The soil fertility and leaf nutrient status in enset gardens in different altitude zones of the Gamo highlands, Ethiopia and inferences for Xanthomonas wilt prevalence

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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. Its production suffers from poor soil fertility management and a bacterial wilt disease caused by the pathogen *Xanthomonas campestris* pv. *musacearum.* The aim of this study was to assess soil-plant-nutrient variation

20 within enset home gardens over three different altitudes (ranging from 2000-3000 masl) in the Chencha catchment of the Gamo highlands and investigate whether this variation affects disease prevalence. Plant available P, Ca and Mg significantly increase with decreasing elevation but significantly decline with distance from the house. In addition, soil pH, conductivity, total organic carbon (TOC), total N, available K, Mn and Fe levels significantly decline with distance from the house. This indicates that soil fertility factors are influenced by both agro-ecology and farmers' management practices. Moreover, most nutrients reach

25 very high levels in the garden whereas the more distant outfields are severely nutrient deprived. Plant nutrient levels are not correlated to soil nutrient levels except for N. Twenty two percent of the studied farms are symptomatic for bacterial wilt and its prevalence increases with decreasing elevation. Symptomatic gardens have a higher soil pH and available P, K and Ca levels. We conclude that soil fertility management in enset gardens should be optimized in relation to agro-ecological conditions and that both elevation and soil nutrient status need to be considered when developing strategies to curb the current

30 Xanthomonas wilt epidemic.

Keywords: *Ensete ventricosum*, fertility gradient, inner, outer and outfield zone, optimum nutrient levels, soil properties, symptomatic and non-symptomatic.

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1. Introduction

Food security is a main target for the global sustainable development goals (Mariño and Banga, 2016).
Moreover, new challenges to food security arise because of climate change (Rosegrant et al., 2003).
Hence, crops with tolerance to marginal conditions and resilience to climatic stress such as cassava,
40   cocoyams, yams, sweet potato, and perennials will play an increasingly important role in food security,
but they are underrepresented in international literature (Allemann et al., 2004; Nayar, 2014). One of
these so-called 'orphan crops' is enset or 'false banana' (*Ensete ventricosum* (Welw.) Cheesman). It is
one of the oldest cultivated plants in Ethiopia (Brandt et al., 1997) feeding about 20 million people
(Merga et al., 2019; Yemataw et al., 2014). Nicknamed the 'tree against hunger', enset can withstand
45   prolonged periods of moisture stress (Brandt et al., 1997; Quimio and Tezera, 1996), 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).

50   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 yield per ha is higher
than of any other crop cultivated in Ethiopia, with the fresh weight of fermented kocho ranging from 19-
55   33 t/ha/year (Tsegaye and Struik, 2001). Moreover, the dense leaf canopy is an asset in reducing land
degradation and sequestering carbon (Brandt et al., 1997; Tamire and Argaw, 2015). Despite its high
economic and socio-environmental importance, enset farming systems remain under-researched
compared to common arable crops (Borrel et al., 2019). ~~Due to limited genetic research~~, there is also no
widely adopted nomenclature for enset varieties~~.~~ Local names exist based on agro-morphological traits
60   and uses, and varieties are also commonly intercropped in complex patterns and associations (Cartledge,
1995, Tesfaye and Lüdders, 2003; Yemataw et al., 2014).

Two particular problems reduce yields and hamper the economic competitiveness of the enset systems. The first one is poor soil fertility management. Enset typically grows on weathered tropical soils, which require organic inputs as part of the soil fertility management strategy (e.g. application of animal manure and household refuse; Elias et al., 1998; Tamire and Argaw, 2015; Tsgaye and Struik, 2002). However, supply of organic inputs is limited and mainly acquired from free-ranging cattle, which puts an additional strain of overgrazing on the already degraded grazing lands and fields (Amede and Taboge, 2007; Assefa and Hans-Rudolf, 2017; Garedew and Ayiza, 2018). Hence, the optimal use of scarce nutrient resources is vital, yet there are no recommendations on optimal nutrient management for enset gardens available to farmers (Amede and Taboge, 2007; Elias et al., 1998; Uloro and Mengel, 1994).

