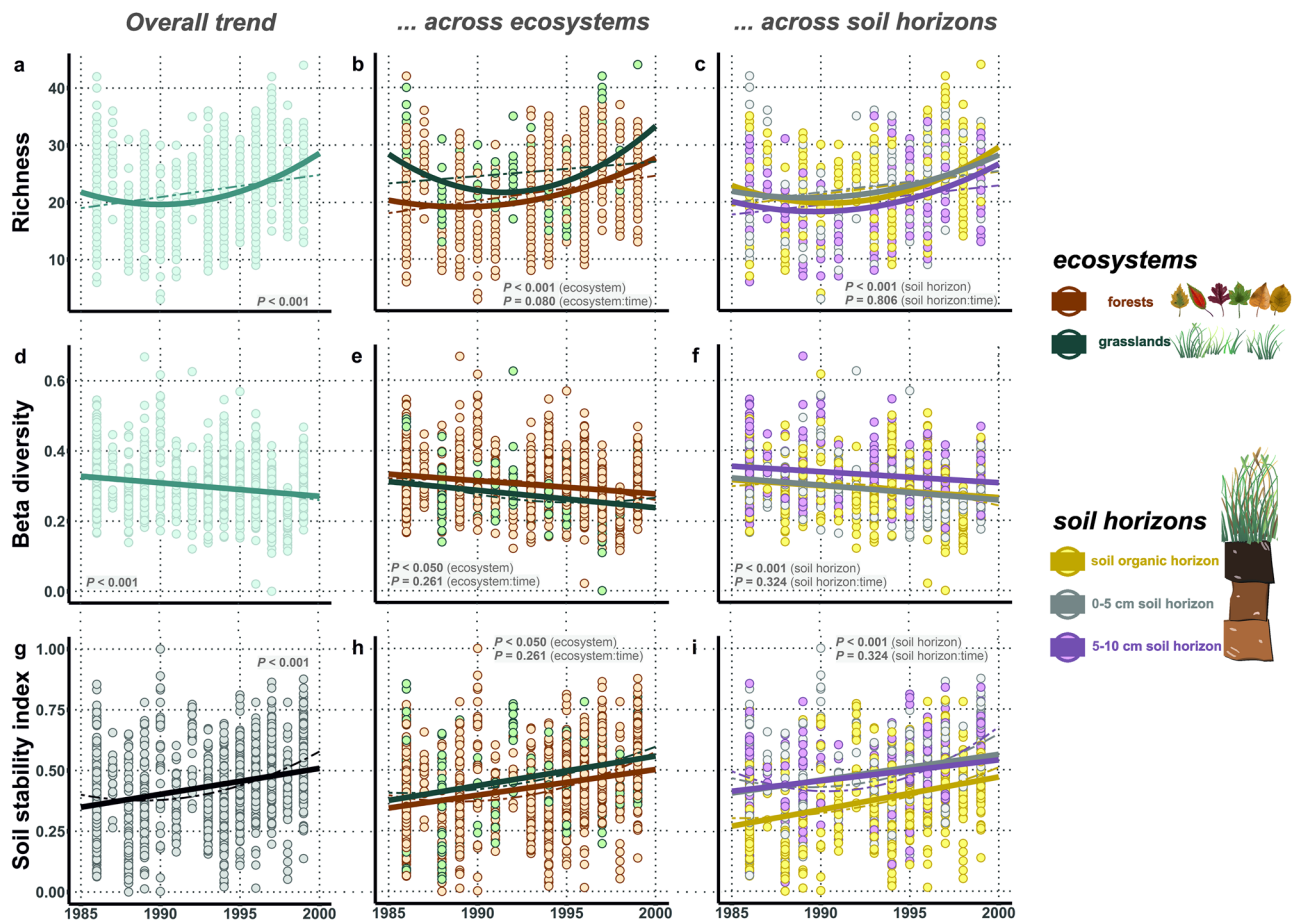


Fig. 2 | Temporal trends in nematode diversity and Soil Stability Index (SSI). Time data analysis of the nematode communities is examined using metrics of alpha diversity with **a–c** nematode taxa richness and with **d–f** beta diversity. SSI (**g–i**) was based on the variation of nematode-based indices for each plot (see more details in

“Methods”). For each year, we plotted the mean and SE of the diversity metrics across all plots, which were then used to fit the regression lines. All curves are fitted by a quadratic polynomial and a linear trend. All fitted trends are significant based on LMMS, and solid lines indicate the best model according to Akaike information criterion (AIC).

correlations were noted among the selection of precipitation-related variables (after forward selection procedure), our analyses revealed a pronounced convergence in the directionality and trend of temporal changes in annual precipitation (P) and interannual variability of precipitation (ΔP). That is, the strong annual increase in precipitation (P ; $P < 0.001$; Fig. 1c) coincided with a substantial increase in precipitation variability (ΔP ; $P < 0.001$; Fig. 1b) over time. Moreover, these models also revealed positive trends in climatic water balance (i.e., higher SPEI, $P < 0.001$; Fig. 1a) and thus increasingly wetter conditions over time. This was accompanied by rather stable temperature-related variables over time (i.e., annual mean temperature (T) and interannual variability of temperature (ΔT); Supplementary Fig. 1). However, the number of extreme cold events declined over time (i.e., lower NSCD; $P < 0.001$; Supplementary Fig. 1).

Temporal trends in nematode diversity and Soil Stability Index (SSI)

In contrast to our expectations, our results revealed opposite temporal trends for nematode alpha and beta diversity (Fig. 2). At the plot scale, while nematode taxa richness increased over time from an average of 19–26 taxa (+37%; $P < 0.001$; Fig. 2a; better adjusted with a quadratic trend), our temporal trend models revealed a gradual decline in the within-site spatial turnover of the nematode community over the period 1986–2000 from an average of 0.313 to 0.255 (–18%; $P < 0.001$; Fig. 2d). Meanwhile, as SSI increased over time (+41%; SSI, $P < 0.001$; Fig. 2g), we did not find any significant connection between an increased SSI with a higher variation in community composition within the site, since beta diversity gradually decreased in the studied time period.

