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

Sabura Shara[1,2], Rony Swennen[3,7], Jozef Deckers[1], Fantahun Weldesenbet[4], Laura Vercammen[1], Fassil Eshetu[2], Feleke Woldeyes[5], Guy Blomme[6], Roel Merckx[1] and Karen Vancampenhout[1]

[1]KU Leuven, Dept. of Earth and Environmental Sciences, Geel and Leuven, Belgium

[2]Arba Minch University, College of Agricultural Sciences, Arba Minch, Ethiopia

[3]KU Leuven, Dept. of Biosystems, Leuven, Belgium

[4]Ethiopian Biotechnology Institute, Addis Ababa, Ethiopia

[5]Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia

[6]Bioversity International, c/o ILRI, Addis Ababa, Ethiopia

[7]International Institute of Tropical Agriculture, Arusha, Tanzania

Correspondence to: Karen Vancampenhout (karenvancampenhout@kuleuven.be)

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 in soils significantly raised with decreasing altitude. 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, total N, and available P, K, Ca, Mg, Mn and Fe levels to significantly decline with distance from the house, yet this decrease in soil nutrients was 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 Xanthomonas wilt disease susceptibility.

Keywords: *Ensete ventricosum*, soil fertility gradient, foliar nutrient levels, home gardens, Ethiopia, Xanthomonas wilt.

1. Introduction

The global sustainable development goals (SDG) 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). 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 (Manners and van Etten, 2018; Naylor et al., 2004). 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 (Brandt et al., 1997; Nurfeta et al., 2008; Tesfaye and Lüdders, 2003). It is one of the oldest cultivated 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 (Andeta et al., 2018; Atlabachew and Chandravanshi, 2008; Tsegaye and Struik, 2002). Nicknamed the '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 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).

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; Elias et al., 1998; Garedew and Ayiza, 2018). 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 might change with altitude.

Enset Xanthomonas wilt (EXW), a bacterial wilting disease caused by *Xanthomonas campestris* pv. *musacearum* (Xcm), causes significant damage to enset gardens (Garedew 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 et al., 2012; Huber and Graham, 1999; Huber and Haneklaus, 2007). We therefore hypothesize that an insight into soil-plant and plant-pathogen interactions at different altitudes might yield a complementing path for EXW control.

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 garden (inner and outer garden) and between the garden and the surrounding fields (outfield). 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.

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 Peoples' 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 1100-3250 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., 1996). Dominant soil types are reddish, deeply weathered Nitisols and Luvisols (Coltorti et al., 2019; IUSS Working Group WRB, 2015).

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).

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, 1999, 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. 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). 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.

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.

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). In these higher, middle and lower altitude zones, enset 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. The subset was selected to obtain similar amounts of farms per agro-ecological zone (i.e. 14 in the higher, 15 in the middle and 11 in the lower zone) and per occurrence of EXW symptoms (i.e. 13 farms with symptomatic enset at the time of sampling, 13 farms with no symptomatic enset at the time of sampling but symptomatic plants were present in the last five years, and 14 farms with no symptomatic enset in the last 5 years; supplementary table St_3) . 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_1 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 *'Mazia'*; 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 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 Mazia'*, '*Chamo', 'Checho', 'Falake', 'Geena', 'Katame', 'Katise', 'Kunka', 'Phello*' and '*Sorghe*'), to expand the dataset on non-symptomatic plants to 218 samples from 58 gardens (supplementary table St_1 provides a summary).