Secondly, the enset Xanthomonas or bacterial wilt (EXW) caused by *Xanthomonas campestris* pv. *musacearum* (Xcm) is ~~wreaking havoc~~ in enset dependent farming communities since its first outbreak in Ethiopia (Garedew and Ayiza, 2018; Yemataw et al., 2017; Yirgou and Bradbury, 1968). It can cause yield losses of up to 100% (Yemataw et al., 2016) and all cultivated varieties are susceptible (Merga et al., 2019), although some variation in level of susceptibility/tolerance has been reported (Welde-Michael et al., 2008a; Wolde et al., 2016). Control methods available to farmers are limited to basic phytosanitary practices involving the removal of the diseased plants, disinfection of farm tools, and use of clean planting materials (Tadesse et al., 2003; Welde-Michael et al., 2008b). So far they have had little effect in curbing the disease. Disease-free plantlets are to date not available to farmers (Negash et al., 2000; Welde-Michael et al., 2008a; Wolde et al., 2016). Hence, alternative disease control strategies need to be explored, based on a better understanding of the relation between disease prevalence, soil fertility management and environmental factors (Francl, 2001; Keane and Kerr, 1997). ~~In general, environmental and edaphic conditions and play an important role in disease control in various crops (Agrios, 2005; Huber and Graham, 1999; Huber and Haneklaus, 2007; Huber et al., 2012; Mwebaze et al., 2006; Welde-Michael et al., 2008b).~~ Also, recent studies on banana have indicated an effect of certain nutrients (N, P, K, Ca, and Si) in reducing bacterial wilt incidence (Atim et al., 2013; Mburu et al., 2016; Ochola et al., 2014). We therefore hypothesize that an insight into these interactions might yield a complementing path for disease control in enset systems as well.

Using an on-farm observation approach, the aim of this study was therefore to contribute to food security
and livelihood improvement of enset dependent farm households by assessing (i) soil properties of enset
gardens in various agro-ecological zones and variations within the garden and (ii) soil-plant-pathogen
interactions by comparing nutrient levels in symptomatic and non-symptomatic gardens and plants. The
Gamo highlands are particularly relevant as they have a long history of enset cultivation (Cartledge, 1999;
Olmstead, 1975) and EXW is common in the area. Moreover, local elevation gradients reflect much of
the Ethiopian agro-ecological diversity. In total, 276 smallholder enset gardens were selected between
2,000-3,000 masl, which corresponds to the main altitudinal enset belt in the Ethiopian highlands. In each
farm, soil properties were assessed nearer and further away from the house and soil and leaf nutrients
were compared. Additionally, the prevalence of EXW disease was assessed, and the relationship between
soil properties and affected farms was investigated. Moreover, the leaf nutrient contents of symptomatic
and non-symptomatic plants were compared.

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. It was located 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 1,100-3,250 masl over a distance of 20 km
(Assefa and Hans-Rudolf, 2017; Coltorti et al., 2019). Located in the western part of the southern
Ethiopian rift valley escarpment, the landscape of the Chencha highlands is characterized by flat plateaus
bordered by steep slopes and dissected by concave valleys and gullies due to erosion of the volcanic
sequence (Coltorti et al., 2019). ~~The bedrock of the area 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).~~ Predominant soil types in the enset growing
highlands are reddish, deeply weathered Nitisols and Luvisols (Coltorti et al., 2019).

In the Gamo Highlands, the local climate is strongly influenced by the complex terrain (Jury, 2014) and
mainly associated with altitudinal gradients (Assefa and Hans-Rudolf, 2017; Berhanu et al., 2013). The
mean annual temperature ranges between 23 °C and 14 °C and the mean annual rainfall between 750 mm

and 1,700 mm, in the lowlands and highlands, respectively. On the basis of local agro-ecological zonation, four climate zones were defined in the Gamo highlands. These were 'kolla' (semi-arid), below 1,500 masl; 'woyna dega' (sub-humid), between 1,500-2,300 masl; 'dega' (humid), between 2,300-3,000 masl, and 'wurch (frost), a cold alpine zone above 3,000 masl (Cartledge, 1999).

2.2 Sampling and data collection

A reconnaissance field visit and discussion with farmers before the start of the actual study indicated that the disease seems more problematic with decreasing elevation. Hence, a stratified random sampling based on the elevation gradient was followed. The larger catchment was divided into three elevation zones: lower (2,000-2,300), middle (2,300-2,600) and higher (2,600-3,000 masl). In each zone 72, 83 and 121 households were randomly chosen, respectively. Inter-varietal differences were not maintained as a primary focus in this study. The data acquisition had to take the existing complexities in the enset management system into account. In affected gardens, symptomatic plants are commonly uprooted; the decision depending on severity stage, enset maturity stage, and variety, the household's workload and awareness of sanitary measures. Hence it was difficult to exactly quantify disease incidence in a garden.

Fig. 1. Symptoms of leaf wilting, leaf yellowing and complete death of the aerial plant part (top row) and
145 internal symptoms of yellow bacterial ooze in the cut leaf petiole and section of leaf sheath (bottom row)
caused by *Xanthomonas campestris* pv. *musacearum*.