Our results further indicate that temporal changes in nematode diversity were mostly consistent across ecosystems and soil horizons, although the value range differed (Fig. 2). This was especially marked for nematode taxa richness, with higher values in grassland, and even increasing differences in comparison to forest in the earliest and latest years (i.e., quadratic trend), leading to almost distinct values between ecosystems ($P < 0.001$; Fig. 2b). However, the temporal trend was different only when distinguishing between distinct forest types and only for beta diversity, since, for example, a smaller decline in within-site species turnover occurred for mixed forests (Supplementary Fig. 2). The temporal change in SSI was also consistent among the two types of ecosystems (i.e., grasslands and forests) and soil horizons (Fig. 2h, i). However, and similar to diversity, we found a significant effect of ecosystem type and soil depth on SSI. For instance, grasslands had consistently higher values for taxa richness and SSI (Fig. 2b, f, i), while the deepest soil horizon had consistently lower taxa richness, but higher beta diversity and soil stability than the organic horizon (Fig. 2c, f, i).

Temporal trends in functional indices and community composition of nematodes

In addition to changes in nematode diversity, the functional composition of soil nematodes also shifted over time (Fig. 3). First, we examined how nematode community structure varied over time by exploring the proportion of each trophic group within the overall nematode community. Fungal feeders and plant feeders were the only groups that showed significant and opposite temporal changes over time (Fig. 3a, b), as plant feeders gradually increased while fungal feeders strongly declined over time

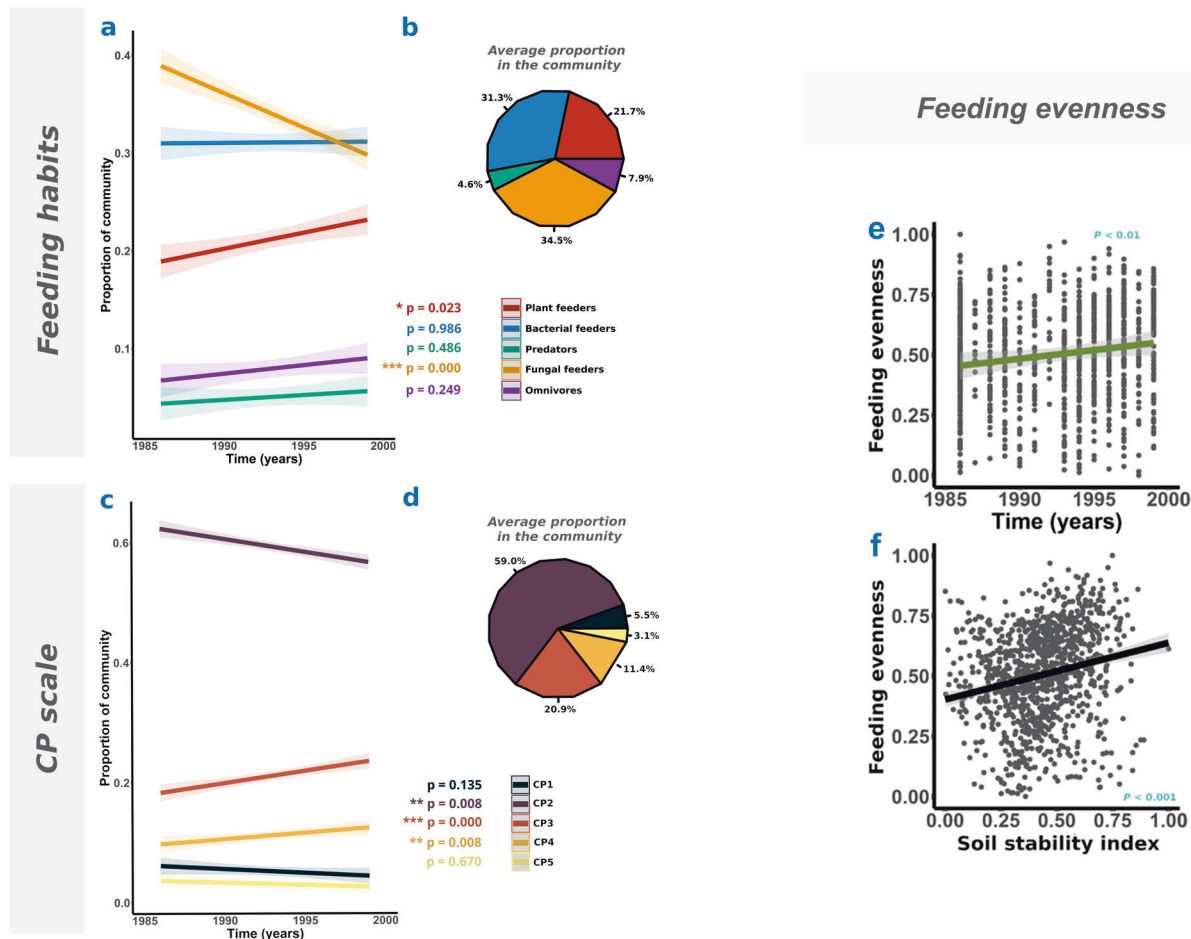


Fig. 3 | Temporal changes in the functional composition of nematode communities. Time data analysis of the functional composition of nematode communities is examined using **a**, **b** feeding habits, **c**, **d** colonizer-persister scale and with **e** feeding evenness. Additionally, the **f** relationship between feeding evenness and SSI is shown.

