Reply to Reviewers

Reviewer 1 –Reply

General Comment: Trenti et al. investigated soil physicochemical properties and the microbial community along a slope gradient related to chestnut ink disease. This is an interesting study, and the results help to understand the factors relating to chestnut ink disease. However, the study is rather descriptive, and more analysis should be added to explore the underlying mechanisms.

We thank Reviewer 1 for the constructive comments and for recognizing the relevance of our study. We appreciate the opportunity to clarify the scope and limitations of our work and to improve the manuscript accordingly.

> 1. The introduction should clearly show the knowledge gaps. Why is slope a key factor affecting chestnut ink disease?

We agree and have revised the Introduction to better highlight the knowledge gaps related to the influence of topography on ink disease dynamics. Specific revisions were made between lines 47-60.

> 2. How does slope affect soil biochemical properties and microbial communities related to chestnut ink disease?

Thank you for this relevant and insightful question. In our study, slope influenced several key soil properties—both physicochemical and biochemical—that in turn affected the microbial communities associated with ink disease symptoms in chestnut trees.

Specifically, the site affected by ink disease (INK1) was located at the **footslope**, where lower slope gradient (3%) and hillslope position favoured **water (redoximorphic features) and SOM accumulation, increased infiltration (high KS), and clay illuviation**, resulting in more **moist and acidic conditions**. These conditions were associated with:

- ✓ higher **water-extractable organic carbon (WEOC)** and **WEOC:WEN ratio**, suggesting altered carbon input likely due to infected plant exudates and higher water availability;
- ✓ increased **specific hydrolytic enzyme activities** in deeper horizons, reflecting microbial adaptation to altered SOM quality and nutrient availability;
- ✓ a shift in **microbial community structure**, with reduced abundance of nitrifying bacteria (e.g., **Nitrospirota**) and higher abundance of **Acidobacteriota** in subsoil horizons;
- ✓ the **presence of Phytophthora DNA** in the BC horizon;

- ✓ a predominance of **ectomycorrhizal fungi** with **contact and medium-distance exploration types**, linked to moist soil conditions and possible nitrogen limitation.

In contrast, the upslope sites (INK2 and INK3), with steeper gradients (9% and 30%, respectively), showed:

- ✓ shallower organic horizons,
- ✓ better aeration and drainage,
- ✓ more balanced microbial communities with higher relative abundance of **Nitrospirota**,
- ✓ and fungal communities dominated by **saprotrophs** or ectomycorrhiza with **short-distance exploration types**.

These findings suggest that slope-driven hydrological dynamics substantially modify soil biochemical environments and microbial ecology, thereby influencing the establishment and progression of chestnut ink disease. We have clarified this relationship in the revised *Discussion* section.

> 3. A comparison of pathogen (relative) abundance should be conducted between healthy and diseased trees, and the soil factors affecting pathogen abundance should also be explored.

We appreciate this insightful comment. However, our study did not aim to quantify pathogen abundance across a population of trees, but to explore soil and microbiological differences along a topographic gradient, using a soil-profile-based approach. The presence of *Phytophthora* DNA was confirmed only in the footslope profile (INK1), which corresponded with visible disease symptoms. Given this binary distribution (presence/absence), **a relative abundance comparison was not feasible**, as *Phytophthora* DNA was not detectable in any of the healthy profiles. However, we do agree that the identification of **soil factors linked to pathogen occurrence** is crucial.

Due to the limited number of sampled trees, a robust comparison of pathogen abundance was not feasible within our design. We now discuss this limitation explicitly in the revised Discussion (lines 365-372).

> 4. More analysis should be added to explore causal relations and the mechanisms leading to chestnut ink disease, e.g., structural equation modelling.

We agree that more advanced statistical approaches could strengthen mechanistic insights. However, our study focused on vertical and lateral soil variability rather than disease prediction or modelling. As we included only three fully characterized profiles, modelling causal relationships (e.g., using SEM) was not statistically justifiable. Nevertheless, we have acknowledged this limitation in the revised Discussion and have clarified that our study is exploratory in nature and offers a methodological framework for future research (lines 365-372).

> 5. The overall writing should be revised to be more concise, logical, and readable.

The entire manuscript has been thoroughly revised to improve clarity, conciseness, and scientific readability. We thank the Reviewer for this valuable recommendation.