

Reviewer 2 –Reply

General Comment: A significant weakness in this study is that it appears to have very limited replication of treatment plots. It appears that a single soil profile was sampled at each of the three locations. If there were independent soil profiles sampled at each location, this would need to be better explained in the text. But if only a single tree was sampled, sampling from the various horizons would appear to be “pseudo-replication” rather than independent samples. Given that a “tree” would be the appropriate sample unit to assess plant disease, some indication of disease severity (e.g., % of roots infected or trunk canker size) at each site would be helpful. In addition, true replication would help resolve the soil site-by-horizon interaction that contributes to high within-site variability. Overall, the Conclusions section seems to go much farther than the limited replication would allow. If the authors can document adequate replication, this paper could be acceptable. But a single soil profile per site is not adequate.

We thank the reviewer for this thoughtful and important comment. Indeed, our study design included **one fully characterized soil profile per site**, selected to represent contrasting plant health conditions (i.e., a symptomatic tree at INK1 and healthy trees at INK2 and INK3). We fully acknowledge that this design **does not provide statistical replication at the site level**, and we have now clarified this limitation in the revised *Discussion* (lines 365-372).

To partially address representativeness, **two additional auger borings** were performed at each site to support the soil morphological interpretation and confirm the kind of genetic horizons and soil type observed in the main profile. These auxiliary observations revealed consistent pedogenetic horizons and support the selection of the main profile as representative of each site. However, we agree with the reviewer that the **sample unit in this study is the site-profile combination**, and that the individual soil horizons do not constitute statistically independent replicates. We have included this clarification in the text and explicitly framed our interpretation within a **site-specific and exploratory context**, following recommendations from the literature (Garten, 1993; Conant et al., 2003).

Regarding disease assessment, INK1 was selected based on evident and persistent **crown symptoms** (i.e., canopy thinning and dieback) consistent with ink disease. In this preliminary phase, we did not perform quantitative evaluations of disease severity (e.g., root necrosis percentage or trunk canker scoring), but we agree that such data would strengthen future studies and should be included in **more replicated designs**. This is now acknowledged in the revised *Discussion*.

Finally, we have carefully revised the *Conclusions* section to better reflect the **exploratory nature of the study**, avoiding overgeneralization, and clearly indicating the need for follow-up studies with true replication. We hope these revisions address the reviewer’s concerns.

> Line 21 – Why would clay “increase” hydraulic conductivity?

Thank you for pointing out this important clarification. We agree that, in general, increasing clay content tends to reduce saturated hydraulic conductivity (KS) due to smaller pore sizes and reduced permeability.

In our study, however, we do **not suggest that higher clay content directly increases KS**. Rather, we hypothesize that **the position of the soil profile (INK1) at the footslope** plays a dominant role in shaping both **clay accumulation** and **hydraulic behaviour**. The footslope position favours:

- **accumulation of water and fine particles** transported from upslope via runoff and subsurface flow,
- **higher inputs of organic matter** due to landscape convergence and vegetation processes,
- and potentially **better soil aggregation** due to higher organic carbon content, which can enhance porosity and water infiltration under certain conditions (as discussed by Hudson, 1994; Rawls et al., 2003). The main structure in the A horizon in INK1 is fine lumpy, with a secondary fine subangular polyhedral structure. The aggregates are soft and brittle.

Therefore, the observed **high KS in INK1** is not attributed to its clay content per se, but rather to the **combined effect of slope position, organic matter enrichment, and soil structure development**, which can offset the restrictive influence of clay. We have revised the *Discussion* to clarify this point.

> Soil porosity or aggregate structure assessed?

Yes, soil structure was assessed during the survey. Aggregates were predominantly subangular and angular blocky, fine to medium in size, with weak structural stability. The main structure in the A horizon in INK1 is fine lumpy, with a secondary fine subangular polyhedral structure. The aggregates are soft and brittle. This information has been included in the Methods.

> Line 100 – Sampling season?

Sampling was conducted in July 2023. This detail has been added to the Methods section.

> Line 112–114 – Sentence unclear

This sentence has been deleted as suggested.

> Line 123–125 – Leaf nutrient levels contradict statement

We thank the Reviewer. The discrepancies in the original statement have been corrected. We clarified that foliar nutrient analysis was reported descriptively and not as a core

focus of the study. A note on pH influence on B and P availability was also added to the Discussion.

> Line 138–140 – [Substrate for alpha- and beta-glucosidase](#)

We corrected the mislabelling and now clearly distinguish between the two enzyme substrates.

> Line 177–179 – [Why infer hydraulic properties instead of measuring?](#)

We agree that direct measurements are preferable. However, due to logistical constraints, we applied validated pedotransfer functions. We referenced recent studies supporting the reliability of these models and added a note regarding historical land use (centuries of chestnut cultivation). These clarifications appear in the revised Methods.

> Line 180 – [Bulk density data not presented](#)

Bulk density data have been added to Table B1, as requested.

> Lines 270–272 – [Ground cover description lacking](#)

We thank the Reviewer. The ground cover has been described in detail: ferns and bramble species with ~40% average coverage, consistent across all sites.

> Lines 311–313 and 348–349 – [Claims about plant effect on organic matter not supported](#)

We appreciate this important observation. The relevant speculative statements have been removed or rephrased for clarity and caution.

> Lines 361–362 – [Anthropomorphizing fungi](#)

We agree and have revised this sentence to remove implications of fungal “intent.”

> Line 378 – [“Inoculation” is incorrect term](#)

Replaced with “presence” as suggested.

> Line 379 – [“Population dynamics” inference invalid](#)

Corrected: replaced with “microbial community composition.”

> Line 382 – [Labile carbon not shown as a disease response](#)

This statement has been deleted.