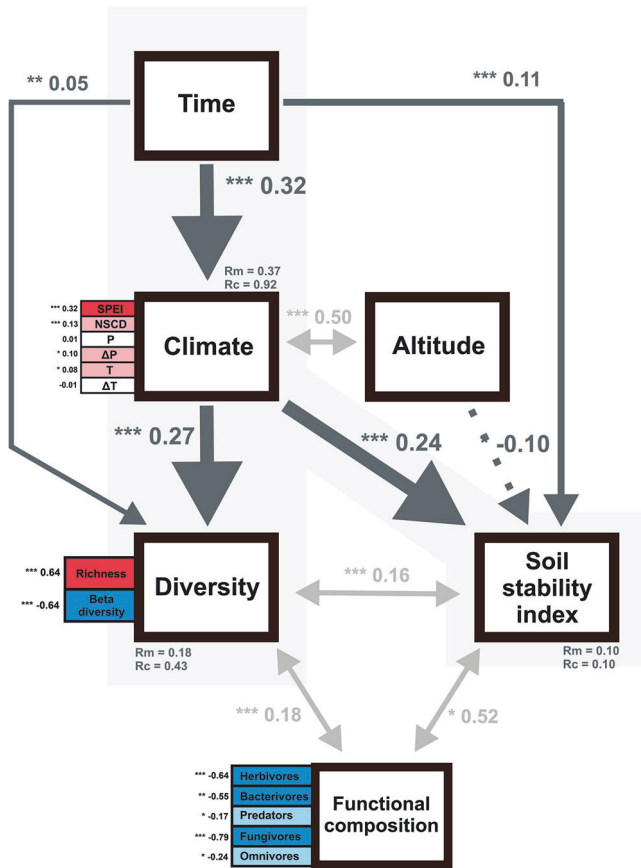
Feeding evenness was based on the uniformity of the proportion of each feeding habits in the overall nematode community. For each year, we plotted the mean and SE of the diversity metrics across all plots, which were then used to fit the regression lines. All curves are fitted by linear trend. All fitted trends are significant based on LMMS.

(Fig. 3a, b). We then investigated whether there are any significant changes in the uniformity of the functional composition of nematode communities over time. In fact, the temporal regression model indicated a steady increase in community functional uniformity over time (i.e., feeding evenness; $P < 0.01$; Fig. 3e). Additionally, we detected a positive relationship between this ratio of uniformity and the SSI ($P < 0.001$; Fig. 3f). We also detected significant changes in nematode communities along the colonizer-persister (cp) scale over time (Fig. 3c, d). More specifically, cp2 nematodes decreased whereas cp3 and cp4 nematodes increased over time, confirming the gain and spread of more stress-sensitive taxa (i.e., mostly larger nematodes that are favored by a less disturbed environment).

Temporal changes in stability and diversity effects

Although our conceptual model was based on multiple potential causal associations (Supplementary Fig. 3), structural equation modeling (SEM) helped to support our hypothesis that temporal changes in nematode diversity are attributed to climatic changes across the 14 years covered by our study. Certainly, this pathway was consistent in the two SEMs evaluated (i.e., in the simplified and complex models with and without habitat types; Fig. 4 and Supplementary Fig. 3, respectively). Although both models were significant, the simplistic model showed a better fit than the complex one ($P < 0.001$; Chi-square Difference Test). SEM first confirmed our temporal climate modeling results, which indicated significant shifts in climate over time (standardized parameter estimates (SE) = 0.32; $P < 0.001$). Likewise, SEM revealed that nematode diversity was positively influenced by temporal changes in climate (SE = 0.27; $P < 0.001$; Fig. 4). Interestingly, temporal

changes in nematode diversity (Fig. 2), largely dominated by positive and negative changes in taxa richness and beta diversity, respectively (SE = 0.64 and -0.64 , $P < 0.001$; see the structure of the composite variable diversity in Fig. 4), were mainly attributed to climatic effects, given the weak relationship between time and diversity components (SE = 0.05; $P < 0.01$). SEM also revealed that SSI was positively influenced by climate variables over time (SE = 0.24; $P < 0.001$), and with a significant association with nematode diversity (SE = 0.16; $P < 0.001$; Fig. 4). As for nematode diversity, we found a significant positive relationship between time and SSI (SE = 0.11; $P < 0.001$), which may indicate an additional unobserved environmental driver. Although multiple significant relationships were found between diversity and SSI with climatic variables (Supplementary Figs. 5 and 6), SEM revealed that the effects of climate on nematode diversity and SSI were mainly attributed to changes in SPEI (SE = 0.32; $P < 0.001$; Fig. 4). That is, increasing SPEI over time (Fig. 1a) positively influenced nematode diversity (i.e., taxa richness) and SSI. On the other hand, nematode diversity and SSI were also positively associated with the functional composition of nematode communities (SE = 0.18, $P < 0.001$; SE = 0.52, $P < 0.05$; respectively) (Fig. 4). The effect of the functional composition may be largely attributed to variation in nematode communities of fungal, bacterial, and plant feeders (Fig. 4), given their high proportion found in the overall communities and the significant temporal trend detected for the trophic groups of fungal and plant feeders (Fig. 3). In fact, a higher proportion of fungivore nematodes was significantly related to low levels of SSI, as opposed to plant feeders (Supplementary Fig. 7). Finally, our SEM including environmental contexts revealed that the habitat significantly influenced both nematode diversity



AIC = 48.1; BIC = 167.4; Fisher's C = 0.04; P-value = 0.978; 2 df

Fig. 4 | Direct and indirect drivers of temporal changes in nematode diversity and the Soil Stability Index (SSI). PiecewiseSEM testing the direct and indirect relationships between climate, nematode diversity, and functional community composition, as well as SSI in a long-term time series (14 years, 1986–1999), considering the functional composition of the nematode communities and altitude simultaneously ($n = 1069$ soil samples, 120 sites). All measured variables are arranged into the following composite variables: climate (annual mean temperature (T) and precipitation (P), inter-annual variability of temperature (ΔT) and precipitation (ΔP), standardized precipitation-evapotranspiration index (SPEI), the number of severe cold days (NSCD, $T_{min} < -10^\circ\text{C}$)), nematode diversity (nematode taxa richness and beta diversity) and functional composition (proportion of plant feeders, bacterial feeders, fungal feeders, predators and omnivores in the overall community). Numeric variables were standardized using z-scores, except for evenness, beta diversity, and SPEI variables, since they are already represented by an index. Four main potential causal pathways were examined (see Supplementary Fig. 3) which were fitted using LMMs, controlling temporal autocorrelation with corAR1 structure and incorporating sampling and month year, as well as the plot ID as crossed random effects on the slope and the intercept in the model. Positive and negative causal relationships are denoted with solid and dotted arrows, respectively; with the width of the arrows proportional to the strength of the path coefficients. Numbers on the arrows are standardized parameter estimates with significance levels denoted using asterisks (i.e., * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Light gray arrows indicate relationships between components linked by correlations. For composite variables, the strength of significant standardized coefficients is represented along a palette of red for positive and blue for negative relationships. Conditional (R^2_c) and marginal (R^2_m) R^2 for response variables for each model are also indicated. AIC, Akaike information criterion.

