**How the Research Questions Connect to Form a Unified Research Topic**

The research topic **"Elevation-Driven Changes in Soil Properties, Climate Variability, and Nematode Functional Diversity: Implications for Ecosystem Stability and Carbon Cycling"** is built upon **four interconnected research questions**, each addressing a key ecological process. These questions form a **logical sequence** that explains how **climate, soil properties, nematode communities, and ecosystem functions interact** along elevation gradients.

**🔗 Connection Between Research Questions**

Each question builds upon the previous one to **progressively explore ecosystem dynamics**:

1️⃣ **Climate and Soil Interactions**  
**Q1:** *How do climate variables (temperature, precipitation seasonality, annual range) interact with soil properties (pH, moisture, conductivity, temperature) along elevation gradients?*

* **Foundation:** Climate sets the stage for soil conditions by influencing **moisture availability, acidity (pH), and temperature**.
* **Why It Matters:** Soil properties **directly shape** the **habitat quality for nematodes**, impacting their composition and activity.

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2️⃣ **Soil and Nematode Community Structure**  
**Q2:** *How do these environmental changes influence nematode trophic structure and functional diversity (e.g., herbivores, bacterivores, fungivores, omnivores, predators)?*

* **How It Connects to Q1:** Changes in soil **pH, moisture, and temperature** impact the distribution of nematode feeding groups.
* **Why It Matters:** The **dominance of certain nematode groups** (e.g., fungivores vs. bacterivores) provides insights into **decomposition pathways and nutrient cycling**.

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3️⃣ **Ecosystem Stability & Soil Functional Indices**  
**Q3:** *What is the role of soil maturity indices (Sigma Maturity Index, Enrichment Footprint, Composite Footprint, Channel Index) in predicting ecosystem responses?*

* **How It Connects to Q2:** Functional indices reflect the **degree of ecosystem stability**, **resilience to environmental change**, and the balance between **opportunistic (colonizer) vs. stress-tolerant (persister) nematodes**.
* **Why It Matters:** These indices **summarize the overall ecosystem condition**, indicating whether climate and soil changes lead to **sustainable or degraded environments**.

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4️⃣ **Nematodes, Carbon Cycling & Nutrient Turnover**  
**Q4:** *How do changes in plant-parasitic nematodes (PP 2-5) and free-living nematodes (CP 1-5) affect belowground nutrient cycling and carbon flux?*

* **How It Connects to Q3:** If nematode diversity and functional stability shift, this will alter **carbon sequestration, decomposition rates, and nutrient cycling efficiency**.
* **Why It Matters:** This question **completes the research framework** by linking environmental shifts (climate → soil → nematodes) to **ecosystem-level processes like carbon and energy flow**.

**🔬 How These Questions Create a Unified Research Topic**

**📌 Core Theme:**

**"How do elevation-driven climate and soil changes affect nematode biodiversity, ecosystem stability, and carbon cycling?"**

**🌿 Logical Flow of the Study:**

✅ **Climate shapes soil properties** → ✅ **Soil properties determine nematode diversity** → ✅ **Nematode diversity reflects ecosystem stability** → ✅ **Ecosystem stability influences carbon cycling**

This stepwise approach ensures a **holistic understanding** of how **soil biodiversity, climate change, and ecosystem processes interact**, making it a **comprehensive research direction**.

**Recommended Plots for Each Research Question**

To effectively visualize relationships between **climate, soil properties, nematode communities, and ecosystem functions**, here are the **best plots for each research question**:

**📌 Research Question 1: Climate and Soil Interactions**

**❓ How do climate variables (temperature, precipitation seasonality, annual range) interact with soil properties (pH, moisture, conductivity, temperature) along elevation gradients?**