Therefore, disease presence/absence data were gathered at the household level to map disease distribution
at the catchment scale. Furthermore, each household was asked about the history of disease presence in
150 the past five years. Symptoms were readily recognized by farmers and the identification was aided by
characteristic visual symptoms, i.e. leaf wilting, leaf yellowing, yellow slimy bacterial ooze inside the
petiole and leaf sheath tissues (Fig. 1). Each home garden was recorded as "symptomatic" (symptoms
present) or "non-symptomatic" (no disease symptoms observed). The study took place from June to
November 2016 to March 2017.

155 Soil properties were compared between farms, but as enset is produced in highly diverse home gardens
around traditional houses (Fig. 2), intra-farm variability may be important as well. Each enset home
garden was therefore divided into two fertility zones (Fig. 2), i.e. inner (IR) and outer zone (OR), hereafter
the infields. In addition, an outfield zone (OF) with annually cropped fields around the enset garden, was
included in the study (Fig. 2). A mixed soil sample of four composites was taken per fertility zone and
160 per garden from 40 enset gardens and 28 outfields. 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).
The number of sampled outfields was lower compared to the IR and OR zones, as not all enset gardens
had outfields. Also, 19 leaf samples were collected from the variety locally identified as *Maze,* a variety
often reported as tolerant to EXW (Weldemichael et al., 2008a; Handoro and Michael, 2007), per fertility
165 zone from 19 gardens to measure leaf nutrient levels in relation to soil fertility status. Moreover, leaf
samples were taken from 20 non-symptomatic and 20 symptomatic plants (same pairs of 10 local
varieties) in 12 gardens. Finally, as no literature exists on typical nutrient concentrations in enset leaves,
leaf nutrient levels were assessed of non-symptomatic plants from an additional 27 gardens, making a
total dataset of 58 enset gardens and 218 samples. Since no standard leaf sampling method is described
170 for enset, the common method for banana was adopted (Martin-Prével analysis, 1977), taking the central

10 cm whole lamina on both sides of the midrib. The second fully open leaf was taken from mature plants of approximately 5-7 years old.

Fig. 2. Typical enset-based farm in the Gamo highlands, showing traditional huts (light blue arrows), and
175 an iron-roofed house (black arrow) surrounded by an enset 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 enset 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

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
185 total organic carbon and total nitrogen were measured by elemental analysis (Carlo Erba EA1110; 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 using inductively coupled plasma mass

spectroscopy (Date and Gray, 1983). Soil texture was determined by Laser diffraction particle size

190 analyzer after pre-treatment with HCl and H_2O_2 (LS 13320-Beckman Coulter; ISRIC, 2002).

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. Then, a one-way

195 analysis of variance was used to determine variation in soil properties among elevations and between
symptomatic and non-symptomatic gardens. General nutrient status in enset leaves was the mean value
of all the samples for each nutrient. On-farm variability in soil properties among the fertility zones was
determined by the linear mixed model. A paired sample t-test was employed to determine the variation in
plant nutrient levels within enset garden and symptomatic and non-symptomatic plants. Multiple

200 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 hoc test. All means were
separated at 5 % probability level. Disease prevalence (%) was compute as
$$\frac{\text{Number of symptomatic gardens}}{\text{Total number of assesssed gardens}} * 100.$$

205 ## 3. Results

3.1 Gradients in soil properties between enset gardens: effect of soil texture and elevation

Factor loadings of the first two principal components explained 56.4 % of the variation of the data (Fig.
3). Component 1 (38.9 % of the variation) showed positive loadings for most soil nutrients, soil carbon,
and pH, whereas Al has negative loadings on this component. Component 2 (17.5 % of the variation) has