and SSI (Supplementary Fig. 4). However, SEM mainly highlighted the significant influence of habitat on the SSI ($SE = -0.18$; $P < 0.001$), given the opposite and weak effect on nematode diversity ($SE = 0.09$; $P < 0.001$). The effect of habitat was equally determined by the type of ecosystem (forest or grassland) and soil horizons but with a small overall effect ($SE = 60.05$ and

$SE = -0.07$; $P < 0.001$; respectively). However, it should be noted that the significant associations found for habitat could be explained by the different range of values observed for forest and grassland ecosystems as well as soil horizon, given the temporal consistency of diversity and SSI across both environmental contexts (Fig. 2). In this case, we also found a positive correlation between nematode diversity and SSI ($SE = 0.23$; $P < 0.001$).

Discussion

The objective of this study emerged from the current global focus on climate change effects on biodiversity and mitigation strategies, as well as the paucity of information on soil biodiversity time-series (but see BioTIME initiative⁵²). Climate and environmental change has multiple effects on above- and belowground organisms in terrestrial ecosystems^{2,3} and is considered a major driver of biodiversity change^{28,30}. To improve the current knowledge on how biodiversity changes, different biodiversity metrics need to be considered in long-term observations at large spatial scale and across different environments^{15,47,53}. Species abundance and richness (alpha diversity) belonging to particular taxa are traditionally used in many studies focused on changes in soil biodiversity, whereas species turnover (beta diversity) remains little explored^{16,47}. The latter biodiversity facet is useful in large-scale studies to assess vulnerability of soil communities to global change⁵⁴, and the relevance of protected areas in conserving soil biodiversity⁵⁵. With all these assumptions to consider, our work is especially suited for the Carpathian Ecoregion due to its highly diverse landscapes, biodiversity^{56–58}, and vulnerability to climate change⁵⁹.

Here, we report on the first study on temporal trends of nematode diversity in forest and grassland ecosystems in the Carpathian Ecoregion of Eastern Europe in a 14-year analysis of different biodiversity facets and the underlying drivers. Temporal climate models revealed a quasi-stable temperature regime, strong annual increase in precipitation, substantial increase in precipitation variability, and increasingly wetter conditions in the Romanian Carpathians and Transylvanian Plateau between 1986 and 1999. Observational records between 1979 and 1999 in the Romanian Carpathians showed a clear and significant warming trend, intensifying after 1994, particularly so after 1999, and especially during winters⁶⁰. A very slight increase in annual precipitation in the Romanian Carpathians was noted over 1961–2010, with wetter years during the late 1960s and 1970s and occasionally after 1990. However, a progressive transition from a wetter to a drier climate was noted in many parts of the Romanian Carpathians, mostly in summer⁶⁰.

Temporal changes in nematode diversity concerned mostly taxonomic richness and were mainly due to climatic effects, in agreement with our hypothesis. More specifically, nematode alpha diversity (taxa richness) and beta diversity changed significantly and divergently over time, with nematode alpha diversity increasing and nematode beta diversity decreasing over time. This contrasting trend between the two-diversity metrics is in line with previous reports across a variety of taxa and ecosystems^{61–63}, suggesting that biotic homogenization could occur despite observing an increase in taxon richness⁴⁷. This pattern might agree with the influence of large-scale environmental filters that cause homogenized conditions across habitats that lead to more similar ecological communities^{2,64}. This is congruent with our findings, where temporal changes in nematode diversity were remarkably consistent across ecosystem types (forests and grasslands) and soil horizons, providing strong evidence for the influence of climatic factors/climate change on soil biodiversity at large spatial scales. At the same time, we observed an increasing functional balance in the proportion of trophic groups within nematode communities over time. A steady increase in functional uniformity of nematode communities, along with a decline in beta diversity indicates more homogenous conditions in soils over time. The above is probably meaningful if we consider that temporal change models revealed increasingly wetter conditions (especially decreasing drought events) in the Carpathian Ecoregion between 1986 and 2000 in a post-disturbance scenario. This disturbance was likely caused by changes in soil conditions due to atmospheric pollution as a result of SO_2 and NO_x emissions, causing acid rains in Romania before 1990^{65,66}.

The SSI (developed to assess homeostasis of soil systems based on functional composition of nematode community) increased over time, suggesting a decline of soil disturbances and more mature and complex soil food webs, confirming our hypothesis on the post-disturbance recovery scenario. This is also supported by the fact that the stabilization of nematode communities over time (increase in the rate of feeding uniformity, balance in the proportion of trophic groups) was positively related to soil stability. The congruent increase of SSI and alpha diversity provides further evidence that more diverse nematode communities in soil are favored by more stable environmental conditions (see also ref. 67). SSI has therefore practical utility in evaluating soil ecological status, alone or in addition to other traditionally nematode-based indices used in biological monitoring. Nevertheless, SSI cannot clearly detect the turning point when soil food web structure and complexity change due to disturbances, but is rather an ecological metric that shows the gradient/intensity of perturbations. Interestingly, the effect of climate on nematode diversity and SSI was mainly attributed to SPEI and not to the variation in annual average precipitation, indicating that water balance in soil is more important than the net amount of precipitation. The pattern of temporal variation in the trophic composition of nematode communities is noteworthy, because it shows contrasting trends of functionally very dissimilar taxa: while the community proportion of plant feeders increased, that of fungal feeders decreased over time, the latter related to lower SSI. This opposite pattern could possibly be a consequence of increasing soil moisture over time due to higher amount of annual precipitation and a subsequent increase in plant biomass, promoting root feeders⁶⁸. On the other hand, we observed an increase of specialized (cp3-5) nematodes and a decline of generalists (cp2) over time, as the proportion of the most prevalent taxa (i.e., generalists) in the community decreased between 1986 and 2000.

