

## **Point by point answer to the reviewers' and editor's comments**

### **1. Editor's Comments to the Author:**

**AC:** The authors wish to thank the editor for his additional comments, that pointed out that several important aspects in the manuscript still lacked clarity. We therefore gave the manuscript a major overhaul, rewriting most sections with the help of a linguist to make them more concise and to the point. We also highlighted the international relevance of the study and focused the discussion more on processes and mechanisms relevant to a wider audience. In what follows, we have put the comments by the editors and reviewers in bold, and answered them directly below (authors comment: AC).

**Editor comment 1: Both reviewers agree that the manuscript presents a well-structured and interesting study, although R2 note that its relevance is mostly local: R1:"The manuscript represented an interesting study about trying to determine how soil physicochemical properties in the Gamo highlands of Ethiopia influenced the development of Xanthomonas wilt of enset (EXM)" R2 "The topic is relevant for the region because of the importance of enset for food security in Ethiopian highlands. In general, the manuscript is clearly written, well structured, and contains observational data that may be technically useful for local farmers and land managers".**

**AC:** We rephrased the title, abstract and the first paragraph of the introduction to indicate that more international scientific attention should be paid to indigenous or nationally important crops such as enset to increase worldwide food security and agroecosystem resilience in future climate conditions. Moreover, enset is important for a huge number of people, as Ethiopia expects to have over 200 million people by 2050, extending the scope beyond 'local'. We added additional references to support this claim (Naylor et al, 2004 in Food Policy, Manners and van Etten, 2018 in Global Environmental Change and Renard et al., 2019 in Nature).

**Editor comment 2: This make the manuscript of possible relevance to the journal, but both reviewers have raised major concerns of the manuscript that can be summarized as: 1-There appears to be many confounding effects, which impact on the results and the conclusions made by the authors. (R1)**

**2- There is no clear discrimination between sites and there is insufficient statistical power to agree with the conclusions made by the authors. (R1)**

**3- The study lacks crop yield data and important details on management practices (e.g., amount and form of nutrients added to soils), which make it hardly useful to develop general strategies to improve crop production, enhance soil sustainability, and fight plant diseases. (R2)**

**AC on concern 1:** It is correct that several factors are confounded, but this is logical given the nature of the interactions studied and therefore should not pose a problem: soil nutrients nearly always co-vary to some extent due to driving factors in the soil – e.g. available Al and pH are typically inversely correlated as a lower pH increases the solubility of Al. Another example is that adding manure is adding a mixture of C and several nutrients, again causing some degree of covariation.

Both management and altitude have an effect on soil nutrients, but the effect of altitude can be clearly related to differences in soil leaching (now stated in the discussion). The effect of management is paramount and the strong contrasts between inner gardens, outer gardens and outfields is highly significant regardless of altitude. This was highlighted better in the discussion.

Both altitude and certain soil parameters have an effect on EXW prevalence, but the effect of these soil parameters remains significant if only farms from the lower altitude (with highest EXW prevalence) are considered. We emphasized this in the discussion.

Hence, even though a certain degree of confounding cannot be excluded, we feel confident about our conclusions and perhaps even more important: we draw the attention to this problem through our suggestions for future research. Both the confounding and the difficulty to determine a simple crop performance statistic like 'yield' (vide infra), aggravate the situation and point to a sense of urgency for this research.

AC on concern 2: We were surprised by this comment, as our farms (sites) were clearly distinguishable, and the altitudinal zones were also unambiguously defined. Hence, we suspect the problem lies with the materials and method section, which was indeed confusing. We rewrote the sampling strategy, adapted figure 1, added a table to the supplementary material and changed the confusing nomenclature used for the different garden 'zones' to solve this problem. Regarding statistical power; our conclusions are based solely on results that were significant. We changed the symbology used in the tables (as suggested) to make this more clear.

AC on Concern 3:

Regarding important details on management practices: a summary of local farming practices and the amount and form of nutrients added to the soil was added to the description of the study area in the materials and methods section.

Regarding the lack of crop yield data:

We agree that the relationship between crop yield and soil nutrients needs much more study in enset, for different varieties, soil types, agro-ecological zones etc., but we are strongly convinced that such data cannot be inferred in a meaningful way from on-farm observations. Hence, agricultural trials are needed, but can only be established in a meaningful way if more information becomes available on actual soil nutrient levels and potential deficiencies in farmers' fields – hence the urgency of the current study. We opted to use nutrient levels in a recent leaf instead, which is a very common agronomical practice for estimating potential nutrient deficiencies and provides an avenue to complement the scanty information that is available from on-station dose-response trials. Therefore, in a preliminary assessment like in this survey, we judged it to be suitable for the aim of this paper. We stated the rationale behind our approach more clearly in the manuscript and elaborate on the arguments for this approach below:

Why we are convinced that it is not possible to infer meaningful yield data from on-farm observations:

- Enset is not grown for fruit bunches: yield encompasses the biomass of the corm and pseudo-stem for food, starch for porridge and leaves for feed. Plants are harvested one at a time based on the needs of the household (or several in case of a wedding or funeral), and some products are eaten immediately while others need to ferment for months. Individual leaves are harvested continuously for the cattle. As farmers don't keep records of their yield in numerical terms, no actual yield data are available on farm.
- Crop yield data estimates for enset can be established using allometric methods for standing biomass (Tsegaye and Struik, 2000 and 2001; Negash et al., 2013) like plant height or plant girth. However, as shown in figure 1 and more clearly on the picture below, younger enset plants are grown further away from the house and they are transplanted closer to the house as they mature, which takes between 4 to 7 years. Also, varieties grown for eating the boiled corm are typically smaller and also grown further away from the house. Hence, the standing biomass is typically lower in the outer garden, but that is related to plant age and variety rather than soil fertility. This information was also added to the manuscript (materials and methods, discussion).
- Younger plants are grown at a much higher density at the rim of the garden than older plants near the house. Besides, the spacing of the older plants near the house is not homogenous: plants may have disappeared e.g. because of EXW or because more were harvested for a wedding or a funeral. Moreover, farmers that focus on subsistence will typically space enset more densely, while farmers with a focus on producing crops for local markets will have a more open canopy that allows for intercropping. Farmers that focus on raising cattle will also prune enset leaves quite intensively for cattle feed, which will also affect plant growth and canopy cover.
- Plant age and variety cannot be corrected for, as farmers don't keep records and age and variety can't accurately be determine on morphology alone, especially in younger plants.

Why information on actual, on-farm conditions is needed to establish meaningful agronomical trials:

- Enset agricultural trials are quintessentially expensive and complicated: they need to be long-term due to the long maturation time and need a lot of space and repetitions due to a very large variability in growth between individuals of the same variety (the reasons for that are still unknown).
- Even in controlled conditions, the complexity of measuring yield or crop performance remains an important hurdle (Tsegaye and Struik, 2000 and 2001; Negash et al., 2013).
- As a consequence, controlled trials are almost nonexistent compared to other crops at present and will only be able to test for a limited number of soil fertility levels, altitudes or varieties in the foreseeable future.
- Hence the urgent need for the data presented in this manuscript: as we now indicated more clearly in the introduction, soil nutrient information on enset farming systems is so scarce that we first need to establish a knowledge base on actual soil nutrient levels and potential deficiencies in farmers' fields, before we can start designing meaningful and well-targeted trials for optimizing them.

Why we choose to relate soil nutrient levels to foliar analysis of a recent leaf, and why this approach will remain relevant even when more controlled trials are established:

- Leaf tissue analysis is a standard agronomical method commonly used to identify potential nutrient responses and deficiencies in plants
- While being aware that nothing such exists for enset, we derived principles and norms from what is known for banana, where actually relationships between foliar data and yield components are well established. (e.g. Martin-Prével, 1977 in Fruits; Turner and Barakus 1981 in Fruits, Lahav and Turner, 1989 in IPI Bulletin).
- The method has been applied to enset (e.g. Amede and Taboge, 2007) and research based on foliar analysis will be necessary to complement the scanty long-term agronomical trials for this crop.



**In addition to this the manuscript needs to be improved in terms of clarity (e.g. the sampling strategy is complex and a bit difficult to follow) and English usage, and have many additional comments.**

AC: the manuscript has been given a major overhaul to improve clarity, including the section on sampling strategy. The manuscript has been checked by a linguist (L. Vancampenhout) for English usage.

## 2. Updated responses to reviewers' comments

### Comments by R1

The authors would like to thank Reviewer 1 for his/her very detailed and comprehensive report and annotated text, which will help us considerably in improving the manuscript. Kindly find our answers (AC) to the comments (RC1) below, along with the final changes made to the manuscript. Kindly note that the line numbers below refer to the original submission.

#### General Comments

**RC1: "Unfortunately, there appears to be many confounding effects, which impact on the results and the conclusions made by the authors".**

AC: See reply to the editor's remark above

Manuscript changes: we've emphasized how we dealt with confounding effects in the discussion

**RC1: While the authors surveyed 276 enset gardens, they based their conclusions on the results from 11 sites, those at lower elevations that had corresponding disease assessment and soil physicochemical data.**

AC: This is only partly correct, as the paper has two aims (line 90-93): (i) a soil fertility assessment and (ii) examining potential correlations of soil and altitude with *Xanthomonas* prevalence, as to gain some insight in the ecological niche of the pathogen. With regard to the first objective, the number of sampled farms is 40 (table 1 and 2; first part of the conclusion, line 419-414). With regard to the second objective, disease prevalence was related to altitude in 276 farms (table 4, conclusion line 414) and to soil properties in 40 farms (table 5). As soil properties and altitude are confounded, we also assessed the zone with the highest incidence separately (11 farms, table 6, line 415 in the conclusion).

Manuscript changes: the number of farms for each assessment is clearly indicated with each table and we added a supplementary table on the sampling strategy. However, we agree with the reviewer that it is unclear in the conclusion. Hence, we removed 'in 276' from line 409 and we removed pH and K in line 414-415 and in the abstract, as those indeed are only based on a small number of farms.

**RC1: Would it be possible that these organic fertilizers were also sources of EXW inoculum adding to reinfection of plants?**

AC: Although no specific information for EXW is available, other strands of *Xanthomonas* are considered to be eliminated during the composting process (e.g. Elorrieta et al., 2003 in *Agriculture, Ecosystems and the Environment* [https://doi.org/10.1016/S0167-8809\(02\)00170-6](https://doi.org/10.1016/S0167-8809(02)00170-6)). We therefore did not consider it as a potential source for inoculum in this study.

Manuscript changes: At line 397 we have added the following information: "An alternative explanation is that the organic composts used to fertilize the garden may be a source of inoculum for EXW and hence explain the correlation between certain soil nutrients and EXW incidence. Yet, although no specific information is available for EXW, other *Xanthomonas* species have been reported to be heat-sensitive and easily eliminated during composting (Elorrieta et al., 2003).

**RC1: Much of the physicochemical data is not significantly different from one another, there is no clear discrimination between sites and there is insufficient statistical power to agree with the conclusions made by the authors.**

AC: See reply to the editor's comments above.

Manuscript changes: we rewrote the methodology and discussion to make this more clear. Also, we've adapted the symbols in the tables to distinguish more easily between significant and non-significant results

**RC1: Currently the manuscript is confusing with some terms like "inner-outer", which are difficult for the reader to follow. Furthermore, there is inconsistent use of tense, with the manuscript changing between past and present tense. This should be revised so that there is consistent use of past tense English. The inclusion of commas needs to be reviewed throughout the manuscript.**

AC: Thank you for the very detailed annotation and linguistic mistakes pointed out. They are noted and will be corrected.