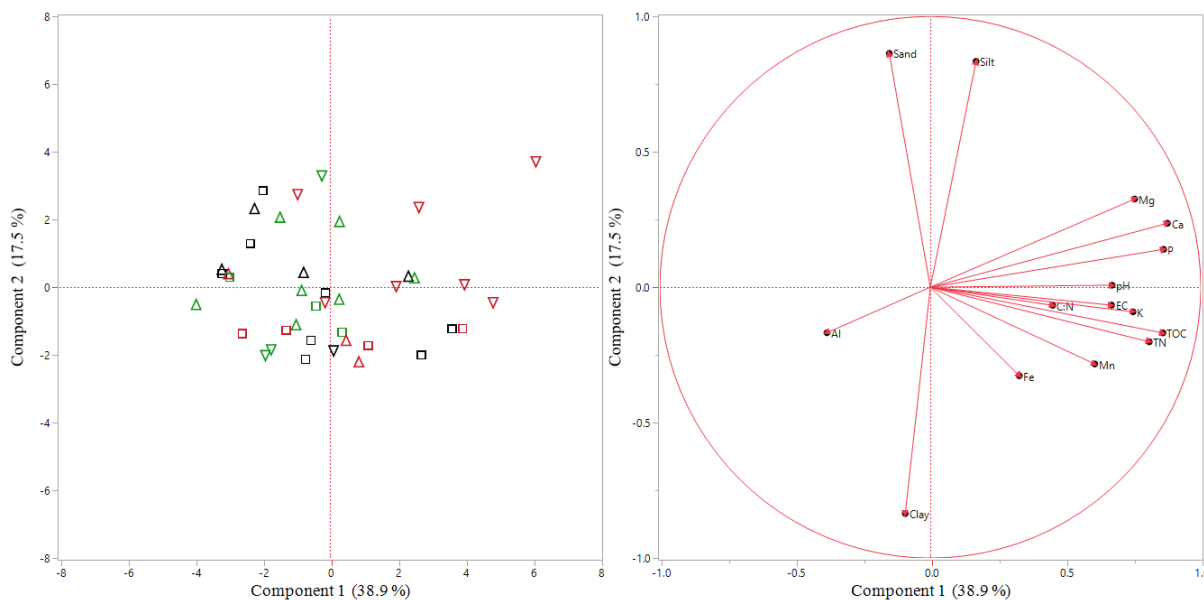
210 positive loadings for sand and silt while negative loadings for clay content. Hence, component 1 mainly
reflects soil nutrient status and component 2 the soil texture. Eleven (64.71 %) of of the 17 gardens on
the positive side of component 1 belonged to the middle and lower elevations. Of the 23 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.

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Fig. 3. A score plot (left) showing the distribution patterns of 40 enset gardens in relation to the loadings plot (right) of soil properties (pooled averages of inner and outer zones) over the two principal components. Symbols in the score plot denote enset gardens at higher (△), middle (▢) and lower (▽) elevations and colours represent currently symptomatic gardens (red), gardens symptomatic in the past 220 five years (black) and gardens with no symptoms (green).

The average and range of values of soil properties against elevation gradient are presented in Table 1. Soil texture in enset gardens did not differ with elevation and the dominant class of the soil texture was heavy clay. Most soil chemical properties showed an increasing trend with decreasing elevation, yet this 225 trend was significant only for plant available P_{av} (p<0.05), Ca_{av} (p<0.001) and Mg_{av} (p<0.01). On the contrary, significantly (p<0.001) higher levels of Al_{av} were observed at a higher elevation compared to the middle and lower elevation. The difference was largest for P_{av} and Ca_{av}, with ranges from 31.8 to 1771.6 mg/kg and 1176.9 to 7865.3 mg/kg, respectively.

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Table 1. Variation in soil factors between enset gardens (inner and outer zones pooled) with respect to elevation (H=Higher: 2600-3000 masl, N=14; M=Middle: 2300-2600 masl, N=15; L=Lower: 2000-2300 masl, N=11). Soil nutrients refer to the available forms (mg/kg).

Elevation	Soil properties	Max	Min	Mean±SD	Soil properties	Max	Min	Mean±SD
H		10.9	1.5	6.1±2.7[a]		354.7	31.8	151.1±105.0[b]
M	Sand (%)	11.0	0.4	3.6±3.1[a]	P_{av}	1072.5	34.8	390.4±316.9[ab]
L		12.0	0.2	5.4±3.6[a]		1771.6	33.6	711.5±660.1[a]
H	Silt (%)	37.5	27.3	32.6±3.2[a]		3609.6	401.6	1726.5±860.2[a]
M		41.7	25.4	30.8±4.2[a]	K_{av}	4012.4	686.0	1742.2±1078.6[a]
L		46.4	26.3	32.3±5.8[a]		4013.1	445.7	1625.8±1006.4[a]
H	Clay (%)	71.2	53.0	61.5±5.6[a]		5678.7	1762.8	3818.3±1902.5[b]
M		73.4	31.0	66.4+5.0[a]	Ca_{av}	6982.7	1176.9	3569.5±1455.3[b]
L		73.5	30.0	58.3+15.8[a]		7865.3	2477.2	5889.5±1902.5[a]
H		7.6	5.1	6.3±0.8[a]		1127.0	529.8	819.2±194.7[ab]
M	pH	7.1	5.1	6.4±0.6[a]	Mg_{av}	1154.3	320.2	701.4±222.3[b]
L		7.7	6.0	6.7±0.4[a]		1368.6	614.5	969.2±202.3[a]
H		0.4	0.1	0.2±0.1[a]		881.0	283.4	552.1±216.0[a]
M	EC (ds/m)	0.5	0.1	0.3±0.1[a]	Mn_{av}	673.1	270.5	459.5±126.6[a]
L		0.5	0.1	0.3± 0.1[a]		778.7	377.2	571.5±116.9[a]
H		4.4	1.9	3.3±0.7[a]		664.8	267.7	487.5±117.9[a]
M	TOC (%)	5.6	2.2	3.7±0.9[a]	Fe_{av}	627.1	252.1	457.9±134.2[a]
L		5.3	2.4	3.9±1.1[a]		573.8	194.6	404.9±96.3[a]
H		0.4	0.2	0.3±0.1[a]		793.1	439.5	560.1±95.8[a]
M	TN (%)	0.5	0.2	0.4±0.1[a]	Al_{av}	647.4	417.9	419.4±112.5[b]
L		0.5	0.2	0.4±0.1[a]		572.4	228.5	396.1±104.4[b]