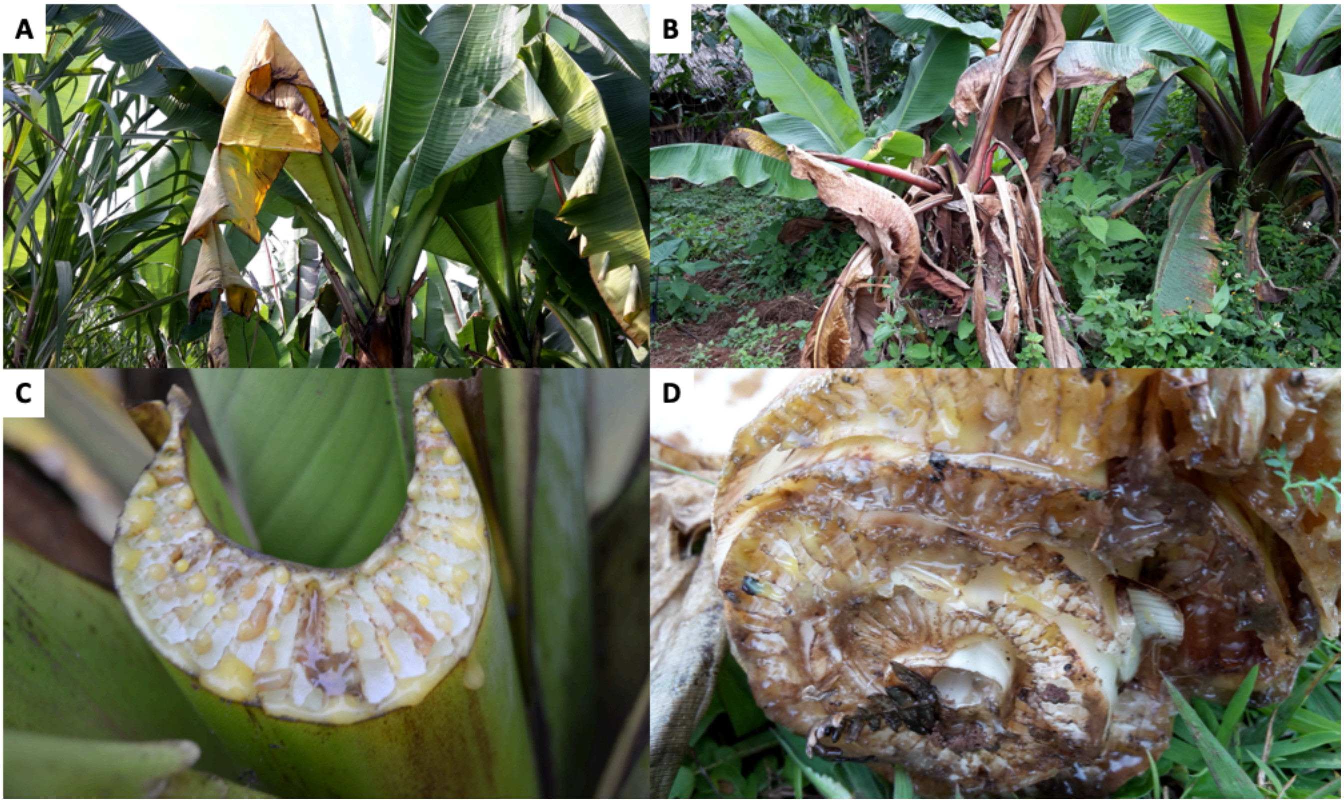

Figure 2. Visual symptoms attributed to infection by *Xanthomonas campestris* pv. *musacearum*: leaf wilting and yellowing (A), complete death of the aerial plant part (B) and yellow bacterial ooze emerging from the cut leaf petiole (C) and pseudo-stem (D)

2.3 Laboratory analysis

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). 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}, Al_{av}, Mn_{av} and P_{av} were extracted from soil samples 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; Kirsten and Hesselius, 1983). Leaf samples were oven-dried at 60-70°C and finely ground. Approximately 50 mg of each ground leaf 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).

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

3. Results

3.1 Variability of soil properties between farms

Factor loadings of the first two principal components (PCs) explained 57 % of the variation of the dataset (figure 3). PC1 (39 % of the variation) showed positive loadings for most soil nutrients, soil carbon, and pH, whereas Al has negative loadings on this component (figure 3A). PC2 (18 % of the variation) has positive loadings for sand and silt and 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; $p < 0.05$).