Manuscript changes: all annotations will be reviewed and we will send the paper for editing to a linguist. We moreover simplified the information on the different zones in an enset farm by rewording them to inner garden, outer garden and outfield. To make this more clear, Figure 1 was adapted and a section on typical farming practices was added to the description of the study area.

### **Specific Comments**

#### **Abstract**

**RC1: This is a summary of what was done and therefore should be in past tense. e.g. increase → increased, are → were etc.**

AC/Manuscript changes: adapted and reviewed by a linguist

**RC1: L28 The authors make a “throw-away” statement that, “enset gardens should be optimized in relation to agro-ecological conditions and that both elevation and soil nutrient status need to be considered. ....”. It would be better if the authors were able to elucidate what these optimum conditions were to reduce EXW and not leave the readers wondering what these conditions were?**

AC: This statement indeed is somewhat obvious. What we want to say is that there are no optimum nutrient recommendations available in literature for Enset, and that, from the data, we can see that currently organic inputs are not used effectively, hence the need for optimization.

Manuscript changes: we rewrote the abstract to highlight more clearly the specific outcomes of the study

#### **Introduction**

**RC1: The introduction needs to be reviewed to make it more concise with information relevant to the study. Currently there is a lot of superfluous information which does not add to the argument being developed in the manuscript.**

AC: Noted

Manuscript changes: the introduction has been restructured and rewritten to make it more concise. Also, a clearer link was made to the international relevance of enset as an orphan crop by adding a section and two references stressing the importance of international scientific attention to indigenous crops.

**RC1: L37 Food security is a main target or is a target?**

AC/Manuscript changes: sentence was rewritten

**RC1: L50 fibre not fiber**

AC/Manuscript changes: adapted as suggested

**RC1: L53 greater of higher?**

AC/Manuscript changes: higher

**RC1: L55-56 How does the dense leaf canopy reduce land degradation and sequester carbon?**

AC: A high canopy cover reduces raindrop impact, interrill erosion and rill erosion (cfr. the Universal Soil Loss Equation) and reduces carbon losses due to erosion. It also provides shade which reduces soil temperature and therefore decomposition rate of organic carbon in the soil. The sentence was adapted and a reference was added. (Lal R., 2003. Environment International 29, 437-450; DOI: 10.1016/S0160-4120(02)00192-7)

Manuscript changes: Sentence changed to : “The dense leaf canopy moreover is an asset in reducing soil erosion and in sequestering carbon (Brandt et al., 1997; Lal, 2003; Tamire and Argaw, 2015).”

RC1: L58 Remove Due to limited genetic research

AC/Manuscript changes: adapted as suggested

**RC1: L62-64 These sentences should be reviewed to be more concise.**

AC/Manuscript changes: that part of the introduction was rewritten

**RC1: L72 Remove wreaking havoc**

AC/Manuscript changes: adapted to “causes significant damage”

**RC1: L82-85 Is this sentence necessary, does it add anything to the argument being developed?**

AC/Manuscript changes: Yes, but we changed it to “Recent studies in banana indicate a promising link between soil fertility management, plant nutrition and bacterial wilt incidence for N, P, K, Ca, and Si (Atim et al., 2013; Mburu et al., 2016) but this avenue remains to be explored for enset.”

**RC1: L88-100 The paragraph outlining the aims and hypothesis needs to be reviewed to make both the hypothesis and aims of the study clearer for the reader.**

AC/Manuscript changes: We highlighted the two main knowledge gaps/hypotheses addressed in this paper by adding the following sentences to the introduction:

“We therefore advocate that soil-plant-nutrient interactions should be studied on-farm first, as to better align agronomical research with farmers’ practices. Moreover, given the considerable ecological amplitude of enset, we hypothesise that these interactions may change with altitude.”

And

“We therefore hypothesize that an insight into soil-plant-pathogen interactions at different altitudes might yield a complementing path for EXW control.”

The aims were rewritten as follows:

“Using an on-farm observational approach, this study therefore aims to increase the knowledge base required to attain the full potential of this ‘orphan crop’ and to improve food security and livelihood of enset dependent farm households, namely by assessing soil-plant-nutrient interactions in typical enset farms and by exploring potential inferences for Xanthomonas wilt prevalence. More specifically, we assessed the impact of altitude and management on soil fertility, by comparing soil properties in enset gardens across different altitudes. We also compared soil properties within the gardens (inner and outer gardens) and between the garden and the surrounding fields (outfields). Furthermore, we related the observed variation in soil nutrients to plant nutrition by comparing soil nutrient levels to leaf nutrient status. Next, we surveyed prevalence and distribution of EXW symptomatic enset gardens and related the distribution of symptomatic gardens to altitude, soil properties and leaf nutrient contents.”

#### Materials and methods

**RC1: L110-113. Is the description of bedrock required?**

AC: Yes, because it is important to understand soil properties later in the manuscript

Manuscript changes: ‘bedrock’ was changed to ‘parent material’ to make this more clear

**RC1: L153-154 Dates are confusing**

AC/Manuscript changes: Adapted to “Observations were made between June 2016 and March 2017”.

**RC1: L159 A mixed soil sample... is a confusing sentence that requires reviewing.**

AC/Manuscript changes: Adapted to “Four bulk soil samples were taken and combined in one composite bulk sample per farm zone and ... “

**RC1: L202 computed not compute**

AC/Manuscript changes: Adapted as suggested

**RC1: L211 of is repeated**

AC/Manuscript changes: Adapted as suggested

**RC1: L212-214 It is unclear what this sentence is trying to explain. Needs revision.**

AC/Manuscript changes: see next comment

**RC1: L211 The number of points on a positive side of the PCA diagram is 64.7%, which means that 35.3% were on the negative side, which does not give a lot of confidence that there is nothing more than chance to where the points occur on the PCA biplot. And RC1: Fig 3 There are no obvious groupings in the PCA diagram. If 95% confidence intervals were placed around elevation or symptomatic gardens there would be a lot of overlap.**

AC: We used the PCA analysis as an exploratory procedure (see line 194), i.e. to get a grip on what explains most of the variation in the dataset and to identify interrelationships among the variables. It shows that most soil nutrients are related (vectors aligned positively along PC1) and that high values for these nutrients imply low values of Al. Moreover, it shows that the variation related to soil nutrient is independent (orthogonal) to soil texture. Subsequently, ANOVA, Mixed models and t-tests were used to establish which of these patterns were significant.

MC: the section was rewritten to make the purpose of the PCA more clear, and significance levels were added to assess the relation between altitude/EXW symptoms and the scores on PC1.

**RC1: The analysis and presentation of the results used should be reviewed to develop a minimum data set that discriminates between the sites and consider the use of box and whisker plots to show how much overlap and variation there is between the different categories.**

AC: This information is in table 1. Putting it into graphs would aid interpretation, but would also result in a very large number of graphs.

**RC1: Table 1 The table is difficult to read and understand as it is presented. Consider using horizontal lines to separate each variable. There is inconsistent placement of the soil property title either in the middle or in centre.**

AC/Manuscript change: Adapted as suggested: a horizontal line drawn between each variable and the soil properties are consistently placed in the middle. Non-significant differences denoted by ns.

**RC1: Consider using ns to denote non-significant difference between elevation categories. This applies to all tables in the manuscript.**

AC/Manuscript change: Adapted as suggested.

**RC1: L243 the description of the zones is very confusing. This should be revised and consistently use throughout the manuscript.**

AC/Manuscript change: Noted. We addressed the description of the zones in the materials and methods section to make it more concise and checked the consistent use of terms throughout the manuscript.

**RC1: Table 2 Consider using ns to denote non-significant difference between elevation categories.**

AC/Manuscript change: Adapted as suggested.

**RC1: L258-266 This section is very confusing, and I am not sure what it is trying to explain. Is it necessary for the data presented within the manuscript?**

AC: The main message of this section is that the difference in soil nutrient levels is not reflected in a difference in leaf nutrient levels, except for N (lines 254-256; Table 3). The information that follows (line 257-266 and figure S1) is not considering fertility zones, but wants to explain that despite high levels of soil nutrients, several (micro)nutrients may be deficient in the plant. Table S1 refers to a bigger dataset than the farms that we have nutrient content for, but as there is very little information about Enset leaf nutrient status available in literature, we decided to add it as supplementary information. We consider this information important for future agronomical research and recommendations, but we agree that the current formulation is confusing.

Manuscript changes: we rewrote the formulation of lines 257-266 accordingly.

**RC1: Table 3 Consider using ns to denote non-significant difference between elevation categories.**

AC: Accepted. The differences outlined in table 3 are between garden zones, not elevation categories

Manuscript change: we will use ns to denote non-significant differences between garden zones and adapt the table caption to make this clearer.

**RC1: Table 3 Does the leaf data add much to the argument?**

AC: One of the main aims of the paper (line 90-93) is a fertility assessment. As there are no standard values or critical ranges described in literature for Enset, we consider this important information for future agronomical research and recommendations.

**RC1: L278-280 This sentence is unclear and requires revision**

AC/Manuscript change: the sentence can be dropped from the manuscript

**RC1: L288-289 This method is not convincing for discrimination between symptomatic and asymptomatic gardens.**

AC/Manuscript change: vide supra (comment on L211): we will use the same approach to see if the factor scores on PC1 and PC2 of symptomatic gardens differ significantly from asymptomatic ones.

**RC1: Table 5 Consider using ns to denote non-significant difference between elevation categories.**

AC: Accepted. The differences outlined in table 5 are between symptomatic and asymptomatic gardens, not elevation categories

Manuscript change: we will use ns to denote non-significant differences between symptomatic and asymptomatic gardens and adapt the table caption to make this clearer.

**RC1: Table 6 It would appear that the entire sample size being used in this table is 11 gardens. How many were symptomatic and how many asymptomatic of EXW? 11 gardens is not a large enough sample size to be meaningful, when 276 were included in the original survey. This would mean that the results are being extrapolated from only 4% of the gardens included in the survey.**

Table 6 should be seen in relation to table 5. Soil properties were measured in 40 gardens to compare symptomatic and non-symptomatic gardens (table 5). The table shows that available P and Ca are significantly different between the two, but as altitude also affected P and Ca levels as well as EXW symptoms, we checked if the influence of these nutrients was still significant if only one elevation zone (the one with the highest prevalence) was considered – which was the case. This is why we added table 6, which is a subset of table 5. (7 symptomatic and 4 non-symptomatic gardens).

Manuscript changes: the number of symptomatic and non-symptomatic gardens will be added in the heading of the table in table 5. Also, we will expand table 6 to include the data of all elevations (also the middle and upper one) – total of 40 farms – and move it to supplementary materials.

**RC1: Table 7 Consider using ns to denote non-significant difference between elevation categories.**



AC: Accepted. The differences outlined in table 7 are between symptomatic and asymptomatic gardens, not elevation categories

Manuscript change: we will use ns to denote non-significant differences between symptomatic and asymptomatic gardens and adapt the table caption to make this clearer.

## **Discussion**

**RC1: Review the use of tense throughout the discussion. E.g. are → were**

AC/Manuscript change: Noted, the manuscript will be adapted and reviewed by a linguist.