Means followed by the same letter within a column and for the same property are not significantly different at p<0.05

3.2 Gradients in soil properties between infields and outfields

Apart from soil texture, the measured soil properties changed significantly with distance from the house (Table 2). The differences in soil pH, conductivity, total N, total C, available P_{av}, K_{av}, Ca_{av}, Mg_{av} and

240 Mn_{av} were significant ($p<0.001$). These soil properties were typically higher closer to the house, while available Al_{av} was significantly ($p<0.01$) lower. Except for texture and Al_{av}, all soil properties were lower in the outfields compared to the infield zones. Generally, the difference in magnitude of soil properties between the three fertility zones appeared in the order: Inner - Outer < Outer - Outfield < Inner - Outfield. Within the infield zones, all the soil properties were significantly higher in the inner zone compared to

245 the outer zone, with the exception of available Al and texture.

Table 2. Variation in soil factors (Mean ± SD) within a farm: infields (the inner and outer zones within the enset garden) and the outfield (annually cropped plot around the enset home garden).

Properties	Inner zone (N=40)	Outer zone (N=40)	Outfield zone (N=28)
Sand (%)	4.2±3.4[a]	6.2±5.6[a]	3.9±3.5[a]
Silt (%)	31.6±4.7[a]	32.2±6.0[a]	29.9±3.3[a]
Clay (%)	64.6±7.3[a]	63.3±8.5[a]	66.2±5.7[a]
pH	6.6 ± 0.7[a]	6.3 ± 0.7[b]	5.7 ± 0.6[c]
EC (dS/m)	0.3 ± 0.2[a]	0.2 ± 0.1[b]	0.1 ± 0.1[c]
TOC (%)	3.9 ± 1.2[a]	3.2 ± 0.8[b]	2.3± 0.4[c]
TN (%)	0.4 ± 0.1[a]	0.3± 0.1[b]	0.2 ± 0.1[c]
C:N	10.5±0.9[a]	10.1±.0.7[a]	9.3±0.6[b]
P_{av} (mg/kg)	477.7± 543.9[a]	305.1± 406.8[b]	33.9± 43.5[c]
K_{av} (mg/kg)	2063.6± 1381.2[a]	1314.8 ± 834.0[b]	587.9± 385.9[c]
Ca_{av} (mg/kg)	4639.9± 2076.7[a]	3816.6 ± 1745.7[b]	2436.8± 1033.2[c]
Mg_{av} (mg/kg)	880.7± 303.8[a]	744.7± 253.6[b]	535.6 ± 230.3[c]
Mn_{av} (mg/kg)	564.9± 187.8[a]	468.1± 176.3[b]	412.9± 144.4[b]
Fe_{av} (mg/kg)	492.8± 153.8[a]	421.6± 126.8[b]	396.6± 92.1[b]
Al_{av} (mg/kg)	443.5± 149.6[b]	486.9± 123.9[ab]	524.3± 136.4[a]

Means followed by the same letter within a row are not significantly different at $p<0.05$.

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3.3 Leaf nutrient status: variation within the garden (infields)

Despite the observed significant differences in soil nutrient levels between inner and outer zone in the garden, there was very little difference in leaf nutrient levels (Table 3). Only leaf total N was significantly ($p<0.01$) higher in the inner zone (3.27 ± 0.33 %) than in the outer zone (2.97 ± 0.43 %). In the results obtained from different varieties and pooled for the fertility zones of larger sample households (Table S1), ranges for foliar macronutrient and Mo levels were relatively narrow. ~~Larger ranges were observed for micro-nutrients (Mn, Fe, Zn, and Cu). Levels of Ca, Mg and Fe in both inner and outer zones had similar values with the pooled average values of the mixed varieties.~~ However, levels of N in the inner zone, and P in both zones were lower and K in both zones was higher than the pooled average. ~~Levels of Mn in the outer zone and Al and Zn in both zones were higher while Mn in the inner zone and Cu in both zones were lower than the pooled average. Moreover, compared to the percentage of leaf samples falling within optimal and deficiency ranges using banana as a reference (Fig. S1), levels of N, Mn and Fe in both fertility zones fall within the optimum range, whereas 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 enset plants of the variety locally named *Maze* in the inner and outer zones within enset gardens.