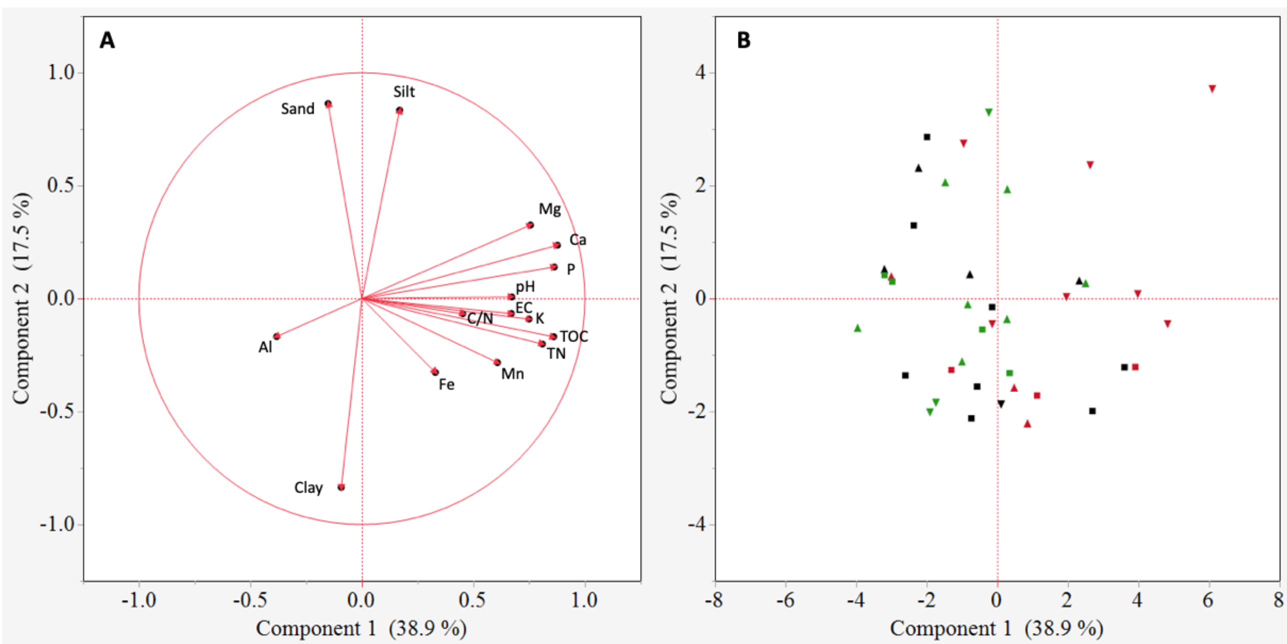

Figure 3. The distribution patterns of 40 enset gardens showing loadings plot (A) in relation to score plot (B) of soil properties (inner and outer garden data pooled) over two principal components. Shapes in B denote enset gardens at higher (△, n=14), middle (□, n=15) and lower (▽, n=11) altitudes, while colours represent gardens that were symptomatic at the time of sampling (red, n=13), were not symptomatic at the time of sampling but had been symptomatic in the past five years (black, n=13) and were not symptomatic in the past 5 years (green, n=14).

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} ($p<0.05$), Ca_{av} ($p<0.001$) and Mg_{av} ($p<0.01$). P_{av} was 65 % higher at the lower than at the higher altitude. Levels of Ca_{av} 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 Al_{av} at the higher altitude were 14 % and 17 % larger than that at the middle and at the lower altitudes, respectively.