**RC1: L342-343. The authors suggest that “continual application of manure and organic waste ...” is responsible for changes in soil nutrient levels. Could the organic waste also be contributing to EXW? If contaminated by-products are disposed into gardens in close proximity to houses there may be an increasing amount of inoculum that is associated with increase in nutrient levels?**

AC: Although no specific information for EXW is available, other strains of Xanthomonas are considered to be eliminated during the composting process (e.g. Elorrieta et al., 2003 in Agriculture, Ecosystems and the Environment [https://doi.org/10.1016/S0167-8809\(02\)00170-6](https://doi.org/10.1016/S0167-8809(02)00170-6)). We therefore did not consider it as a potential source for inoculum in this study.

Manuscript changes: At line 397 we will add the following information: “An alternative explanation is that the organic composts used to fertilize the garden may be a source of inoculum for EXW and hence explain the correlation between certain soil nutrients and EXW incidence. Yet, although no specific information is available for EXW, other Xanthomonas species have been reported to be heat-sensitive and easily eliminated during composting (Elorrieta et al., 2003).

**RC1: L352 The authors indicated that soil nutrients were greater than anticipated. The differences in soil nutrients due to proximity from the house is the main outcome from this study and could be expanded with greater analyses showing differences in nutrients due to garden zones.**

AC: The garden zones are actually distance classes to the house, so they reflect proximity to the house.

Manuscript changes: line 155-159 will be rewritten to make this more clear.

**RC1: L356 “liming effect” or neutralising effect?**

**AC:** The term ‘liming effect’ is commonly used in literature to denote the effect of organic residues on raising soil pH (e.g. Mokobate et al. 2002 in Biology and Fertility of Soils; 10.1007/s00374-001-0439-z)

**RC1: L364-367 The authors suggest that foliar nutrients were excessive based on limited literature? Was there any productivity measures that would support that productivity had plateaued with the increase in leaf nutrient levels or is this purely speculative due to lack of data?**

AC/Manuscript change: The manuscript does not state that the foliar nutrients were excessive, only that there is a lack of variation between foliar nutrient levels. This statement is evidenced in our own data. We assume that the confusion is caused by the use of ‘enset gardens’ on line 361. We propose to change that to ‘enset garden soils’ instead.

AC/Manuscript changes: Observed soil nutrient levels were very high as compared to values for Ethiopia mentioned in literature (see line 353 for references), and to the outfields. Also, if an increase in soil nutrients is not mirrored in an increase in foliar nutrients (hence: plant uptake) it can be considered a sign of inefficient soil nutrient management.

Manuscript change: We will add following information to line 366: “ (...) status. If an increase in soil nutrients is not mirrored in an increase in foliar nutrients, it can be considered a sign of inefficient plant nutrient uptake and therefore non-optimal soil nutrient management. (...)”

## **Conclusion**

**RC1: It is hard to agree with the conclusion as some of the statements are misleading, such as 276 gardens used in the study. While 276 may have been used in the study the conclusions are based on 4% of these sites, only 11, which would appear to be insufficient to be a robust number to draw conclusion of the physicochemical properties that lead to EXW. The authors acknowledge there are many confounding effects in the study which could lead to differences in disease incidence and with only 11 sites on which the physicochemical results are based on, it does not give the reader a lot of confidence in the findings from the survey.**

AC: This is only partly correct, as the paper has two aims (line 90-93): (i) a soil fertility assessment and (ii) examining potential correlations of soil and altitude with Xanthomonas prevalence, as to gain some insight in the ecological niche of the pathogen. With regard to the first objective, the number of sampled farms is 40 (table 1 and 2; first part of the conclusion, line 419-414). With regard to the second objective, disease prevalence was related to altitude in 276 farms (table 4, conclusion line 414) and to soil properties in 40 farms (table 5). As soil properties and altitude are confounded, we also assessed the zone with the highest incidence separately (11 farms, table 6, line 415 in the conclusion).

Manuscript changes: the number of farms for each assessment is clearly indicated with each table. However, we agree with the reviewer that it is unclear in the conclusion. Hence, we have rewritten the conclusion.

**RC1: L414 less instead of lower**

AC/Manuscript changes: adapted as suggested

### **Suggestions**

**RC1: There appears to be four elements to this study of Disease incidence of EXW of Nutrient status of enset gardens iC g With elevation iC g With distance from houses of Leaf nutrient status of enset. These could be analysed separately.**

AC: That is correct. They were analysed in separate paragraphs (nutrient status with elevation in 3.2, nutrient status with distance from the house in 3.3; leaf nutrient status in 3.4 and disease incidence of EXW in 3.5). However, as literature suggests nutrient levels and elevation can influence EXW incidence, we also combined the data in sections 3.6. We made a change to the introduction to make this clearer (see the comment on L88-100)

**RC1: To me, the most interesting aspect of this study is how soil nutrients levels change with distance from the houses. This could be teased out further and reanalysed to determine groups or trends across a gradient from the house?**

AC: As many enset gardens are relatively small (about 0,5 ha) we did not consider it relevant to divide the garden into more than 2 zones.

Manuscript changes: We will add information about the average size of an enset farm to the Materials and Methods section.

**RC1: The manuscript needs to be revised to improve clarity and conciseness throughout. More care is required in the grammar, particularly the use of past tense and the use of commas.**

AC/MC: Noted, the manuscript will be adapted and reviewed by a linguist.

## Comments by R2

The authors would like to thank Reviewer 2 for his/her detailed report and many very relevant remarks. The reviewer very accurately points out the main challenges we experienced with studying an 'orphan crop' as Enset, with limited scientific literature available to this day. Kindly find our answers (AC) to the comments (RC2) below, along with the final changes made to the manuscript. Kindly note that the line numbers below refer to the original submission

### General Comments

**RC2: This manuscript reports an observational study on soil fertility, leaf nutrient content, and Xanthomonas wilt disease incidence in enset gardens of the Ethiopian Gamo highlands in three different altitude zones. In general, the authors found that fertility levels in gardens were higher than in surrounding outfields, increased with decreasing the distance from the house in the garden, and tended to increase with decreasing elevation; except for N, nutrient contents of leaves and soils were not correlated; and disease incidence increased with decreasing elevation. The topic is relevant for the region because of the importance of enset for food security in Ethiopian highlands. In general, the manuscript is clearly written, well structured, and contains observational data that may be technically useful for local farmers and land managers. At the same time, the study lacks crop yield data (...)**

AC: see reply to the editor's comment above

**RC2: At the same time, the study lacks (...) important details on management practices (e.g., amount and form of nutrients added to soils), which make it hardly useful to develop general strategies to improve crop production, enhance soil sustainability, and fight plant diseases.**

AC: We did interviews to learn about the management practices in all 276 visited farms. However, as there is no guide for good practices or extension material available to the farmers, we observed a large variability in practices between farms. Describing it into detail would require a separate manuscript. We summarized the most common and consistent aspects (e.g. on how that plants further away from the garden receive less inputs) and added it to the description of the study area (materials and methods section).

Manuscript changes: we added this information to the materials and methods section.

**RC2: Also because of the observational nature of the study and sampling strategy, it fails to provide clear new insights into processes and mechanisms related to enset nutrition and Xanthomonas wilt disease, in as much as the effects of the soil fertility variables and elevation on disease cannot be not separated.**

AC: To the best of our knowledge, the contrast between the garden zones has not been described in literature before. Moreover, the link between altitude and Xanthomonas diseases in members of the banana family remain an issue of debate, and no data on the effect of nutrient levels on bacterial wilt diseases in Enset is available. We rewrote the discussion and focused more on potential mechanisms, to highlight more clearly the value of our data for targeting future research.

### Specific comments and technical corrections

**RC2: L. 42. Is "(Welw.) Cheesman" needed here?**

AC: Technically, full scientific names of plants require mentioning the author, so we added that information the first time we mention the plant in the manuscript.

**RC2: L. 51-53. "The major food..." This sentence seems to be irrelevant for the present study and should be removed.**

AC/manuscript changes: we will remove the Ethiopian names of the product but will mention that the processed pseudostem and corm are consumed rather than the fruit, to highlight the difference with better known members of the banana family.

**RC2: L. 58- 59. "Due to limited genetic research, there is also no widely adopted nomenclature for enset varieties." Again, this sentence seems to be irrelevant for the study.**

AC/manuscript changes: We removed “due to limited genetic research” and moved the information on varieties/landraces to the description of the study area

**RC2: L. 67- 70. The authors state that there are no recommendations on nutrient management for enset. I do not have access to the full text of the references provided here by the authors (Amede and Taboge, 2007; Elias et al., 1998; Uloro and Mengel, 1994), but they do seem to deal with nutrient management for enset. The present manuscript describes an observational study, and thus the optimal nutrient requirements of enset remain unresolved (L. 366-367).**

AC: It is correct that the papers mentioned deal with nutrients in observational (Amede and Taboge, 2007; Elias et al., 1998) and experimental (Uloro and Mengel, 1994) studies on Enset nutrient requirements. They however state that more research is required, notably per agro-ecological zone. Hence the current study. More experimental research is indeed required to resolve the issue further, as stated on line 416.

Manuscript changes: we mean that there are no official/generally accepted recommendations or extension materials available to enset farmers in the region. We changed the section accordingly to make this clearer.

**RC2: L. 84. Remove “and.”**

AC/manuscript changes: the sentence was rewritten .

**RC2: L. 91. Specify here which agroecological zones you are referring to.**

AC/manuscript changes: as agroecology mainly changes with altitude for enset, we refer to elevation zones. This was stated more clearly in the text.

**RC2: L. 97. Change “further” to “farther”.**

AC/manuscript changes: adapted as suggested.

**RC2: L. 98. I suggest revising to “the relationship between soil properties, leaf nutrient contents, and affected farms was investigated.”**

AC/manuscript changes: the sentence was adapted.

**RC2: L. 99-100. This sentence is redundant and can be removed.**

AC/manuscript changes: adapted as suggested.

**RC2: L. 105. Change to “state of Ethiopia, between 6...”**

AC/manuscript changes: adapted as suggested.

**RC2: L. 112-113. Provide a reference for the soil classification system used.**

AC/manuscript changes: we used World Reference Base for Soil Resources, version 2015. We added the reference.

**RC2: L. 117-120. The zones below 2000 and above 3000 m were not addressed in the study. Why?**

AC: In the study area, there are nearly no enset farms below 2000 m or above 3000m.

**RC2: L. 126. Why these particular sample sizes? Provide the total number of households and gardens in the zones.**

AC: Sample sizes are a consequence of the number of households we found that were willing to participate in the study. We aimed to have a comparable number of farms in each elevation zone. Moreover, we added additional farms in the highest elevation zone, as the relative number of symptomatic farms is much lower there. We were not able to report on the total number of households/garden in each zone, as these data are only available per ‘kebele’ (smallest administrative unit in Ethiopia). However, a kebele typically does not coincide with a single elevation zone.

**RC2: L. 127. Are there inter-varietal differences? This information should be provided if available. The authors mention in the Introduction that previous studies have reported inter-varietal differences in the level of tolerance to Xanthomonas wilt disease.**

AC/manuscript changes: Literature indeed have reported differences in their tolerance to EXW, although none is resistant. Inter-varietal differences on leaf nutrients are not well studied. The main problem is that most varieties are landraces rather than standardized breeds and have only local names that moreover differ between communities (the same variety often has a different name in different villages). Hence, it was impossible to compare the names of the varieties in our study area to the names of varieties mentioned in the literature. On top of that, not all varieties can be accurately distinguished in the field. Due to this level of insecurity, we decided not to consider inter-varietal differences in this study but we mentioned the names of the varieties that we sampled.