While this study provides novel and important insights into the temporal change of taxonomic and functional diversity of nematode fauna across a rapidly changing environment, the underlying data is not based on a soil biodiversity monitoring initiative with repeated assessments of the same locations over time. Caveats of the present database include some variation in the time of sampling (most sites (101) sampled between May and July), and altitude (most sites (93) located between 500 and 1600 m), as well as mostly genus-level taxonomic information. However, the consistent findings of alpha and beta diversity trends presented here offer a strong argument for the urgent need for long-term soil biodiversity and function monitoring^{47,69–71}, and highlights the importance of nematodes as powerful indicators of change in soil systems at large spatial scales³⁷. Moreover, our study underlines the importance of considering different biodiversity facets to appreciate changes and implications of biodiversity shifts driven by environmental change. The information presented here and the results are extremely valuable as reference data for the nematode fauna of the Carpathian Ecoregion, which may be used in the future for biomonitoring purposes and climate change-integrated conservation strategies at regional scale.

Conclusions and implications

Our large-scale, temporally-resolved dataset across the Carpathian Ecoregion provides novel evidence that climatic variability in the context of climate change is an important contributory factor in driving the diversity of nematodes across ecosystem types and soil horizons. Notably, we found opposite trends of alpha and beta nematode diversity and a consistent stabilizing role of nematode diversity. Changes in nematode diversity were associated with increasingly wetter conditions over the 14 years of study, and SPEI was found to be a powerful climate predictor for nematode diversity change. The observed community homogenization may have significant consequences for the functioning, resistance, and resilience of this climate-vulnerable ecoregion^{70,72}. However, future concerted whole-ecosystem monitoring approaches are required to more comprehensively assess the causes and consequences of soil biodiversity change^{69,70}. Given that we saw opposing trends for soil nematodes being mostly beneficial for several ecosystem services like fungal feeders and those that provide disservices like plant-feeding nematodes, climate change might modulate the fine balance

between the net effects of these soil health indicators. While the climate became more beneficial for soil nematodes during the study time in the Romanian Carpathians, other regions of the world experience a deterioration of climatic conditions including more severe and frequent droughts⁷³. Next to pure monitoring approaches, further research also needs to address adequate strategies to mitigate climate change effects and conserve soil biodiversity and health. Moreover, long-term monitoring of different biodiversity facets of soil biota at the regional level may improve predictions of biodiversity change at policy-relevant scales, for better adaptive conservation responses¹⁵.

Methods

Site description

A total of 120 sites distributed across in the Romanian Carpathians (114 sites) and the Transylvanian Plateau (6 sites), both part of the Carpathian Ecoregion, were investigated (Supplementary Fig. 8). Ecoregions are defined as relatively large units of land containing a distinct assemblage of natural communities and species⁷⁴. The Carpathian Ecoregion is situated in the Eastern part of Europe and encompasses the Carpathian Mountains and adjacent highlands across Poland, Czech Republic, Slovakia, Ukraine, Austria, Hungary, and Romania^{46,75} and is particularly sensitive to climate change (see e.g., refs. 44,45,76).

Forests dominate the Carpathian Ecoregion, constituting about 60% of the landscape⁴⁵. However, past intensive land use has impacted most woodlands, transforming the landscape into a mosaic of forests (some of them subsequently used for timber production), grasslands, and arable fields, with patterns varying significantly between regions and countries^{77–79}. Our sampling sites were situated in forests ($n = 91$) and in grasslands ($n = 29$). We included coniferous ($n = 25$), deciduous ($n = 41$), and mixed ($n = 25$) forests. Coniferous forests were primarily dominated by Norway spruce (*Picea abies* L.). Deciduous forests consisted of European beech (*Fagus sylvatica* L.), either alone or in combination with hornbeam (*Carpinus betulus* L.), sessile oak (*Quercus petraea* (Matt.) Leibl), and sweet chestnut (*Castanea sativa* Mill.). Mixed forests were composed of Norway spruce, beech, and sporadically silver fir (*Abies alba* Mill.). Sampling sites were situated at altitudes ranging from 180 to 2350 m above sea level, some of them located in natural protected areas, characterized by low to negligible anthropogenic pressures⁸⁰.

Sampling design and nematode data collection

The soil collection was performed in almost all locations by the same nematologist for 14-years (1986–1999) (Supplementary Table 1). Soil samples were collected during multiple independent projects carried out in several mountain ranges across the Romanian Carpathians, aiming to describe and characterize the nematode fauna associated with the most relevant ecosystems of each individual mountain unit⁸¹. Samples were taken between May and October; 119 sites were sampled once; one site was sampled twice (May and July in the same year). At each site, three to seven plots of 100 m², considered representative for the ecosystem type based on expert knowledge, were randomly selected for soil sampling. The distances between plots were at least 10 m, reason for considering sample replicates as pseudoreplicates and treated as such. In each plot, up to 10 cores with an inner diameter of 2.2 cm were separately taken from the organic (25 cm² in surface) and mineral soil horizons (core of 3.8 cm² in surface, divided into 0–5 cm and 5–10 cm depth). In total 1069 samples were collected and analyzed, most of them (669) between 1993 and 1999 (Supplementary Tables 1 and 2). Forest soil samples prevailed (905) as compared to those collected in grasslands (164), as well as those collected from the organic soil horizon (litter, fermentation, and humification layers of forest soils, and the superficial turf layer with matted roots of grassland soils). The variation in the number of nematode samples according to the soil horizon (Supplementary Table 2) was due to the soil depth, as mineral layers were not always developed on parent rock.