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[1]	Soil property	Max	Min	Mean±SD[1]
Higher		10.9	1.5	6.1±2.7^{ns}		354.7	31.8	151.1±105.0^{b}
Middle	Sand (%)	11	0.4	3.6±3.1^{ns}	P_{av} (mg/kg)	1072.5	34.8	390.4±316.9^{ab}
Lower		12	0.2	5.4±3.6^{ns}		1771.6	33.6	711.5±660.1^{a}
Higher		37.5	27.3	32.6±3.2^{ns}		3609.6	401.6	1726.5±860.2^{ns}
Middle	Silt (%)	41.7	25.4	30.8±4.2^{ns}	K_{av} (mg/kg)	4012.4	686	1742.2±1078.6^{ns}
Lower		46.4	26.3	32.3±5.8^{ns}		4013.1	445.7	1625.8±1006.4^{ns}
Higher		71.2	53	61.5±5.6^{ns}		5678.7	1762.8	3818.3±1902.5^{b}
Middle	Clay (%)	73.4	31	66.4+5.0^{ns}	Ca_{av} (mg/kg)	6982.7	1176.9	3569.5±1455.3^{b}
Lower		73.5	30	58.3+15.8^{ns}		7865.3	2477.2	5889.5±1902.5^{a}
Higher		7.6	5.1	6.3±0.8^{ns}		1127	529.8	819.2±194.7^{ab}
Middle	pH (H_2O)	7.1	5.1	6.4±0.6^{ns}	Mg_{av} (mg/kg)	1154.3	320.2	701.4±222.3^{b}
Lower		7.7	6	6.7±0.4^{ns}		1368.6	614.5	969.2±202.3^{a}
Higher		0.4	0.1	0.2±0.1^{ns}		881	283.4	552.1±216.0^{ns}
Middle	EC (ds/m)	0.5	0.1	0.3±0.1^{ns}	Mn_{av} (mg/kg)	673.1	270.5	459.5±126.6^{ns}

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

[1]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'.

3.3 Variation in soil properties between inner gardens, outer gardens and outfields

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_{av}, K_{av}, Ca_{av}, K_{av} and Mg_{av} 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_{av} and Fe_{av} were significantly higher in the inner garden as compared to the rest of the farm. Al_{av} was significantly higher in the outfields as compared to the inner garden.

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 enset garden).

Soil properties	Inner garden (IG; n=40)	Outer garden (OG; n=40)	Outfield (OF; n=28)
Sand (%)	4.2 ± 3.4^{ns}	6.2 ± 5.6^{ns}	3.9 ± 3.5^{ns}
Silt (%)	31.6 ± 4.7^{ns}	32.2 ± 6.0^{ns}	29.9 ± 3.3^{ns}
Clay (%)	64.6 ± 7.3^{ns}	63.3 ± 8.5^{ns}	66.2 ± 5.7^{ns}
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}

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

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 ($p<0.01$) higher (6%) in leaves from plants from the inner compared to the outer garden. 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_2). 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_1), they generally fall 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/Mazia'* enset plants in the inner and outer garden.

Nutrients	n	Inner garden (IG)	Outer garden (OG)
C (%)	19	44.2±1.1^{ns}	43.7±0.9 ^{ns}
N (%)	19	3.3±0.3^{a}	2.9±0.4^{b}
P (%)	19	0.4±0.1^{ns}	0.4±0.1^{ns}
K (%)	19	5.9±0.8^{ns}	5.9±0.8^{ns}
Ca (%)	19	0.6±0.2^{ns}	0.5±0.2^{ns}
Mg (%)	19	0.3±0.1^{ns}	0.3±0.0^{ns}
Mn (mg/kg)	18	233.8±134.7^{ns}	282.9±192.5^{ns}

Fe (mg/kg)	17	145.9 ± 54.9^{ns}	154.0 ± 75.0^{ns}
Al (mg/kg)	18	102.2 ± 36.6^{ns}	94.5 ± 27.9^{ns}
Cu (mg/kg)	19	6.3 ± 2.3^{ns}	5.8 ± 1.6^{ns}
Zn (mg/kg)	19	36.1 ± 37.5^{ns}	51.7 ± 71.9^{ns}
Mo (mg/kg)	18	1.8 ± 1.4^{ns}	2.2 ± 2.4^{ns}

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 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_3).