Leaf nutrient	N	Inner zone	Outer zone
C (%)	19	44.2±1.1[a]	43.7±0.9[a]
N (%)	19	3.3±0.3[a]	2.9±0.4[b]
P (%)	19	0.4±0.1[a]	0.4±0.1[a]
K (%)	19	5.9±0.8[a]	5.9±0.8[a]
Ca (%)	19	0.6±0.2[a]	0.5±0.2[a]
Mg (%)	19	0.3±0.1[a]	0.3±0.0[a]
Mn (mg/kg)	18	233.8±134.7[a]	282.9±192.5[a]
Fe (mg/kg)	17	145.9±54.9[a]	154.0±75.0[a]
Al (mg/kg)	18	102.2±36.6[a]	94.5±27.9[a]
Cu (mg/kg)	19	6.3 ± 2.3 [a]	5.8 ± 1.6[a]
Zn (mg/kg)	19	36.1 ± 37.5[a]	51.7 ± 71.9[a]

Mo (mg/kg)	18	1.8± 1.4		2.2± 2.4

270   Means followed by the same letter within a row are not significantly different at p<0.05.

3.4 Prevalence and distribution of symptomatic enset gardens

Of the studied 276 enset gardens (including the 40 gardens in which soil properties were studied), 60 gardens (21.7 %) were currently symptomatic, whereas 96 gardens (34.78 %) had disease symptoms in

275   the recent past (Table 4). Disease prevalence increased with decreasing elevation irrespective of time periods. At present, the number of symptomatic gardens in the middle and lower elevations was 2.6 to 3.6 fold that in the higher elevation. Moreover, in the 40 enset gardens where soils were sampled, a similar trend with elevation was observed (Table S2). Of these 40 gardens, 14 (35.0 %) were currently symptomatic with the prevalence of 21.4 %, 26.7 %, and 63.6 % at higher, middle and lower elevations,

280   respectively.

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

Elevation	Nº of assessed gardens	Nº of symptomatic gardens		Prevalence (%)	
		Present	Past	Present	Past
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

285   ## 3.5 Variation in soil fertility management and disease symptom presence
3.5.1 Effect of soil fertility status

Patterns in the principal component analysis showed that symptomatic gardens were spread in all the quadrants. However, at present, 64 % out of the 14 symptomatic gardens were found on the positive side of component 1 axis (Fig. 3, score plot). These gardens have relatively higher levels of Ca_{av}, P_{av}, TOC,

290   TN, Mg_{av}, and pH (Fig. 3, loadings plot). Of these nine symptomatic gardens, seven (78 %) belonged to low lying (middle and lower) elevations. From the gardens with negative scores on component 1, fewer

(i.e. only five) were symptomatic. This part of the factor space was associated with lower levels of nutrients and higher levels of Al_{av}.

Analysis of variance showed that symptomatic gardens have significantly ($p<0.05$) higher levels of P_{av} and Ca_{av} 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 elevations were pooled. However, analysis for each of the three elevations showed that currently symptomatic gardens have significantly ($p<0.05$) higher pH, P_{av}, K_{av}, and Ca_{av} levels compared to non-symptomatic gardens in the lower elevation only (Table 6). When considering the last five years, significant difference ($p < 0.05$) for TOC and TN was also observed between symptomatic and non-symptomatic gardens. Furthermore, P_{av}, K_{av}, Ca_{av} and Mg_{av} were slightly higher, and Al_{av} was lower in the symptomatic gardens, but the effect was not significant.

Table 5. Comparison of soil factors between symptomatic and non-symptomatic enset gardens (N=40) from the Chencha catchment, Gamo highlands. The average elevation of the symptomatic and non-symptomatic gardens was 2,304.9±257.6 and 2,565.1±274.4 masl, respectively.

Soil factors	Symptomatic	Non-symptomatic
Sand (%)	3.9±3.2[a]	4.9±3.5[a]
Silt (%)	31.2±5.2[a]	32.0±4.0[a]
Clay (%)	58.3±16.8[a]	57.3±16.0[a]
pH	6.7±0.5[a]	6.3±0.7[a]
EC (dS/m)	0.3±0.2[a]	0.3±0.1[a]
TOC (%)	3.9±0.9[a]	3.4±0.8[a]
TN (%)	0.4±0.1[a]	0.3±0.1[a]
C:N	10.5 ±0.4[a]	10.2±0.7[a]
P_{av} (mg/kg)	642.3±612.3[a]	270.6±272.5[b]
K_{av} (mg/kg)	2037.8±1023.2[a]	1521.4±905.6[a]
Ca_{av} (mg/kg))	5088.8±1918.6[a]	3813.9±1390.8[b]
Mg_{av} (mg/kg)	881.3±258.4[a]	779.6±211.1[a]
Mn_{av} (mg/kg)	547.5±161.6[a]	501.7±165.9[a]
Fe_{av} (mg/kg)	430.5±114.2[a]	470.0±126.8[a]

Al$_{av}$ (mg/kg)	417.8±103.5^a	487.9±126.2^a

Means followed by the same letters within a row are not significantly different at p<0.05.