**RC2: L. 131. “it was difficult to exactly quantify...” How did this difficulty affect the results?**

AC: as stated on line 131, we decided not to quantify disease incidence (number of plants affected by the disease) as this cannot accurately be done (it is recommended to remove diseased plants, but farmers don't keep records of how many plants they removed during a certain time period). Instead, we decided to use presence/absence data, i.e. if the farm had symptomatic plants or not during the study period and in the last five year.

Manuscript changes: we stated this more clearly in the manuscript.

**RC2: L. 157. Change to “two fertility zones: inner (IR) and outer zone (OR)” Why are these two zones called “fertility” zones?**

AC/manuscript changes: We agree that the names of the zones are confusing. We changed them to inner garden, outer garden and outfields, added a section to the study area description and adapted figure 1 to make this more clear.

**RC2: L. 160. Did you take 40 samples per elevation zone? Please clarify.**

AC: we sampled 40 gardens. In each garden we took a composite sample per garden zone (2 samples per garden, so a total of 80 in the infields). Additionally, we took 28 samples in the outfields, as not all gardens had outfields (some were neighboring other gardens).

Manuscript changes: we rewrote the section on the sampling strategy to make it less confusing. We also added a supplementary table (St1) summarizing the number of gardens and plants sampled.

**RC2: L. 163. Why 19?**

AC: Within the 40 gardens that were intensively sampled for soil nutrients, only 19 had plants of the selected variety (Maze) of a comparable age in both garden zones (IG) and (OG).

Manuscript changes: the section on sampling was rewritten to make this clearer.

**RC2: L. 155-170. The sampling strategy is complex and I found this text a bit difficult to follow. For the sake of clarity, I suggest providing a supplementary table summarizing the number of plant and soil samples, gardens, zones and households.**

AC/manuscript changes: We agree that the section on sampling strategy was difficult to follow. We rewrote it to make it more concise. We added a supplementary table (St1) as suggested.

**RC2: L. 183. Why didn't you analyze other nutrients (e.g., Cu, Zn. . .)?**

AC: We measured those only in plant samples, not in soil samples. This is because the nutrients mentioned on line 183 can all be measured in the same ammonium lactate extract for soil samples, while analyzing the others would require a lot of additional extractions and lab expenses. Hence, as the PCA showed that most soil nutrients are strongly correlated, we decided to focus our limited resources on other aspects of the study.

**RC2: L. 185. “by elemental analysis” is redundant. Please reword.**

AC/manuscript changes: changed to 'total combustion'.

**RC2: L. 200-201. What test did you used for heteroscedasticity? Were normality assumptions checked and tested?**

AC/manuscript changes: Levene's test was used to test for heteroscedasticity. Shapiro-Wilk test and the Normal Quantile plot were used to check normal distribution. Data were log-transformed if necessary. This information was added to the manuscript

**RC2: Figure 3. Axis labels are too small.**

AC/manuscript changes: adapted as suggested.

**RC2: L. 227-228. "... with ranges from 31.8 to ..." These ranges are provided in Table 1 and not needed here.**

AC/manuscript changes: removed as suggested.

**RC2: Table 1. I suggest providing only the most relevant information in a figure, raw data as supplementary information material.**

AC: Ranges for soil properties in enset gardens are rare in literature, especially with regard to differences between altitudes. Hence, we would like to keep the table in the main body of the text.

Manuscript changes: we will use ns to denote non-significant results, to make the table easier to read.

**RC2: L. 239-240. "The differences in soil..." This sentence can be removed.**

AC/manuscript changes: adapted as suggested.

**RC2: L. 254-266. All this info is in Table 3, and this paragraph can be shortened by highlighting only relevant results.**

AC/manuscript changes: adapted as suggested.

**RC2: L. 359-365. Any discussion on soil fertility without on-site data on crop yields remains highly speculative.**

AC: Kindly refer to our response to the general comment on the same issue. In this study, we decided to compare soil fertility to leaf nutrients, which is a standard practice in agronomy to determine nutrient deficiencies.

**RC2: L. 368-374. This discussion is largely based on nutrient contents previously reported for banana, which are not necessarily comparable to enset. This is explicitly recognized by the authors in L: 377-379, "optimal enset nutrient levels may differ substantially from those reported for banana."**

AC: Indeed, more information specific for enset would be ideal. We however only found only two relevant references dealing with leaf nutrient content in enset (Uloro and Mengel (1994) and Nurfeta et al. (2008)), so we also added reference values for banana, which is of the same family and obviously much better studied. As the reviewer states, we explicitly stated in the text that this comparison should be made with caution.

Manuscript changes: we will mention more clearly in table 7 which values are from studies done on enset, and which ones are on banana.

**Altitude and management affect soil fertility, leaf nutrient status and Xanthomonas wilt prevalence in enset gardens.**

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**Abstract**

Enset (*Ensete ventricosum*) is a productive, drought-tolerant and multipurpose food security crop grown in the densely populated Ethiopian highlands. It is a so-called 'orphan crop' and its production suffers from a lack of information on proper soil fertility management and its interaction with bacterial wilt disease, caused by the pathogen *Xanthomonas campestris* pv. *musacearum*. The aim of this study was therefore to assess soil-plant-nutrient variation within enset home gardens at three altitudes (ranging from 2000-3000 masl) in the Gamo highlands and investigate whether this variation affects disease prevalence. Altitude in the rift valley covaries with soil leaching, and plant available P, Ca and Mg significantly raised with decreasing altitude. Moreover, soil carbon and most nutrients reached very high levels in the gardens, whereas the more distant outfields were severely nutrient deprived. Differences in management intensity within the garden caused soil pH, conductivity, total organic carbon (TOC), total N, and available P, K, Ca, Mg, Mn and Fe levels to significantly decline with distance from the house, yet an increase in soil nutrients is not mirrored in a response of foliar nutrient content except for N. Hence, over-fertilization is likely and establishing evidence-based nutrient recommendations for enset would benefit soil quality and productivity both in the gardens as in the outfields. Disease prevalence was high in the study area, with one third of the farms affected in the recent past. Although more experimental work is needed to exclude confounding factors, our data indicate that effects of altitude, P-fertilization, micronutrients and K-Ca-Mg balance are promising avenues for further investigation into EXW disease susceptibility.

**Keywords:** *Ensete ventricosum*, fertility gradient, foliar nutrients, garden zones, optimum nutrient levels, soil properties, symptomatic, non-symptomatic, *Xanthomonas wilt*.

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## 1. Introduction

The global sustainable development goals aim for zero hunger and stress the urgency of combatting climate change impacts on agriculture (SDG 2 and 13, Mariño and Banga, 2016; Rosegrant et al., 2003).

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Indigenous crops with tolerance to marginal conditions and resilience to climatic stress are therefore regarded as an increasingly important avenue for achieving food security and agro-ecosystem resilience in future tropical climate conditions (Allemann et al., 2004; Nayar, 2014; Renard and Tilman, 2019). A large discrepancy exists globally between the potential and the current use of such crops, which is partly attributed to limited international scientific attention and investments (Naylor et al., 2004; Manners and

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van Etten, 2018). One of these so-called 'orphan crops' is enset or 'false banana' (*Ensete ventricosum* (Welw.) Cheesman).

Enset is a perennial multipurpose crop grown for food, feed, fibre and medicine (Bezuneh et al., 1967; Brandt et al., 1997; Nurfeta et al., 2008; Tesfaye and Lüdders, 2003). It is one of the oldest cultivated

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plants in Ethiopia (Brandt et al., 1997), feeding about 20 million people (Brandt et al., 1997; Merga et al., 2019; Yemataw et al., 2014). Unlike other plants from the banana family, enset takes five to seven years to mature, performs best from 2000 to 2750 masl (Brandt et al., 1997) and is not grown for fruit bunches. Instead, the processed pseudo-stem and corm are consumed and leaves are fed to the cattle (Atlabachew and Chandravanshi, 2008; Tsegaye and Struik, 2002; Andeta et al., 2018). Nicknamed the

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'tree against hunger', enset can withstand prolonged periods of moisture stress (Brandt et al., 1997; Quimio and Tezera, 1996) and the food yield per ha is higher than any other crop cultivated in Ethiopia, with the fresh weight of the fermented product ranging from 19-33 t/ha/year (Tsegaye and Struik, 2001).

The dense leaf canopy moreover is an asset in reducing soil erosion and in sequestering carbon (Brandt et al., 1997; Lal, 2003; Tamire and Argaw, 2015). Despite their potential for increasing agricultural

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resilience in future climates, enset farming systems remain under-researched, leaving issues in soil fertility management and disease control largely unresolved (Borrel et al., 2019).

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Deleted: and is an important resource for the farming systems of the densely populated highlands of south, southwestern and central parts of Ethiopia (Brandt et al., 1997; Merga et al., 2019; Yemataw et al., 2014). Early reports mention that enset is generally grown between 1,100 to above 3,000 masl but thrives best from 2,000 and 2,750 masl (Brandt et al., 1997).

Enset is a multipurpose crop used as food and animal forage, and for fiber and medicinal purposes (Bezuneh et al., 1967; Brandt et al., 1997; Nurfeta et al., 2008; Tesfaye and Lüdders, 2003). The major food products are 'kocho' and 'bulla' (both processed products from pseudostem and corm), and 'amicho' (boiled corm; Andeta et al., 2018; Brand et al., 1997; Tsegaye and Struik, 2002). Its

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Enset typically grows in gardens on weathered tropical soils, and animal manure and compost from household refuse are used as soil amendments (Elias et al., 1998; Tamire and Argaw, 2015; Tsgaye and Struik, 2002). However, the supply of these organic inputs is limited and mainly acquired from free-ranging cattle, which puts an additional strain of overgrazing on the already degraded communal lands (Amede and Taboge, 2007; Assefa and Bork, 2017; Garedeew and Ayiza, 2018, Elias et al., 1998). Hence, the optimal use of scarce nutrient resources is vital, yet there are no generally accepted recommendations to the enset farmers in the region. Moreover, information is scanty on how current management has affected soil fertility in existing enset farms. We therefore advocate that soil-plant-nutrient interactions should be studied on-farm first, as to better align agronomical research with farmers' practices. Moreover, given the considerable ecological amplitude of enset, we hypothesize that these interactions may change with altitude.

Enset *Xanthomonas wilt* or bacterial wilt (EXW) caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) also causes significant damage to enset gardens (Garedeew and Ayiza, 2018; Yemataw et al., 2017; Yirgou and Bradbury, 1968). It can cause yield losses up to 100% (Yemataw et al., 2016), and all cultivated varieties are susceptible (Merga et al., 2019), albeit some variation in tolerance occurs (Handoro and Michael, 2007; Welde-Michael et al., 2008a; Wolde et al., 2016). Basic phytosanitary practices involve the removal of diseased plants, disinfection of farm tools, and use of clean planting material (Tadesse et al., 2003; Welde-Michael et al., 2008b). Yet without access to disease-free plantlets, these measures have little effect in curbing the disease and alternative disease control strategies need to be established (Negash et al., 2000; Welde-Michael et al., 2008a; Welde-Michael et al., 2008b; Wolde et al., 2016). Recent studies in banana indicate a promising link between soil fertility management, plant nutrition and bacterial wilt incidence (Atim et al., 2013; Mburu et al., 2016) but this avenue remains to be explored for enset (Huber and Graham, 1999; Huber and Haneklaus, 2007; Huber et al., 201). We therefore hypothesize that an insight into soil-plant and plant-pathogen interactions at different altitudes might yield a complementing path for EXW control.