**Recommended Plots:**

1. **Scatter Plot (Correlation Plot)**
   * **X-axis:** Elevation
   * **Y-axis:** Soil properties (**pH, moisture, conductivity, temperature**)
   * **Color or Facet:** Transects (Bakori, Budhal, Darhal, Thanamandi)
   * **Purpose:** Shows how **elevation affects soil characteristics** across different sites.
2. **Heatmap (Climate vs. Soil)**
   * **X-axis:** Climate variables (**Precipitation Seasonality, Temperature Annual Range**)
   * **Y-axis:** Soil properties
   * **Color Gradient:** Strength of correlation
   * **Purpose:** Highlights **which climate factors have the strongest effect on soil**.
3. **Boxplot (Variation by Elevation)**
   * **X-axis:** Elevation (categorized: low, mid, high)
   * **Y-axis:** Soil properties
   * **Purpose:** Examines how **soil pH, moisture, and conductivity change at different elevation levels**.

**📌 Research Question 2: Soil and Nematode Community Structure**

**❓ How do soil property changes influence nematode trophic structure and functional diversity (herbivores, bacterivores, fungivores, omnivores, predators)?**

**Recommended Plots:**

1. **Stacked Bar Plot (Nematode Composition by Elevation)**
   * **X-axis:** Elevation
   * **Y-axis:** Relative abundance (%) of nematode groups (**Herbivores, Bacterivores, Fungivores, Omnivores, Predators**)
   * **Purpose:** Shows how **nematode trophic structure shifts with elevation and soil factors**.
2. **Scatter Plot (Soil Properties vs. Nematode Groups)**
   * **X-axis:** Soil properties (**pH, moisture, conductivity, temperature**)
   * **Y-axis:** % of each nematode group (Herbivores, Bacterivores, etc.)
   * **Purpose:** Examines which **soil variables are the strongest predictors of nematode diversity**.
3. **NMDS (Non-metric Multidimensional Scaling)**
   * **Axes:** NMDS1 vs. NMDS2
   * **Data:** Nematode community composition
   * **Color or Shape:** Elevation levels
   * **Purpose:** Clusters sites based on **community similarity, showing whether nematode composition differs significantly by soil and climate factors**.

**📌 Research Question 3: Ecosystem Stability & Soil Functional Indices**

**❓ What is the role of soil maturity indices (Sigma Maturity Index, Enrichment Footprint, Composite Footprint, Channel Index) in predicting ecosystem responses?**

**Recommended Plots:**

1. **Boxplot (Soil Indices Across Elevation Levels)**
   * **X-axis:** Elevation categories (low, mid, high)
   * **Y-axis:** Sigma Maturity Index, Enrichment Footprint, Composite Footprint, Channel Index
   * **Purpose:** Shows how **ecosystem stability changes with elevation and soil conditions**.
2. **Scatter Plot (Soil Functional Indices vs. Soil/Nematode Variables)**
   * **X-axis:** Soil properties (**pH, moisture, conductivity, temperature**)
   * **Y-axis:** Functional indices (**Maturity Index, Channel Index, etc.**)
   * **Purpose:** Identifies **key soil variables influencing ecosystem stability**.
3. **Regression Plot (Enrichment Footprint vs. Bacterivores & Fungivores)**
   * **X-axis:** % of **Bacterivores & Fungivores**
   * **Y-axis:** Enrichment Footprint
   * **Purpose:** Determines whether **bacterial or fungal pathways dominate nutrient cycling**.

**📌 Research Question 4: Nematodes, Carbon Cycling & Energy Flow**

**❓ How do changes in plant-parasitic nematodes (PP 2-5) and free-living nematodes (CP 1-5) affect belowground nutrient cycling and carbon flux?**

**Recommended Plots:**

1. **Scatter Plot (Nematode Groups vs. Soil Indices)**
   * **X-axis:** % of **CP (1-5) or PP (2-5) nematodes**
   * **Y-axis:** Functional indices (**Channel Index, Enrichment Footprint, Sigma Maturity Index**)
   * **Purpose:** Determines how **nematode guilds influence carbon and nutrient dynamics**.
2. **Heatmap (Carbon Cycling & Energy Flow)**
   * **X-axis:** Nematode groups (**Bacterivores, Fungivores, CP/PP categories**)
   * **Y-axis:** Soil indices (**Channel Index, Enrichment Footprint, Maturity Index**)
   * **Color Gradient:** Strength of correlation
   * **Purpose:** Shows **which nematode functional groups play the biggest role in energy transfer**.
3. **Boxplot (Plant-Parasitic Nematodes vs. Elevation)**
   * **X-axis:** Elevation categories
   * **Y-axis:** % of **PP 2-5 nematodes**
   * **Purpose:** Examines whether **plant-parasitic nematodes decrease at higher elevations due to changing plant-soil interactions**.