Nematodes were extracted using the centrifugation method⁸² and then fixed with TAF (triethanolamine, formaldehyde) or 4% formaldehyde

solution heated at 65 °C. The centrifugal method has been widely reported as the most versatile technique showing extraction efficiency for larger numbers of nematodes, especially of the less mobile forms (please see referenced studies^{83–85}). Temporary mass-slides were prepared from each sample and used for examination. At least 150 well-preserved nematode specimens were randomly identified to genus (adults and most juveniles) or family level (some juveniles of which no adult morphotype and/or genus could be assigned) according to^{86–88}. For reliable comparisons, all the specimens were identified by the same nematologist using a Carl Zeiss microscope, at 40–1,250× magnification, and the results were used to estimate the relative abundance (% of the total nematode individuals identified) of each taxon in each sample. Additionally, we focused on the temporal changes of trophic groups, providing more information on the functional role of nematode-based food webs. To achieve this, all identified nematodes were assigned to one of five main trophic groups: plant feeders, bacterial feeders, fungal feeders, omnivores, and predators⁸⁹, and along the colonizer–persister (cp) scale⁹⁰. The classification of taxa according to cp values and their proportional representation in the nematode community provides a useful expression of environmental condition, as taxa within a cp class are similar in their response to disturbances^{80,82}. In a sequential scenario of response to disturbance, for example, the nematode community would become dominated by fast-growing nematodes (i.e., nematodes that feed on bacteria within cp1), which then would shift to a more diverse community including bacterivores, fungal feeders and plant feeders (i.e., cp2–3), and would ultimately mature due to slow-growing nematodes (i.e., predators, omnivores and large herbivorous nematodes within cp5^{40,67,91,92}). Ultimately, the functional structure of nematode communities could be an important starting point as a diagnostic tool for food webs affected by anthropogenic disturbances and/or climatic factors related to climate change examined in our study^{40,49}.

Nematode diversity indices

We examined temporal changes in nematode biodiversity across different spatial scales by partitioning regional (gamma) diversity into local (alpha) and spatially variable (beta) diversity. This approach allowed us to discern patterns in nematode community dynamics over time. Alpha diversity was evaluated through nematode richness to explore the loss or gain of taxa in each local community over time. Temporal turnover in the nematode community was assessed using the beta diversity metric, derived from the Sørensen dissimilarity matrix obtained through the recorded diversity and abundance of nematodes at each sampling point⁹³. More details on the procedure for calculating beta diversity is provided in the part referring to data analysis.

Nematode-based indices

We used nematode-based indices for exploring the temporal changes of soil ecosystem health in response to climatic variables related to climate change. These indices capture changes in the complexity of life history strategies and trophic groups of nematode communities (i.e., cp values and feeding habits, respectively) while monitoring the structure, function, and state of the food web in relation to environmental disturbance^{42,67,90,94}. Here, we developed an index to examine the state of soil disturbance (i.e., a nematode-based SSI), derived from the functional variation in nematode communities that captures the functional level, maturity, and structural complexity of the soil food web resulting from perturbations. To explore these functional attributes, we accounted for two widely used nematode-based indices such as the Maturity Index (MI) and Structure Index (SI). Both different but complementary indices have been shown to express the level of stability of soil community food webs, reflecting the soil quality^{91,92}. MI represents the proportions of nematodes in various functional guilds based on the colonizer–persister (cp) continuum, excluding the plant-feeding nematodes⁸⁷. This index has been used to indicate the state of the soil food web along successional gradients resulting from environmental disturbance^{42,94–96}. MI was computed as the weighted mean of the proportion of free-living nematodes in each cp group⁹⁰, with high values (>3) indicating a well-structured and complex soil

food web resulting from low levels of disturbance^{42,94}. However, MI does not measure the magnitude of the functions performed by the structural components of community assemblages⁴⁹. To resolve this, we used SI which is an independent yet complementary index providing information on environmental disturbance and the recovery of soil health, based on the complexity of the soil food web^{42,49,94}. SI reflects the structure, complexity, and connectedness of the soil food web, representing a complex community prompted by the presence of persister nematodes (i.e., high cp and/or medium-to-large predators and omnivores). Lower SI values indicate perturbed soil food webs, while higher values indicate a structured soil food web and high resilience. SI was calculated following Ferris et al.⁴⁹ and range from 0 to 100, with low (0–30), intermediate (30–60), and high (60–100) values corresponding to level of soil food web complexity^{42,94}. Therefore, with the calculation of SSI, we encompassed a wider representation of the state of soil health through the consideration of the functional dimension derived from the structure and complexity level of the nematode communities by including these two complementary indices with different ecological meaning^{40,42,49,67,97}. Undoubtedly, these indices enabled us to test temporal changes in soil perturbation levels (i.e., soil ecosystem stability in terms of low levels of environmental disturbance and resilience to disturbance^{98–100}) under climate change, using observed shifts in the diversity–structure–complexity of nematode communities over time. Our SSI was therefore calculated by aggregating the two indices as follows:

$$SSI = aMI + bSI$$

where MI is the Maturity Index component and SI is the Structure Index for each sample, *a* and *b* are the loadings of the first axis of the PCA performed on a matrix of the normalized MI and SI values calculated for each sample. We then used the scores derived from the first PCA axis as the gradient defining SSI. The resulting index is standardized between 0 and 1 to show a monotonic increase and to facilitate its interpretability. Higher values indicate equivalent levels of a high well-functioning, structured, and complex soil food web, while lower values mean a perturbed soil food web derived from a high level of soil disturbance.

Climatic change-related variables

To predict the vulnerability of nematode biodiversity and related soil ecosystem health to climate change, we considered temporal changes in eight climatic variables. We focused on temperature and precipitation, as two key variables related to the climate change¹⁰¹ and important drivers of soil nematode diversity^{37,43,102–104}. For this, we compiled historical climate data from WorldClim (v.2)¹⁰⁵ (<http://www.worldclim.com>) and the CARPAT-CLIM—Climate of the Carpathian Region online database¹⁰⁶ (www.carpatclim-eu.org). All variables were recorded as continuous data in raster layers, and individual values for each sampling site were extracted using the raster to point tool in QGIS.