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Using an on-farm observational approach, this study therefore aims to increase the knowledge base required to attain the full potential of this 'orphan crop' and to improve food security and livelihood of enset dependent farm households, namely by assessing soil-plant-nutrient interactions in typical enset farms and by exploring potential inferences for Xanthomonas wilt prevalence. More specifically, we assessed the impact of altitude and management on soil fertility, by comparing soil properties in enset gardens across different altitudes. We also compared soil properties within the gardens (inner and outer gardens) and between the garden and the surrounding fields (outfields). Furthermore, we related the observed variation in soil nutrients to plant nutrition by comparing soil nutrient levels to leaf nutrient status. Next, we surveyed prevalence and distribution of EXW symptomatic enset gardens and related the distribution of symptomatic gardens to altitude, soil properties and leaf nutrient contents. The Gamo highlands were chosen as a particularly relevant study area, as they have a long history of enset cultivation (Cartledge, 1999; Olmstead, 1975) and EXW is common. Moreover, local altitude gradients represent much of the agro-ecological diversity found in the Ethiopian rift area. In total, EXW prevalence and altitude were recorded in 276 smallholder enset gardens, selected between 2000-3000 masl, which corresponds to the main altitudinal enset belt in the Ethiopian highlands. Soil and leaf nutrients were assessed for a subset of 40 farms.

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**Deleted:** 1 Study area ... [5]

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## 2. Materials and methods

### 2.1 Study area

The study was carried out in the Chencha catchment of the Gamo Highlands in Southern Nations, Nationalities and People's regional state of Ethiopia, (between 6°05'N-6°35'N and 37°30'E-37°45'E). The area rises up from the base of Lake Abaya at 100-3250 masl over a distance of 20 km (Assefa and Bork, 2017; Coltorti et al., 2019). Located in the western part of the southern Ethiopian rift valley escarpment, this landscape is characterized by flat plateaus bordered by steep slopes and dissected by concave valleys and gullies due to erosion (Coltorti et al., 2019). The parent material is mainly made up of continental flood basalts, buried under thick ignimbrites, rhyolites and trachytic flows comprising of

lava flows, pyroclastic and lacustrine deposits (Ayalew et al., 2002; Tefera et al., 1990). Dominant soil types are reddish, deeply weathered Nitisols and Luvisols (IUSS Working Group WRB, 2015; Coltorti et al., 2019).

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360 The local climate is strongly influenced by the complex terrain and mainly associated with altitudinal gradients (Assefa and Bork, 2017; Berhanu et al., 2013; Jury, 2014; Minda et al., 2018). Mean annual temperature ranges between 23 °C and 14 °C and the mean annual rainfall between 750 mm and 1700 mm, in the lowlands and highlands respectively. On the basis of local agro-ecological zonation, four climate zones were defined. These were 'kolla' (semi-arid), below 1500 masl; 'woyna dega' (sub-humid), between 1500 - 2300 masl; 'dega' (humid), between 2300 - 3000 masl, and 'wurch' (frost), a cold alpine zone above 3000 masl (Cartledge, 1999).

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370 In the study area, enset is grown in traditional home gardens surrounding the house (figure 1). Each garden typically comprises a multitude of enset varieties or landraces that are unevenly aged and commonly intercropped with coffee, vegetables, pulses, maize, trees, bamboo or sugar cane in complex patterns and associations (Cartledge, 1995, Tesfaye and Lüdders, 2003; Yemataw et al., 2014). Plantlets are multiplied locally and young plants are densely planted predominantly at the outer rim of the garden. Plants are transplanted several times and moved wider apart and closer to the house as they mature, yet practices vary considerably for different varieties and between farms. Enset gardens are fertilized with animal manure and composted plant and household waste. Fertilizer is rarely used in gardens yet common in the outfields that surround the gardens and are used for arable cropping. Urea and diammonium phosphate (DAP) are most common. The amount of amendments that is applied varies considerably between gardens, depending on financial resources and the amount of land and cattle owned by the farmer. Within the garden, plants near the house (inner garden) receive inputs almost constantly, while plants farther away from the house (outer garden) receive less inputs. A typical amount for the outer garden would be a bamboo basket of ca. 10 kg of composted plant waste and cattle manure per 1-3 enset plants per year (own interviews, performed in 276 visited farms in 2016-2017).

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Figure 1. (A) Typical enset-based farm in the Gamo highlands, surrounding a traditional hut. (B) Illustration of the different farm zones: the area closest to the house is called the inner garden (IG) and receives more organic inputs while the remaining part of enset garden (outer garden - OG ) receives less organic inputs. The garden is often surrounded by a plot devoted for cultivation of annuals, that is fertilized mainly with chemical fertilizer (outfield - OF). (C) Schematic illustration of the spatial arrangement of enset gardens in a landscape. In denser populated areas, gardens are closer together and may not have outfields.

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## 2.2 Sampling and data collection

Based on reconnaissance field visits and discussions with farmers, the larger catchment was divided into three altitude zones: higher (2600 – 3000 masl), middle (2300 – 2600 masl) and lower (2000-2300 masl).

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In these higher, middle and lower altitude zones, gardens were randomly chosen (121, 83 and 72 respectively). Each garden was recorded as “symptomatic” (EXW symptoms present) or “non-symptomatic” (no EXW symptoms observed) and the altitude was noted. Symptoms attributed to EXW were leaf wilting, leaf yellowing and slimy yellow bacterial ooze inside the petiole and leaf sheath tissues (figure 2). The observations were made between June 2016 and March 2017. Based on interviews with the farmer, the presence or absence of symptomatic plants in the previous five years was also registered. It was not possible to accurately assess the number or location of affected plants in the garden, as farmers commonly uproot and remove symptomatic plants and do not keep records.

Soil samples were acquired for a subset of 40 farms, i.e. 14 in the higher, 15 in the middle and 11 in the lower zone. To address intra as well as inter-garden variability, each garden was divided into an inner garden (IG) and an outer garden (OG; figure 1). Further division of these zones was not opportune as the average size of an enset garden is about 0.13 ha. If present, the outfield (OF) zone of the farm surrounding the enset garden was also sampled (this was the case for 28 farms, see supplementary table St<sub>1</sub> for a summary). Four bulk soil samples were taken and combined into one composite bulk sample per farm zone. Sampling depth comprised the upper 25 cm of soil, where most of the enset cord roots are typically distributed (Blomme et al., 2008; Zewdie et al., 2008).

Finally, three sets of leaf samples were taken in order to (i) compare soil nutrient status to leaf nutrient concentrations, (ii) compare nutrient concentrations in leaves of symptomatic and non-symptomatic plants and (iii) document typical nutrient concentrations in enset leaves, as this information is currently not available in literature. Since no standard leaf sampling method is available for enset, the common method for banana was adapted (Martin-Prével, 1977): the central 10 cm of the whole lamina was collected on both sides of the midrib in the second fully open leaf. The first set was collected in the same gardens as the soil samples, if mature (5-7 years old) and non-symptomatic plants from the most common enset variety (locally named ‘Maze’ or ‘Mazeya’; enset varieties do not have standardized names) were present in the inner as well as the outer garden. This was the case for 19 of the 40 gardens in the subset (2 samples per garden, i.e. 38 samples in total). For the second set, leaf samples from 20 pairs of

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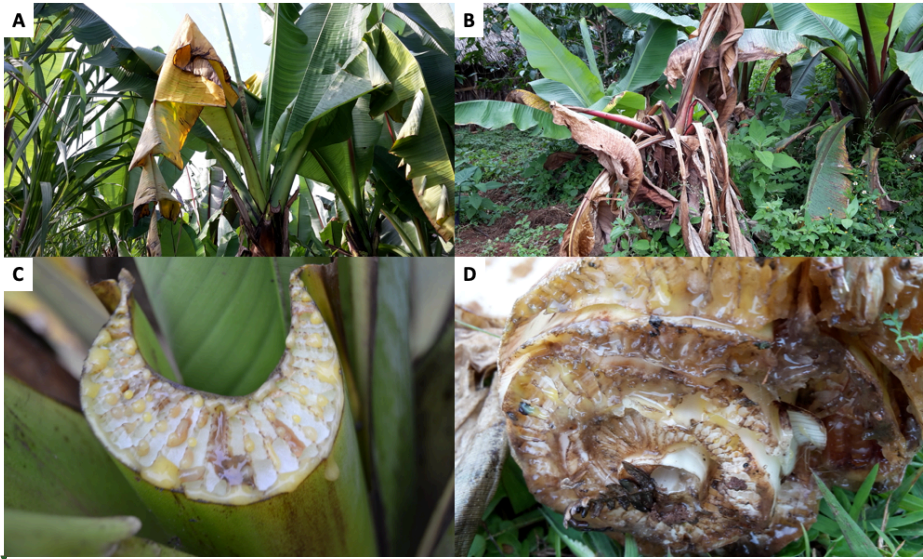
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575 symptomatic and non-symptomatic plants, each pair belonging to the same garden and variety (total of 40 samples) were sampled in 12 gardens. Finally, additional leaf samples of non-symptomatic plants were collected from a range of local varieties (locally named 'Maze or Mazeya', 'Chamo', 'Checho', 'Falake', 'Geena', 'Katame', 'Katise', 'Kunka', 'Maze', 'Phello' and 'Sorghe'), to expand the dataset on non-symptomatic plants to 218 samples from 58 gardens (supplementary table St<sub>1</sub> provides a summary).



580 Figure 2. Visual symptoms attributed to infection by *Xanthomonas campestris* pv. *Musacearum*: leaf wilting and yellowing (A), complete death of the aerial plant part (B) and yellow bacterial ooze emerging from the cut leaf petiole (C) and pseudo-stem (D).

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Fig. 2. Typical onset-based farm in the Gamo highlands, showing traditional huts (light blue arrows), and an iron-roofed house (black arrow) surrounded by an onset home garden (infields) and demonstrating different fertility zones: the area shown by the yellow arrows, with an about 4-6 m radius depending on the size of a garden, represents the area closest to the house and is called the inner zone (IR). The red ellipse, i.e. the remaining part of onset garden surrounding the IR, represents the outer zone (OR) and part of the farm devoted for cultivation of annual crops is the outfield (green arrows).