Table 6. Comparison of soil factors (Mean±SD) between currently symptomatic and non-symptomatic enset gardens in the lower elevation (N=11), units as in table 5.

Soil factors	Symptomatic	Non-symptomatic	Nutrients	Symptomatic	Non-symptomatic
pH	6.9±0.4^a	6.3±0.2^b	K	1927.4±544.4^a	767.8±262.0^b
EC	0.4±0.1^a	0.3±0.1^a	Ca	6743.8±1452.9^a	4394.5±1777.7^b
C/N	8.9±3.9^a	9.9±0.5^a	Mg	1035.3±173.1^a	853.6±220.3^a
TOC	3.5±2.1^a	3.7±0.9^a	Mn	572.4±139.9^a	569.9±80.1^a
TN	0.3±0.2^a	0.4±0.1^a	Fe	416.2±80.3^a	385.3±131.1^a
P$_{av}$	1034.1±612.3^a	146.8±185.4^b	Al	369.1±58.0^a	443.5±157.8^a

310 Means followed by the same letters within a row are not significantly different at p<0.05.

3.5.2 Effect of leaf nutrient status

Comparison of foliar nutrient levels between symptomatic and non-symptomatic plants was significant (p < 0.05) only for K and Cu (Table 7). K and Cu were higher in symptomatic and non-symptomatic
315 plants, respectively.

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Table 7. Pairwise comparison of leaf nutrient status (Mean ± 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 ± 0.8[a]	44.5 ± 0.8[a]
N (%)	20	3.2 ± 0.5[a]	3.2 ± 0.5[a]
P (%)	20	0.4 ± 0.1[a]	0.4 ± 0.1[a]
K (%)	19	4.6 ± 0.4[b]	4.9 ± 0.5[a]
Ca (%)	20	0.5 ± 0.2[a]	0.5 ± 0.2[a]
Mg (%)	19	0.3 ± 0.1[a]	0.3 ± 0.0[a]
Mn (mg/kg)	19	232.2 ± 261.7[a]	203.4 ± 178.6[a]
Fe (mg/kg)	19	125.3 ± 47.5[a]	125.4 ± 36.4[a]
Al (mg/kg)	19	93.8 ± 35.7[a]	88.2 ± 25.2[a]
Cu (mg/kg)	19	6.0 ± 1.2[b]	5.4 ± 1.7[a]
Zn (mg/kg)	19	15.7 ± 3.8[a]	16.2 ± 5.1[a]
Mo (mg/kg)	19	1.8 ± 1.4[a]	1.8 ± 1.5[a]
Co (mg/kg)	20	0.1 ± 0.0[a]	0.1± 0.0[a]
Ni (mg/kg)	19	1.3 ± 0.5[a]	1.4 ± 0.6[a]

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

4. Discussion

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

Agro-ecological zones in the study area are mainly determined by altitude, which also affects temperature
335 and rainfall, as precipitation decreases with decreasing elevation in tropical highlands (Cartledge, 1999; Berhanu et al., 2013; Minda et al., 2018). Strong leaching at the highest elevations is reflected by the elevated Al_{av} content and decreased soil bases (Ca_{av} and Mg_{av}). Lower P_{av} levels at higher elevations could be attributed to slower decomposition of soil organic matter, but is more likely related to soil loss and land degradation or by P fixation on the acid and Al rich Nitisols (Elias, 2017; Shigaki et al., 2007;

340   Vancampenhout et al., 2006). For most of the measured soil properties however, intra-garden variability
was more prominent than inter-garden variability, reflecting the paramount influence of management on
soil properties in the study area. Continual application of manure and organic waste is 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; Haileslassie et al., 2006;
345   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). Applying more organic inputs
closer to the house obviously is more practical, but also serves a purpose. Enset varieties grown for
'kocho' processing are planted in the fertile inner zone so they grow vigorously to have higher pseudostem
and corm biomass. On the other hand, varieties meant for 'amicho' are planted in the outer less fertile
350   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.