We included the following variables: annual mean temperature (*T*) and precipitation (*P*), i.e., mean values for the sampling year; as well as the inter-annual variability of temperature (ΔT) and precipitation (ΔP), i.e., the climatic variability based on the temporal variation of the mean annual temperature and precipitation of the sampling year with respect to the reference period 1970–2000. The equations used for calculating ΔT and ΔP are:

$$\Delta T = T_{av}(i) - T_{av(1970,2000)}$$

$$\Delta P = P_{av}(i) - P_{av(1970,2000)}$$

where $T_{av}(i)$ is the annual mean temperature for the sampling year, $T_{av}(1970, 2000)$ is the annual mean temperature for the time period 1970–2000, $P_{av}(i)$ is the annual mean precipitation for the sampling year, and $P_{av}(1970, 2000)$ is the annual mean precipitation for the time period 1970–2000. Values higher mean an increase in temperature and precipitation relative to the long-term climate conditions of a given site, respectively.

In addition to these variables, we included others describing extreme climate events. Extreme temperature events are likely to have significant impacts soil nematode diversity patterns⁴³. Therefore, we included the following variables: the number of severe cold days (NSCD, $T_{\min} < -10^{\circ}\text{C}$) and extreme hot days (NSHD, $T_{\max} \geq 35^{\circ}\text{C}$) in the last 24 months preceding sampling, excluding the month of sampling. Previous studies have indicated that periods of drought significantly influence soil nematode communities¹⁰⁷. To capture the intra-annual drought variation, we used the SPEI, which measures the temporal variation of the water balance. This index rates the onset, magnitude, and duration of water stress events relative to long-term historical precipitation records¹⁰⁸. Specifically, we selected the 12-SPEI from the available measures^{22,109} and considered the water balance rate during the 12 months before sampling, excluding the month of sampling. Previous studies have indicated that altered precipitation patterns, leading to periods of drought, significantly influence soil microbial and nematode community patterns¹⁰⁶.

Finally, we include a temperature-related variable indirectly describing extreme climate events (i.e., no freezing conditions): the Growing Season Length (GSL)¹¹⁰. While GSL directly affects plant performance, its influence on soil nematode communities is hypothesized to be mediated by soil microbial interactions¹¹¹. The GSL was measured as the annual count of days between the first span of at least 6 days with a daily mean temperature $>5^{\circ}\text{C}$ and the first span after July 1st of 6 days with a daily mean temperature $<5^{\circ}\text{C}$ (averaged over the last 10 years before the year of sampling).

Calculations and data analysis

All data analyses were done with the R version 4.2.2¹¹² (<https://www.R-project.org>). We used the following packages: *vegan* (version 2.6.4)¹¹³; *adespatial* (version 0.3.20)¹¹⁴; *nlme* (version 3.1.160)¹¹⁵; *MuMIn* (version 1.47.5)¹¹⁶; and *piecewiseSEM* (version 2.1.2)¹¹⁷.

Nematode diversity. Alpha diversity expressed as nematode taxa richness was estimated with the *diversity* function, in the *vegan* package. Following the recommendations of previous studies on the appropriate use of large nematode datasets containing zeros and extreme values^{118–121}, a Hellinger transformation was performed on the nematode abundance matrix before estimating beta diversity¹¹⁸. Beta diversity was assessed and estimated using two complementary and sequential measures: the Sørensen dissimilarity of community data and the multivariate homogeneity of groups dispersions^{93,122}. First, we analyzed the differences in taxonomic composition between different sites based on the Sørensen index following the methodology from Legendre⁹³ and using the *beta.div.comp* function implemented in the *adespatial* package. The resulting dissimilarity distances matrix was then captured to compute the multivariate dispersion beta (mean distance to centroid) for each site surveyed in this study using the *betadisper* function in *vegan*. This procedure allowed us to assess differences in the multivariate dispersion of nematode communities between samples within each site surveyed. Therefore, we assigned to each sample the distance in principal coordinate space between it and its respective group centroids (i.e., sites) as beta diversity metrics for further data analysis¹²².

Selection of climate variables. To avoid any redundancy and potential multicollinearity in the effect of the climate on temporal changes in nematode diversity and soil stability, we used a forward selection procedure^{123,124}. This was done because collinearity may affect (i.e., exclude) one or more important variables that drive the spatial and temporal patterns of nematode communities. Specifically, we used a modified forward selection method assessing the effect of climate on the nematode abundance matrix, which is based on a permutation procedure (using 9999 random permutations¹²⁴). To do this, we used the *forward.sel* function in the *adespatial* package.

Time trends analysis. We used multiple regression models to assess temporal trends in climate data, nematode diversity, and SSI, as well as

the effect of climate on nematode diversity and SSI. We conducted the temporal analyses in a 14-year time window (1986–1999) covering the soil and climate data of the sampling design (i.e., 1069 samples in 120 sites). To do this, we analyzed the time data using linear models with the *nlme* package. In time series studies focused on determining the ecological impacts of shifting average environmental conditions in shaping the structure and functioning of ecosystems under climate change (i.e., community dynamics), it is crucial to consider and quantify the temporal structure or autocorrelation of environmental variables^{125–127}. This is because autocorrelation is simply defined by the correlation of adjacent time points, where its negative effects have been well-established regardless of the model and system¹²⁸. Thus, in all cases, we controlled for temporal autocorrelation in response variables among successive years with a first-order autoregressive structure (*corAR1*)¹²⁸. The selection of the structure and order of the autocorrelation function (ACF) to be included in the models was based on the combined understanding and testing of the ACF and partial autocorrelation function (PACF) plots of the time series¹²⁹. In parallel, model performance was assessed by comparing different possible models through the reduction of the Akaike information criterion (AIC) values, which allowed us to select the best fit by dropping non-significant terms from these models before interpreting the results¹²⁹. We performed this multimodel selection and diagnosis processes with the *MuMIn* package.