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### 2.3 Laboratory analysis

610 Soil pH and electrical conductivity (EC) were measured by using 1:5 soil to water ratio. Plant-available  $K_{av}$ ,  $Mg_{av}$ ,  $Ca_{av}$ ,  $Fe_{av}$ ,  $Mn_{av}$  and  $P_{av}$  were extracted by ammonium lactate solution (Egner et al., 1960) and analyzed by inductively coupled plasma optical emission spectroscopy (Winge et al., 1979). Soil and leaf total organic carbon and total nitrogen were measured by total combustion (Carlo Erba EA1110; 615 Kirsten and Hesselius, 1983). Leaf samples were oven-dried at 60-70°C and finely ground. Approximately 50 mg of each ground sample was extracted by 1 ml  $HNO_3$  in acid washed glass tube. Quantification of P, K, Ca, Mg, Zn, Cu, Fe, Mn, Al, Mo, Ni, and Co was made by inductively coupled plasma mass spectroscopy (Date and Gray, 1983). Soil texture was determined by Laser diffraction particle size analysis after pre-treatment with HCl and  $H_2O_2$  (LS 13320-Beckman Coulter; ISRIC, 2002). 620

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### 2.4 Statistical analysis

Data analysis was executed using the JMP Pro 14 statistical software package (SAS Institute Inc., 2018). First, an explorative principal component analysis was computed on soil properties obtain a first appreciation of what explains most of the variation in the dataset and to identify interrelationships among the variables. Then, a one-way analysis of variance was used to determine variation in soil properties among altitudes and between symptomatic and non-symptomatic gardens. On-farm variability in soil properties among the garden zones was determined by a linear mixed model. A paired sample t-test was employed to determine the variation in plant nutrient levels within enset gardens and symptomatic and 630 non-symptomatic plants. Levene's test was used to test for heteroscedasticity. Shapiro-Wilk test and the Normal Quantile plot were used to check for normality assumptions. Data were log-transformed except for pH and EC. Multiple comparisons of significant means were determined using the Tukey-Kramer HSD post hoc test. When unequal variance was observed, Wilcoxon test was used with Steel-Dwass post

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hoc test. All means were separated at 5 % probability level. Disease prevalence (%) was computed as

$$\frac{\text{Number of symptomatic gardens}}{\text{Total number of assessed gardens}} * 100 \%$$

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### 3. Results

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#### 3.1 Multivariate analysis of variability between farms

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Factor loadings of the first two principal components (PCs) explained 56% of the variation of the data (figure 3). PC1 (39 % of the variation) showed positive loadings for most soil nutrients, soil carbon, and pH, whereas AI has negative loadings on this component (figure 3A). PC2 (18% of the variation) has positive loadings for sand and silt while negative loadings for clay content. Hence, a higher score on PC1 reflects lower exchangeable soil acidity and higher soil nutrient status, while the score of a plot on PC2 reflects its soil texture. Garden scores (figure 3B) on PC1 are negatively correlated with farm altitude, yet this correlation is only marginally significant (Spearman rank correlation;  $p < 0.1$ ). Symptomatic gardens have significantly higher scores on PC1 as compared to non-symptomatic gardens (Kruskal-Wallis;  $p < 0.05$ ).

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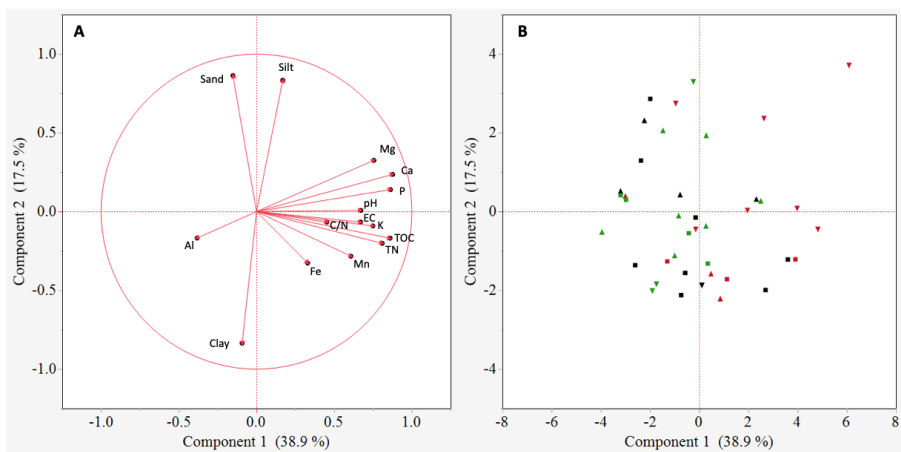
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**Deleted:** Eleven (64.71 %) of the 17 gardens on the positive side of component 1 belonged to the middle and lower elevations. Of the 23 gardens on the negative side of the axis, 18 (78.26 %) belonged to middle and higher elevation; only five (21.74 %) to the lower elevation.

Figure 3. The distribution patterns of 40 enset gardens showing loadings plot (A) in relation to score plot (B) of soil properties (inner and outer garden data pooled) over two principal components. Shapes in B denote enset gardens at higher ( $\Delta$ ), middle ( $\square$ ) and lower ( $\nabla$ ) altitudes, while colours represent gardens currently symptomatic (red), symptomatic in the past five years (black) and had no symptoms (green).

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### 3.2 Effect of altitude on soil properties of enset gardens

Soil texture in enset gardens did not differ with altitude, and the dominant class of the soil texture was clay (table 1). In line with the PCA results, most soil chemical properties showed an increasing trend with decreasing altitude, yet this trend was significant only for  $P_{av}$  ( $p < 0.05$ ),  $C_{dav}$  ( $p < 0.001$ ) and  $Mg_{av}$  ( $p < 0.01$ ).  $P_{av}$  was 65 % higher at the lower than at the higher altitude. Levels of  $C_{dav}$  and  $Mg_{av}$  were 25 % and 16 % larger at the lower than at the middle altitude. In contrast, significantly ( $p < 0.001$ ) higher levels of  $Al_{av}$  were observed at the higher altitude, compared to the middle and lower altitudes. Levels of

$A_{av}$  at the higher altitude were 14 % and 17 % larger than that at the middle, and at the lower altitudes, respectively.

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720 Table 1. Variation in soil properties between enset gardens (IG and OG zones pooled) with respect to altitude (Higher: 2600 -3000 masl, n=14; Middle: 2300-2600 masl, n=15; Lower: 2000-2300 masl, n=11). Soil nutrients refer to available fractions.

Altitude	Soil property	Max	Min	Mean±SD <sup>1</sup>	Soil property	Max	Min	Mean±SD <sup>1</sup>
Higher		10.9	1.5	6.1±2.7 <sup>ns</sup>	$P_{av}$ (mg/kg)	354.7	31.8	151.1±105.0 <sup>b</sup>
Middle	Sand (%)	11	0.4	3.6±3.1 <sup>ns</sup>		1072.5	34.8	390.4±316.9 <sup>ab</sup>
Lower		12	0.2	5.4±3.6 <sup>ns</sup>		1771.6	33.6	711.5±660.1 <sup>a</sup>
Higher		37.5	27.3	32.6±3.2 <sup>ns</sup>	$K_{av}$ (mg/kg)	3609.6	401.6	1726.5±860.2 <sup>ns</sup>
Middle	Silt (%)	41.7	25.4	30.8±4.2 <sup>ns</sup>		4012.4	686	1742.2±1078.6 <sup>ns</sup>
Lower		46.4	26.3	32.3±5.8 <sup>ns</sup>		4013.1	445.7	1625.8±1006.4 <sup>ns</sup>
Higher		71.2	53	61.5±5.6 <sup>ns</sup>	$Ca_{av}$ (mg/kg)	5678.7	1762.8	3818.3±1902.5 <sup>b</sup>
Middle	Clay (%)	73.4	31	66.4±5.0 <sup>ns</sup>		6982.7	1176.9	3569.5±1455.3 <sup>b</sup>
Lower		73.5	30	58.3±15.8 <sup>ns</sup>		7865.3	2477.2	5889.5±1902.5 <sup>a</sup>
Higher		7.6	5.1	6.3±0.8 <sup>ns</sup>	$Mg_{av}$ (mg/kg)	1127	529.8	819.2±194.7 <sup>ab</sup>
Middle	pH (H <sub>2</sub> O)	7.1	5.1	6.4±0.6 <sup>ns</sup>		1154.3	320.2	701.4±222.3 <sup>b</sup>
Lower		7.7	6	6.7±0.4 <sup>ns</sup>		1368.6	614.5	969.2±202.3 <sup>a</sup>
Higher		0.4	0.1	0.2±0.1 <sup>ns</sup>	$Mn_{av}$ (mg/kg)	881	283.4	552.1±216.0 <sup>ns</sup>
Middle	EC (ds/m)	0.5	0.1	0.3±0.1 <sup>ns</sup>		673.1	270.5	459.5±126.6 <sup>ns</sup>

Lower		0.5	0.1	0.3±0.1 <sup>ns</sup>		778.7	377.2	571.5±116.9 <sup>ns</sup>
Higher		4.4	1.9	3.3±0.7 <sup>ns</sup>		664.8	267.7	487.5±117.9 <sup>ns</sup>
Middle	TOC (%)	5.6	2.2	3.7±0.9 <sup>ns</sup>	Fe <sub>av</sub> (mg/kg)	627.1	252.1	457.9±134.2 <sup>ns</sup>
Lower		5.3	2.4	3.9±1.1 <sup>ns</sup>		573.8	194.6	404.9±96.3 <sup>ns</sup>
Higher		0.4	0.2	0.3±0.1 <sup>ns</sup>		793.1	439.5	560.1±95.8 <sup>a</sup>
Middle	TN (%)	0.5	0.2	0.4±0.1 <sup>ns</sup>	Al <sub>av</sub> (mg/kg)	647.4	417.9	419.4±112.5 <sup>b</sup>
Lower		0.5	0.2	0.4±0.1 <sup>ns</sup>		572.4	228.5	396.1±104.4 <sup>b</sup>

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735 <sup>1</sup>Different letters within a column and for the same soil property indicate significant differences in mean soil property values between altitudinal zones (p<0.05). Soil properties that were not significantly different are marked 'ns'.

740 3.3 Gradients in soil properties between inner gardens, outer gardens and outfields.

745 Apart from soil texture, all measured soil properties change significantly (p<0.01) from the garden to the outfields (table 2) and pH, electrical conductivity (EC), TOC, TN, P<sub>av</sub>, K<sub>av</sub>, Ca<sub>av</sub>, K<sub>av</sub> and Mg<sub>av</sub> decrease significantly from the inner garden to the outer garden and from the outer garden to the outfields. The ratio C/N decreased significantly from the garden to the outfields and Mn<sub>av</sub> and Fe<sub>av</sub> were significantly higher in the inner garden as compared to the rest of the farm. Al<sub>av</sub> was significantly higher in the outfields as compared to the inner garden.

750 Table 2. Variation in soil properties (Mean ± SD) within a farm showing average levels and standard deviations for the gardens (inner and outer garden) and the outfield (annually cropped plot surrounding the onset garden).