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

Altitude	N° of assessed gardens	N° 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

Currently symptomatic gardens had 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 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_{av}, K_{av}, and Ca_{av} levels compared to non-symptomatic gardens (supplementary table St_{4-6}). 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).

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

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

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

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.

Table 6. 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 ^{ns}	44.5 ± 0.8 ^{ns}
N (%)	20	3.2 ± 0.5 ^{ns}	3.2 ± 0.5 ^{ns}
P (%)	20	0.4 ± 0.1 ^{ns}	0.4 ± 0.1 ^{ns}
K (%)	19	4.6 ± 0.4^{b}	4.9 ± 0.5^{a}
Ca (%)	20	0.5 ± 0.2 ^{ns}	0.5 ± 0.2 ^{ns}
Mg (%)	19	0.3 ± 0.1 ^{ns}	0.3 ± 0.0 ^{ns}
Mn (mg/kg)	19	232.2 ± 261.7 ^{ns}	203.4 ± 178.6 ^{ns}
Fe (mg/kg)	19	125.3 ± 47.5 ^{ns}	125.4 ± 36.4 ^{ns}
Al (mg/kg)	19	93.8 ± 35.7 ^{ns}	88.2 ± 25.2 ^{ns}
Cu (mg/kg)	19	6.0 ± 1.2^{a}	5.4 ± 1.7^{b}
Zn (mg/kg)	19	15.7 ± 3.8 ^{ns}	16.2 ± 5.1 ^{ns}
Mo (mg/kg)	19	1.8 ± 1.4 ^{ns}	1.8 ± 1.5 ^{ns}
Co (mg/kg)	20	0.1 ± 0.0 ^{ns}	0.1± 0.0 ^{ns}
Ni (mg/kg)	19	1.3 ± 0.5 ^{ns}	1.4 ± 0.6 ^{ns}

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

4. Discussion

4.1 Soil fertility in relation to agro-ecological zones and management practices

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 (Berhanu et al., 2013; Cartledge, 1999; Minda et al., 2018). In rift situations, the most weathered soils typically occur highest in the landscape, which is evidenced in the elevated Al_{av} content and decreased soil bases (Ca_{av} and Mg_{av}; figure 3, table 1). Acid and Al rich Nitisols tend to strongly fix P, which is in line with lower P_{av} 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 nutrient levels covaried with TOC levels (table 2), reflecting the paramount influence of management on soil properties in the study area. 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; Haileslassie 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 enset gardens is comparable to the optimum range suggested for enset, 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; Agbede 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 (Elias et al., 1998; Zelleke et al, 2010). Levels of TOC, TN, P_{av}, K_{av}, Ca_{av}, Mg_{av}, Mn_{av} and Fe_{av} in the enset gardens were 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., 2006) and in 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 Sf_1) 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 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 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 (Negash et al., 2013; Tsegaye and Struik, 2000 and 2001), research based on foliar analysis will be especially important to complement the scanty long-term agronomical trials for this crop.

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

P(mg/kg)	K(mg/kg)	Ca(mg/kg)	Mg(mg/kg)	Mn(mg/kg)	Cu(mg/kg)	Zn(mg/kg)	Fe(mg/kg)	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
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., 2008
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 (banana)
0.2	3.3	0.8	0.4	1476.0	12.1	17.6	150	Turner and Barkus, 1981 (banana)

4.2 Effects of altitude and nutrients on Xanthomonas wilt disease incidence

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.

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 soil 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 susceptibility to Fusarium wilt in banana (Hecht-Buchholz et al., 1998). 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 Sf1). 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 St4-6). 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 where 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).

5. 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 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.

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