First, the temporal trend of the selected climate variables was studied with linear generalized least squares regression¹²⁹ (GLS), using the *gls* function in the *nlme* package. GLS regression is a classical method for quantifying time trends because, unlike ordinary linear regression models, it allows to correct estimate model variance and quantify heteroscedasticity and autocorrelation of residuals in the temporal climate data^{125,130}. GLS was run separately on each selected climate variable (i.e., T, P, ΔT , ΔP , SPEI and NSCD), where model agreement and robustness of trends were statistically assessed following the GLS fits¹²⁹. Second, we fitted linear mixed-effects models (LMM) to examine temporal changes in nematode diversity (i.e., taxa richness and beta diversity) and SSI and their consistency across soil horizons and ecosystems types. We conducted individual models for each index (i.e., nematode taxa richness, beta diversity and SSI) with sampling year as continuous variable and the interactive effects between sampling year with ecosystem type and soil horizon using the *lme* function in the *nlme* package. We used LMM to ensure data independence and to control for pseudo-replication due to multiple sampling plots at the same site (using the sampling year as random effects on the slope, and month as well as plot ID as crossed random effects the intercept in the model)¹²⁹. We selected models according to the penalized log-likelihood (Akaike information criterion) using maximum likelihood (method = ML) while subsequently the coefficients and 95% confidence intervals were calculated from the resulting model with the restricted estimates maximum likelihood method (method = REML)¹²⁹. Before running the models, we encoded the categorical data of ecosystem types for binary classification (forest and others), and we ordered the categorical data of soil horizons based on increasing depth (organic, 0–5 cm, and 5–10 cm layers of the mineral horizon). Furthermore, we used LMM to evaluate the effect of climate data on nematode diversity (taxon richness and beta diversity) and SSI. Using the same model term composition specified above, we ran individual models for all relationships between diversity and stability indices and the selected climatic variables. In this case, each model was fitted with the selected climate variable as a continuous variable without considering interactions and categorical variables.

Finally, temporal changes in the functional community composition were also examined. Functional community traits, including the proportion of trophic groups (i.e., feeding habits) and cp groups (the colonizer-persister (cp) scale) over the overall nematode community were selected to assess the role of temporal changes in functional composition. Additionally, we also evaluated changes of the nematode-based indices (MI and SI) over time. This allowed to test the suitability of our SSI in evaluating temporal changes

in the soil disturbance level of our data. The temporal changes in the functional data were tested with LMMs using the same composition of model terms as indicated above.

Structural equation modeling. We used piecewise structural equation models (piecewise SEM¹¹⁷) to provide an ecosystem-level understanding of the effects of climate change on soil nematodes and, in turn, the nematode-based index indicating the soil stability level. SEM allowed us to determine how much of temporal changes of nematode diversity is due to climate change-related variables, considering different ecosystems. To do so, we designed a conceptual model accounting for a series of causal assumptions based on our observational data (Supplementary Fig. 3). Specifically, we evaluated the relationships and correlation assumptions between nematode diversity and functional community composition on Soil Stability Index (SSI) in a 14-year trend scenario, considering multiple ecosystem factors, including altitude, ecosystem types (forests or grasslands) and soil horizons (organic, 0–5 cm and 5–10 cm layers of mineral horizon) simultaneously. Excepting time and altitude, all measured variables considered were previously arranged into composite variables for subsequent input to the SEM. The composite variables were then climate (T, P, ΔT , ΔP , SPEI, and NSCD), nematode diversity (taxa richness and beta diversity), functional composition (proportion of plant feeders, bacterial feeders, fungal feeders, predators, and omnivores in the overall community), and habitat (ecosystem types, and soil horizons). An a priori model was constructed to examine the direct and indirect relationships among time, altitude, climate, diversity, functional composition, habitat, and SSI. Four main potential causal pathways were investigated in the SEM analyses, namely: (1) climate change-related variables (temporal variability of climate data), (2) functional composition trend (habitat effects on temporal changes in functional composition), (3) diversity trend (effects of climate and habitat on temporal changes in diversity), and (4) SSI trend (effects of climate and habitat on temporal changes in soil stability); it should be noted that nematode diversity, functional composition and SSI, as well as climate and altitude, are linked by correlations because the dependence between these variables (Supplementary Fig. 4). Each pathway was implemented as an independent LMM model in the piecewise SEM. For time data analysis, we controlled for temporal autocorrelation with a corAR1 structure¹²⁶. To confirm the robustness of the relationships between SEM components in LME models, we also incorporated sampling year as random effect on the slope and the plot ID as random effects on the slope in the model¹²⁹. This allowed us to provide the marginal and conditional contribution of climate, diversity, habitat, and functional composition in driving soil stability in a time-trend scenario. Categorical data was encoded as time-data monitoring, i.e., the ecosystem type in a binary classification (0 for forests and 1 for grasslands), and soil horizons in an increasing depth order (0 for organic horizon, 1 for 0–5 cm, and 2 for 5–10 cm layer of mineral horizon). We used standardized values of numeric data (using z -scores) to improve predictive accuracy of the SEM, except for SPEI which was already represented by an index. Finally, temporal changes in composite variables were tested with LMMs using the same composition of model terms as stated above.

An additional SEM model was run in order to display more readable outcomes by simplifying the complexity of the initial conceptual model (Supplementary Fig. 3), while excluding the habitat component. We then compared the two models using a Chi-squared difference test and AIC. In each model separately, the goodness-of-fit of the SEM was evaluated through a multivariate information framework using χ^2 , P value, Fisher's C test, and AIC¹¹⁷. More specifically, we used the Fisher's C -test (when $0.05 < P < 1.00$) to confirm that the model is consistent with the data¹⁰⁹. We used the Shipley's test of d -separation to find potentially missing or overlapping paths in each piecewise SEM and thus modified the model according to the significance ($0.05 < P < 1.00$)^{117,131}. For time data analysis, LMM models were performed with the *nlme* package, whereas SEM was constructed and evaluated using the piecewise SEM package.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data generated or analyzed during this study are included in this article and its supplementary information files. Original data is available in Dryad.

Code availability

R code is available in Dryad.

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Author contributions

N.E. conceived the study based on preliminary concepts. M.C. compiled and provided the data base, with significant input from P.K. A.A.-Y. and N.E. proposed the stepwise approach of data analysis. A.A.-Y. performed the statistical analyses, provided interpretation of the output, elaborated figures and organized supplementary information. M.C. wrote the early version of the manuscript, which was then improved with substantial contributions from A.A.-Y., N.E. and P.K.

Competing interests

The authors declare no competing interests.

Additional information

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