Soil properties	Inner garden (IG; n=40)	Outer garden (OG; n=40)	Outfield (OF; n=28)
-----------------	-------------------------	-------------------------	---------------------

Sand (%)	4.2±3.4 <sup>ns</sup>	6.2±5.6 <sup>ns</sup>	3.9±3.5 <sup>ns</sup>
Silt (%)	31.6±4.7 <sup>ns</sup>	32.2±6.0 <sup>ns</sup>	29.9±3.3 <sup>ns</sup>
Clay (%)	64.6±7.3 <sup>ns</sup>	63.3±8.5 <sup>ns</sup>	66.2±5.7 <sup>ns</sup>
pH	6.6 ± 0.7 <sup>a</sup>	6.3 ± 0.7 <sup>b</sup>	5.7 ± 0.6 <sup>c</sup>
EC (dS/m)	0.3 ± 0.2 <sup>a</sup>	0.2 ± 0.1 <sup>b</sup>	0.1 ± 0.1 <sup>c</sup>
TOC (%)	3.9 ± 1.2 <sup>a</sup>	3.2 ± 0.8 <sup>b</sup>	2.3± 0.4 <sup>c</sup>
TN (%)	0.4 ± 0.1 <sup>a</sup>	0.3± 0.1 <sup>b</sup>	0.2 ± 0.1 <sup>c</sup>
C:N	10.5±0.9 <sup>a</sup>	10.1±0.7 <sup>a</sup>	9.3±0.6 <sup>b</sup>
P <sub>av</sub> (mg/kg)	477.7± 543.9 <sup>a</sup>	305.1± 406.8 <sup>b</sup>	33.9± 43.5 <sup>c</sup>
K <sub>av</sub> (mg/kg)	2063.6± 1381.2 <sup>a</sup>	1314.8 ± 834.0 <sup>b</sup>	587.9± 385.9 <sup>c</sup>
Ca <sub>av</sub> (mg/kg)	4639.9± 2076.7 <sup>a</sup>	3816.6 ± 1745.7 <sup>b</sup>	2436.8± 1033.2 <sup>c</sup>
Mg <sub>av</sub> (mg/kg)	880.7± 303.8 <sup>a</sup>	744.7± 253.6 <sup>b</sup>	535.6 ± 230.3 <sup>c</sup>
Mn <sub>av</sub> (mg/kg)	564.9± 187.8 <sup>a</sup>	468.1± 176.3 <sup>b</sup>	412.9± 144.4 <sup>b</sup>
Fe <sub>av</sub> (mg/kg)	492.8± 153.8 <sup>a</sup>	421.6± 126.8 <sup>b</sup>	396.6± 92.1 <sup>b</sup>
Al <sub>av</sub> (mg/kg)	443.5± 149.6 <sup>b</sup>	486.9± 123.9 <sup>ab</sup>	524.3± 136.4 <sup>a</sup>

Different letters denote significant differences within a row. Non-significant differences are denoted ns (p<0.05).

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### 3.4 Leaf nutrient status: variation within the garden

Despite the observed significant differences in soil nutrient levels between inner and outer garden, there was very little difference in leaf nutrient levels (table 3). Only leaf total N was significantly (p<0.01) 6% higher in the inner zone than in the outer zone. Ranges for foliar macronutrient and Mo levels were relatively narrow, whereas larger ranges were observed for micro-nutrients (Mn, Fe, Zn, and Cu; supplementary table St<sub>2</sub>). When levels of N, Mn and Fe in both garden zones were compared to optimal and deficiency ranges based on banana as a reference (supplementary figure Sf<sub>1</sub>), they generally fall

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within the optimum range, whereas levels of Ca, Mg, and Cu in both zones fall within the deficiency range. However, P, K and Zn levels in both zones were above the optimum.

Table 3. Leaf nutrient status of 'Maze/Mazeya' enset plants in the inner and outer garden.

Nutrients	n	Inner garden (IG)	Outer garden (OG)
C (%)	19	44.2±1.1 <sup>ns</sup>	43.7±0.9 <sup>ns</sup>
N (%)	19	3.3±0.3 <sup>a</sup>	2.9±0.4 <sup>b</sup>
P (%)	19	0.4±0.1 <sup>ns</sup>	0.4±0.1 <sup>ns</sup>
K (%)	19	5.9±0.8 <sup>ns</sup>	5.9±0.8 <sup>ns</sup>
Ca (%)	19	0.6±0.2 <sup>ns</sup>	0.5±0.2 <sup>ns</sup>
Mg (%)	19	0.3±0.1 <sup>ns</sup>	0.3±0.0 <sup>ns</sup>
Mn (mg/kg)	18	233.8±134.7 <sup>ns</sup>	282.9±192.5 <sup>ns</sup>
Fe (mg/kg)	17	145.9±54.9 <sup>ns</sup>	154.0±75.0 <sup>ns</sup>
Al (mg/kg)	18	102.2±36.6 <sup>ns</sup>	94.5±27.9 <sup>ns</sup>
Cu (mg/kg)	19	6.3 ± 2.3 <sup>ns</sup>	5.8 ± 1.6 <sup>ns</sup>
Zn (mg/kg)	19	36.1 ± 37.5 <sup>ns</sup>	51.7 ± 71.9 <sup>ns</sup>
Mo (mg/kg)	18	1.8± 1.4 <sup>ns</sup>	2.2± 2.4 <sup>ns</sup>

Means followed by a different letter within a row are significantly different (p<0.05). Non-significant differences are denoted ns.

### 3.5 Prevalence and distribution of symptomatic enset gardens

Of the 276 enset gardens (including the 40 gardens in which soil properties were studied), 60 gardens (22%) were currently symptomatic, whereas 96 gardens (35%) had disease symptoms in the recent past (table 4). Disease prevalence increased with decreasing altitude irrespective of time periods. At present, the number of symptomatic gardens in the middle and lower altitudes was 2.6 to 3.6-fold higher

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than in the higher altitude. Moreover, in the 40 enset gardens where soils were sampled, a similar trend of disease prevalence with altitude was observed (supplementary table St<sub>3</sub>).

Table 4. Prevalence of EXW and the altitudinal distribution of symptomatic enset gardens (n=276) in the Chencha catchment, Gamo highlands.

Altitude	N <sup>o</sup> of assessed gardens	N <sup>o</sup> of symptomatic gardens		Prevalence (%)	
		Present	Past 5 years	Present	Past 5 years
Higher	121	12	26	9.9	21.5
Middle	83	22	30	26.5	40.9
Lower	72	26	36	36.1	50.0
Overall	276	60	96	21.7	34.8

### 3.6 Association between of soil and leaf nutrients vs disease prevalence

In line with the significantly higher scores on the PC1 axis, currently symptomatic gardens had significantly (p<0.05) higher levels of P<sub>av</sub> and Ca<sub>av</sub> compared to non-symptomatic gardens (Table 5). The other nutrients and texture could not be linked to the presence or absence of EXW symptoms when all altitudes were pooled. When the gardens of the lower altitude zone (where the incidence is highest) are analyzed separately, currently symptomatic gardens have significantly (p<0.05) higher pH, P<sub>av</sub>, K<sub>av</sub>, and Ca<sub>av</sub> levels compared to non-symptomatic gardens (supplementary table St<sub>4</sub>). When the last five years were considered, TOC and TN were also significantly (p<0.05) higher for symptomatic than for non-symptomatic gardens (data not shown).

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Table 5. Comparison of soil **properties** between symptomatic and non-symptomatic onset gardens (n=40) from the Chencha catchment, Gamo highlands.

Soil <b>properties</b>	Symptomatic <b>gardens (n=14)</b>	Non-symptomatic <b>gardens (n=26)</b>
Sand (%)	3.9±3.2 <sup>ns</sup>	4.9±3.5 <sup>ns</sup>
Silt (%)	31.2±5.2 <sup>ns</sup>	32.0±4.0 <sup>ns</sup>
Clay (%)	58.3±16.8 <sup>ns</sup>	57.3±16.0 <sup>ns</sup>
pH	6.7±0.5 <sup>ns</sup>	6.3±0.7 <sup>ns</sup>
EC (dS/m)	0.3±0.2 <sup>ns</sup>	0.3±0.1 <sup>ns</sup>
TOC (%)	3.9±0.9 <sup>ns</sup>	3.4±0.8 <sup>ns</sup>
TN (%)	0.4±0.1 <sup>ns</sup>	0.3±0.1 <sup>ns</sup>
C:N	10.5±0.4 <sup>ns</sup>	10.2±0.7 <sup>ns</sup>
P <sub>av</sub> (mg/kg)	642.3±612.3 <sup>a</sup>	270.6±272.5 <sup>b</sup>
K <sub>av</sub> (mg/kg)	2037.8±1023.2 <sup>ns</sup>	1521.4±905.6 <sup>ns</sup>
Ca <sub>av</sub> (mg/kg)	5088.8±1918.6 <sup>a</sup>	3813.9±1390.8 <sup>b</sup>
Mg <sub>av</sub> (mg/kg)	881.3±258.4 <sup>ns</sup>	779.6±211.1 <sup>ns</sup>
Mn <sub>av</sub> (mg/kg)	547.5±161.6 <sup>ns</sup>	501.7±165.9 <sup>ns</sup>
Fe <sub>av</sub> (mg/kg)	430.5±114.2 <sup>ns</sup>	470.0±126.8 <sup>ns</sup>
Al <sub>av</sub> (mg/kg)	417.8±103.5 <sup>ns</sup>	487.9±126.2 <sup>ns</sup>

Means followed by the same letters within a row are not significantly (**ns**) different (**p**<0.05).

Comparison of foliar nutrient levels between symptomatic and non-symptomatic plants was significant (**p**<0.05) only for K and Cu (table 6). K and Cu were higher in symptomatic and non-symptomatic plants, respectively.

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Table 6. Pairwise comparison of leaf nutrient status (Mean  $\pm$  SD) between symptomatic and non-symptomatic plants, each the same pairs of 10 local varieties ('Chamo', 'Checho', 'Falake', 'Geena', 'Katame', 'Katise', 'Kunka', 'Maze', 'Phello' and 'Sorghe').

Leaf nutrient	n	Symptomatic plants	Non-symptomatic plants
C (%)	20	44.6 $\pm$ 0.8 <sup>ns</sup>	44.5 $\pm$ 0.8 <sup>ns</sup>
N (%)	20	3.2 $\pm$ 0.5 <sup>ns</sup>	3.2 $\pm$ 0.5 <sup>ns</sup>
P (%)	20	0.4 $\pm$ 0.1 <sup>ns</sup>	0.4 $\pm$ 0.1 <sup>ns</sup>
K (%)	19	4.6 $\pm$ 0.4 <sup>b</sup>	4.9 $\pm$ 0.5 <sup>a</sup>
Ca (%)	20	0.5 $\pm$ 0.2 <sup>ns</sup>	0.5 $\pm$ 0.2 <sup>ns</sup>
Mg (%)	19	0.3 $\pm$ 0.1 <sup>ns</sup>	0.3 $\pm$ 0.0 <sup>ns</sup>
Mn (mg/kg)	19	232.2 $\pm$ 261.7 <sup>ns</sup>	203.4 $\pm$ 178.6 <sup>ns</sup>
Fe (mg/kg)	19	125.3 $\pm$ 47.5 <sup>ns</sup>	125.4 $\pm$ 36.4 <sup>ns</sup>
Al (mg/kg)	19	93.8 $\pm$ 35.7 <sup>ns</sup>	88.2 $\pm$ 25.2 <sup>ns</sup>
Cu (mg/kg)	19	6.0 $\pm$ 1.2 <sup>a</sup>	5.4 $\pm$ 1.7 <sup>b</sup>
Zn (mg/kg)	19	15.7 $\pm$ 3.8 <sup>ns</sup>	16.2 $\pm$ 5.1 <sup>ns</sup>
Mo (mg/kg)	19	1.8 $\pm$ 1.4 <sup>ns</sup>	1.8 $\pm$ 1.5 <sup>ns</sup>
Co (mg/kg)	20	0.1 $\pm$ 0.0 <sup>ns</sup>	0.1 $\pm$ 0.0 <sup>ns</sup>
Ni (mg/kg)	19	1.3 $\pm$ 0.5 <sup>ns</sup>	1.4 $\pm$ 0.6 <sup>ns</sup>