Levels of TOC, TN, P_{av}, K_{av}, Ca_{av}, Mg_{av}, Mn_{av} and Fe_{av} were ~~surprisingly high~~ inside the enset gardens,
much higher than typical values reported in literature (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.,
355   2006). The pH measured in the enset gardens is comparable to the optimum range suggested for enset,
i.e. 5.6-7.3 (Brandt et al., 1997) and significantly higher than in the outfields, most likely due to the ~~liming~~
effect of the manure, compost and ashes applied in the gardens (Whalen et al., 2000; Abdala et al., 2015;
Agbebe and Adekiya, 2012). The outfields frequently receive urea and DAP, which are more likely to
further acidify the soil (Eliyas et al., 1998; Zelleke et al, 2010). For soil nutrients, recommended levels
360   are not available for enset gardens, although farmers typically expect an increase in growth with higher
organic inputs (Amede and Taboge, 2007). Nutrient levels observed in enset gardens were much higher
compared to typical levels in banana farms (Ndabamenye et al., 2013; Nyombi et al, 2010). Nevertheless,
for our study area, data suggest that more inputs than required are applied in the gardens, while the
outfields suffer from a lack of soil fertility. This hypothesis is supported by the lack of variation observed
365   in foliar nutrient levels between the inner and outer garden zone, despite significant differences in soil
nutrient status. Hence, agronomical research to determine optimal enset nutrient requirements is urgently
needed to optimize input use in the infields and curb soil degradation and 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 (Fig.S1) contents to available literature
370   for enset and standards in banana (Table 8). Our results were largely comparable to Nurfeta et al. (2008)
but P and K levels were higher than all the literature for enset and 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 such
as Cu and Zn. Our results suggest a potential additional drawback of over-fertilization, as low Ca and Mg
375   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 a deficiency in micronutrients may be induced by excessive
rates of P (Huang et al., 2000; Singh et al., 1988; Soltangheisi et al., 2013). These results however need
to be interpreted with caution, as optimal leaf sampling methods for enset leaves are not known and
optimal enset nutrient levels may differ substantially from those reported for banana.

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Table 8. Comparison of mean leaf nutrient levels in our study against reported values for enset and
banana, units as in table 7.

P	K	Ca	Mg	Mn	Cu	Zn	Fe	Reference
0.4	5.3	0.9	0.3	484	10	17.4	552	Nurfeta et al., 2008
0.2	3.1	2.2	0.3	188	2.5	19.7	-	Mohammed et al., 2013
0.4	4.1	1.1	0.3	194	2.2	10.8		Nurfeta et al., 2009
0.2-0.3	3.1-4.0	0.8-1.2	0.3-0.46	100-2200	7-10	21-35	70-200	Reuter and Robinson, 1997
0.2	3.3	0.8	0.4	1476.0	12.1	17.6	150	Turner and Barkus, 1981
0.4	5.6	0.6	0.3	257.6	8.6	22.5	148.2	Our study

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

385   In our study area, 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 typically attributed to faster

disease progression in the warmer climate at lower elevation (Berhanu et al., 2013; Cartledge, 1999). On the contrary, 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 elevations than

390 bananas, elevation may become a more determining factor for enset.

A plant's susceptibility to disease is often reported to increase when nutrients in plants are at a low concentration or deficient (Graham, 1983; Thongbai et al., 1993), yet an excessive amount of nutrients has also been reported to have negative effects, particularly in the case of high nitrogen levels (Dordas, 2008; Huber and Graham, 1999). In our study area, symptomatic gardens were mostly associated with

395 increased levels of available P and Ca in the soil. However, as nutrients were also highest at the lowest altitudes, these factors may be confounded. When assessing disease prevalence at the lower elevation only, soils of symptomatic farms still have significantly higher levels of these nutrients. Hence, especially in view of the observed over-fertilisation, the potential of soil nutrients to amplify the current EXW epidemic may not be overlooked. Contrary to our findings, higher K, Ca and N levels reduced bacterial

400 wilt incidence in banana (Atim et al., 2013) and NPK levels affected Xanthomonas wilt incidence and severity in banana (Ochola et al., 2014). Yet the effect of nutrients on a plant's response to disease is often species specific (Ghorbani et al., 2009; Spann et al., 2009) and results obtained in banana are not necessarily applicable to enset. Zn deficiency increased susceptibility to Fusarium wilt in banana (Hecht-Buchholz et al., 1997) and although we suspect Zn deficiency in enset in our study area, it cannot be

405 substantiated based on this dataset alone.

Conclusion

In this study, we conducted a reconnaissance observational study into soil fertility and EXW prevalence in enset gardens in the Gamo highlands in 276 smallholder enset gardens. Our results indicated that soil

410 fertility was strongly influenced by 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, with little response of foliar nutrient content to an increase in soil nutrients. 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 lower at higher altitude, and

415 in fields with lower soil pH and available P, K and Ca levels. This suggests that effects of environment and nutrients on the current EXW epidemic may not be overlooked, although more experimental work is needed to exclude confounding of factors.

Author contributions. K.V, R.S, F.Wo. and F.We. designed the observational setup, K.V. and R.M

420 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.

Data availability:

425   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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