**🔍 Summary: Best Plot for Each Research Question**

| **Research Question** | **Best Plot Type** | **Purpose** |
| --- | --- | --- |
| **Climate & Soil Interactions** | **Scatter Plot, Heatmap, Boxplot** | How climate shapes soil properties |
| **Soil & Nematode Structure** | **Stacked Bar Plot, Scatter Plot, NMDS** | How soil affects nematode communities |
| **Ecosystem Stability (Functional Indices)** | **Boxplot, Scatter Plot, Regression Plot** | How soil indices predict ecosystem health |
| **Carbon Cycling & Energy Flow** | **Scatter Plot, Heatmap, Boxplot** | How nematodes regulate belowground carbon flux |

**Optimized Plot Selection for Each Research Question**

Here’s a streamlined **"one best plot"** approach while keeping optional supporting plots if deeper insights are needed.

**📌 Research Question 1: Climate and Soil Interactions**

✅ **Best Plot:** **Scatter Plot (Elevation vs. Soil Properties)**

* **Why?** Shows how soil properties (pH, moisture, conductivity, temperature) change with elevation, the key driver of climate variation.
* **Optional Supporting Plot:** Heatmap (if we want to see how multiple climate variables affect soil properties).

**📌 Research Question 2: Soil and Nematode Community Structure**

✅ **Best Plot:** **Stacked Bar Plot (Nematode Composition by Elevation)**

* **Why?** Clearly shows how nematode trophic groups (Herbivores, Bacterivores, Fungivores, etc.) shift at different elevations.
* **Optional Supporting Plot:** Scatter Plot (Soil Properties vs. Nematode Groups) – If we want to analyze **which soil property influences specific nematode groups the most**.

**📌 Research Question 3: Ecosystem Stability & Soil Functional Indices**

✅ **Best Plot:** **Boxplot (Soil Indices Across Elevation Levels)**

* **Why?** Directly compares how **ecosystem stability indicators** (Sigma Maturity Index, Enrichment Footprint, Channel Index) change with elevation.
* **Optional Supporting Plot:** Regression Plot (if we need to confirm a **strong correlation between soil indices and specific nematode groups**).

**📌 Research Question 4: Nematodes, Carbon Cycling & Energy Flow**

✅ **Best Plot:** **Scatter Plot (Nematode Groups vs. Soil Functional Indices)**

* **Why?** Best for showing whether **certain nematode groups (e.g., CP 1-5, PP 2-5) correlate with key carbon cycling indicators (Enrichment Footprint, Channel Index, etc.)**.
* **Optional Supporting Plot:** Heatmap (if we want to visualize correlations between **multiple nematode groups and multiple soil indices at once**).

**📌 Summary: Just One Plot Per Question is Enough for the Main Analysis**

| **Research Question** | **One Essential Plot** | **Optional Supporting Plot (if needed)** |
| --- | --- | --- |
| **Climate & Soil** | **Scatter Plot (Elevation vs. Soil Properties)** | Heatmap (if analyzing multiple climate-soil interactions) |
| **Soil & Nematodes** | **Stacked Bar Plot (Nematode Composition by Elevation)** | Scatter Plot (if linking soil properties to specific nematodes) |
| **Ecosystem Stability** | **Boxplot (Soil Indices Across Elevation Levels)** | Regression Plot (if we want statistical validation) |
| **Carbon Cycling** | **Scatter Plot (Nematode Groups vs. Soil Functional Indices)** | Heatmap (if analyzing complex relationship. |

Elevation- soil type

Soil type- nematode trophic guild

Nematode- enrichment