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190 **4. Discussion**

**4.1 Soil fertility in relation to agro-ecology and management practices**

195 Agro-ecological zones in the study area were mainly determined by altitude, which also affects temperature and rainfall, as precipitation decreases with decreasing altitude, in tropical highlands (Cartledge, 1999; Berhanu et al., 2013; Minda et al., 2018). In rift situations, the most weathered soils typically occur highest in the landscape, which is evidenced in the elevated Al<sub>av</sub> content and decreased soil bases (Ca<sub>av</sub> and Mg<sub>av</sub>; figure 3, table 1). Acid and Al rich Nitisols tend to strongly fix P, which is in line with lower P<sub>av</sub> levels at higher altitudes. Slower decomposition of soil organic matter, erosion, and land degradation can also contribute to this effect (Elias, 2017; Shigaki et al., 2007; Vancampenhout et al., 2006). Soil texture typically varies with localized differences in Si content of the volcanic parent material, which explains the lack of correlation with altitude or distance from the garden. For most of the measured soil properties however, intra-farm variability was more prominent than inter-farm variability and strongly linked to TOC levels (figure 3), reflecting the paramount influence of management on soil properties in the study area (table 2). Continuous application of manure and organic waste was common within the gardens but not in the outfields and decreases with distance from the house. This explains the clear intra-garden soil fertility gradient (Amede and Taboge, 2007; Elias et al., 1998; Hailelassie et al., 2006; Tensaye et al., 1998), and the sharp contrast between gardens and outfields. Similar observations have been made for banana in Kenyan smallholder farms (Okumu et al., 2011). The pH measured in the onset gardens is comparable to the optimum range suggested for onset, i.e. 5.6-7.3 (Brandt et al., 1997) and is significantly higher than in the outfields, most likely due to the liming effect of organic resources such as manure, compost and ashes applied in the gardens (Abdala et al., 2015; Agbebe and Adekiya, 2012; Mokolobate, and Haynes, 2002; Whalen et al., 2000). On the other hand, the outfields only receive urea and DAP, which can lower soil pH (Eliyas et al., 1998; Zelleke et al., 2010). Levels of TOC, TN, P<sub>av</sub>, K<sub>av</sub>, Ca<sub>av</sub>, Mg<sub>av</sub>, Mn<sub>av</sub> and Fe<sub>av</sub> in the onset gardens were much higher

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than typical values reported in literature for enset (Ayenew et al., 2018; Elias, 2017; Hengl et al., 2017; Mamo et al., 2014; Mamo et al., 2002; Moges and Holden, 2008; Nabhan et al., 1999; Roy et al., 2006) and banana farms (Ndabamenye et al., 2013; Nyombi et al., 2010). For soil nutrients, recommended levels are not available for enset gardens, although farmers typically expect an increase in growth with higher organic inputs (Amede and Taboge, 2007). A shift from free-ranging to on-stable cattle due to increasing population densities is a trend observed in our study area, and may amplify the flux of nutrients to the inner gardens (own interviews).

Applying more organic inputs closer to the house obviously is more practical, but also serves a purpose. Enset varieties grown for the fermented product of the pseudo-stem are transplanted to the fertile inner zone. As a result, they grow vigorously and produce a higher pseudo-stem and corm biomass. On the other hand, varieties meant for eating the cooked corm remain in the outer, less fertile zone and receive manure only during their earlier growth stages, as slower growth is said to improve the texture and taste of the cooked product (own interviews). Nevertheless, in our study area, the high nutrient levels suggest

that more inputs than required are applied in the gardens, while the outfields suffer from a lack of soil carbon and nutrients (table 2). This hypothesis is supported by the lack of variation observed in foliar nutrient levels between the inner and outer garden zone (table 3), despite significant differences in soil nutrient status; if an increase in soil nutrients is not mirrored in an increase in foliar nutrients, it can be considered a sign of inefficient plant nutrient uptake and therefore non-optimal soil nutrient management.

Hence, agronomical research to determine optimal enset nutrient requirements is needed to optimize input use in the infields and curb soil degradation as well as low arable yields in the outfields.

Foliar N, P and K in our study were comparable to earlier reports by Uloro and Mengel (1994) for enset grown with inorganic NPK. We further compared our leaf nutrient contents (supplementary figure Sf1) to available literature for enset and standards in banana (table 7). Our results were largely comparable to Nurfeta et al. (2008), but P and K levels were higher for enset than banana (Lahav and Turner, 1989; Reuter and Robinson, 1997; Turner and Barkus, 1981). Considering standards in banana, our results were comparable for N, Mn, and Fe, above optimum for P, K and deficient for Ca, Mg and micronutrients

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315 such as Cu and Zn. Our results suggest a potential additional drawback of over-fertilization, as low Ca  
 and Mg could be linked to a reduced absorption caused by high K levels (Baker and Pilbeam, 2007,  
 Hiltunen and White, 2002; Lahav and Turner, 1989) and deficiency in micronutrients may be induced  
 by excessive rates of P (Huang et al., 2000; Singh et al., 1988; Soltangheisi et al., 2013). A comparison  
 to reported enset leaf nutrients in literature confirms the high K and low Ca levels in our study area (table  
 7). Nevertheless, these results need to be interpreted with caution, as optimal leaf sampling methods for  
 320 enset leaves are not known and optimal enset nutrient levels may differ substantially from those reported  
 for banana. Hence, dedicated research to infer optimal foliar nutrient status in enset would be an  
 important scope for future agronomical research. Considering the complexity of establishing yield or  
 crop performance for enset (Tsegaye and Struik, 2000 and 2001; Negash et al., 2013), research based on  
 foliar analysis will be especially important to complement the scanty long-term agronomical trials for  
 325 this crop.

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330 Table 7. Comparison of mean foliar nutrient levels in our study as against reported values for enset  
 (Nurfeta et al., 2009; Mohammed et al., 2013; Nurfeta et al., 2008) and banana (Reuter and Robinson,  
 1997; Turner and Barkus, 1981).

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P(mg/k g)	K(mg/ kg)	Ca(mg/k g)	Mg(mg/k g)	Mn(mg/k g)	Cu(mg/kg )	Zn(mg/k g)	Fe(mg/k g)	Reference
0.4	5.6	0.6	0.3	257.6	8.6	22.5	148.2	This study
0.4	5.3	0.9	0.3	484	10	17.4	552	Nurfeta et al., 2009 Mohammed et al., 2013
0.2	3.1	2.2	0.3	188	2.5	19.7	-	Nurfeta et al., 2008
0.4	4.1	1.1	0.3	194	2.2	10.8	-	Reuter and Robinson, 1997 (banana)
0.2-0.3	3.1-4.0	0.8-1.2	0.3-0.46	2200	7-10	21-35	200	

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#### 4.2 Effects of altitude and nutrients on Xanthomonas wilt disease incidence

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In our study area, Xanthomonas wilt incidence was high: over 1/3 of the visited farms had lost plants to the disease in the last 5 years (table 4). As enset takes between 5-7 years to mature, these losses are an important threat to food security in the area (own interviews). Lower lying areas had a significantly higher prevalence of affected gardens, which is in line with results reported by Wolde et al. (2016) and Zerfu et al. (2018) and can be typically attributed to faster disease progression in the warmer climate at lower altitude (Berhanu et al., 2013; Cartledge, 1999). In contrast, Ocimati et al. (2019) did not find a significant effect of altitude and temperature on Xanthomonas wilt spread in banana farms. However, as enset is cultivated at higher altitudes than bananas, our results indicate that altitude may be a more determining factor for enset.

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The relation between plant nutrition and Xanthomonas wilt is typically more difficult to infer: when plants are nutrient deficient, their susceptibility to diseases may increase (Graham, 1983; Thongbai et al., 1993), yet an excessive amount of some nutrients has also been reported to have negative effects (Dordas, 2008; Huber and Graham, 1999). In our study area, a first complication is that both disease incidence and nutrients levels were highest at the lowest altitudes, so these factors seem confounded. Nevertheless, when assessing disease prevalence at the lower, most affected altitude only, soils of symptomatic farms still have significantly higher levels of certain nutrients. From our observations, two potential mechanisms on how nutrient levels may influence Xanthomonas susceptibility are in line with the data. First, excessive rates of P may interfere with micronutrient uptake (Huang et al., 2000; Singh et al., 1988; Soltangheisi et al., 2013) and micronutrient deficiencies have been shown to increase

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susceptibility to Fusarium wilt in banana (Hecht-Buchholz et al., 1997). In our study area, a significantly higher  $P_{av}$  was observed in the soils of symptomatic farms, while an imbalance in micronutrients is likely based on the leaf analysis reported (tables 3 and 7; supplementary figure Sf<sub>1</sub>). Nevertheless, as the effect of nutrients on a plant's response to disease is often species specific (Ghorbani et al., 2009; Spann et al., 2009), the role of micronutrients in enset is an important avenue for future research. Second, interferences in the uptake between K, Mg and Ca have been evidenced to influence plant health in banana (e.g. Atim et al., 2013; Freitas et al., 2015 and 2016). In our study, symptomatic gardens could be linked to increased levels of  $Ca_{av}$  in the soil of all 40 farms and also to both  $Ca_{av}$  and  $K_{av}$  in the subset of the lower altitudes (table 5, supplementary table St<sub>4-6</sub>). Leaf analysis indicates that plants in our study area had the highest K values reported and K content was significantly different in symptomatic plants, while Ca levels in the leaves were the lowest so far reported (table 6, table 7). The dynamics of those cations in the soil and plant should therefore be further researched in enset, especially in view of the observed over-fertilization with compost and manure.

An alternative explanation is that the organic composts used to fertilize the enset garden may be a source of inoculum for EXW and hence explain the correlation between certain soil nutrients and EXW incidence. Although no specific information is available for EXW, other Xanthomonas species have been reported to be heat-sensitive and have been easily eliminated during composting (Elorrieta et al., 2003; Mwebaze et al., 2006; Silva et al., 2012; Wichuk et al., 2011).

## Conclusion

In this study, we conducted a reconnaissance observational study into soil fertility and EXW prevalence in enset gardens in the Gamo highlands. Our results indicate that soil fertility was strongly influenced by altitude as well as management, with sharp contrasts within enset gardens, and between enset gardens and outfields. Gardens in the study area show very high levels for most nutrients, yet an increase in soil nutrients is not mirrored in a response of foliar nutrient content except for N. Hence, over-fertilization is likely and establishing evidence-based nutrient recommendations for enset would benefit soil quality

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440 and productivity both in the gardens as in the outfields. Disease prevalence was high in the study area, with one third of the farms affected in the recent past. Although more experimental work is needed to exclude confounding factors, our data indicate that effects of altitude, P-fertilisation, micronutrients and K-Ca-Mg balance are promising avenues for further investigation into EXW disease susceptibility.

445 **Author contributions.** K.V, R.S, F.Wo., J.D., G.B. and F.We. designed the observational setup, K.V. and R.M designed the soil and nutrient components of the research, R.S. designed the plant and disease related components, S.S. and L.V. collected and analyzed the data and S.S. compiled the manuscript, supervised by K.V., F.E and R.S. All authors contributed to the interpretation and discussion of the results.

**Competing interests.** The authors declare that they have no conflict of interest.

450 Data availability: DOI: <https://doi.org/10.25502/apce-ng55/d>,  
CKAN link: <http://data.iita.org/dataset/reconnaissance-study-on-ecological-niche-of-ensete-ventricosum>.

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