

Sustainable Urban Agriculture using Compost and an Open-pollinated Maize Variety

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1 Abstract

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5 2 Global urbanization leads to the loss of periurban farming land and increases dependency on
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7 3 distant agriculture systems. This provokes greenhouse gas emissions associated with
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10 4 transportation and storage while disconnecting nutrient cycles, as urban organic waste is not
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12 5 recycled into the agricultural system. Urban food production based on composted local
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14 6 biomass could reduce these problems, but currently used hybrid crops rely strongly on
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17 7 inorganic fertilizers. On the contrary, open-pollinated varieties were bred for productivity
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19 8 under organic fertilization, such as compost. Hypothesising that open-pollinated varieties
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22 9 retain high nutritional value under low nutrient conditions, a commercial hybrid and a local
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24 10 open-pollinated variety of maize were cultivated in non-fertilized soil and under two compost
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27 11 applications: Municipal compost as high nutrient input or locally produced green waste
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29 12 compost and municipal compost mix, as medium nutrient input. Unfertilized plots exhibited
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32 13 low grain production (1.9 t/ha), but yields under green waste compost/municipal compost (6.1
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34 14 t/ha) and municipal compost (7.8 t/ha) treatments were comparable to observations from
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36 15 maize under inorganic fertilization. Contrary to the commercial variety, the open-pollinated
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39 16 variety exhibited higher grain micronutrient concentrations, e.g. 220 % higher zinc
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41 17 concentrations and lower accumulation of heavy metals, e.g. 74 % lower nickel
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44 18 concentrations. This variety-related effect was found in all treatments and was independent of
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46 19 soil micronutrient concentrations. In conclusion, both compost mixes were effective in
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49 20 increasing grain yield in both maize varieties. However, the open-pollinated variety produced
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51 21 grain with higher nutritional values in soil and all treatments, indicating it is potentially better
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54 22 suited for compost-based sustainable urban agriculture.
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1. Introduction

Global society reached a turning point in 2007 when urban populations exceeded the population living on the countryside (United Nations, 2010), a trend which is expected to continue as cities become polycentric and new peri-urban centres emerge close to existing urban conurbations (Satterthwaite *et al.*, 2010). This development has far reaching consequences for the lives of people in the urban environment. In general, urbanization leads to improved living standards and life expectancy, however, it also becomes increasingly challenging to create resilient urban food supply systems, with subjects such as malnourishment as well as food and nutrition insecurity being pending issues in urban environments (Knorr *et al.*, 2018).

Peri-urban farming might be a viable option to produce food close to urban agglomerations and relieve some of the problems of urban nutrition, however, the rate of expansion of most cities worldwide exceeds urban population growth, which has intensified the competition for nearby agricultural land (Seto and Ramankutty, 2016). The increasing distance between food production and urban consumption sites gives rise to a vast infrastructure needed to distribute and store food while increasing greenhouse gas emissions (GHG) and food waste (Bloem and de Pee, 2017). Another large part of urban waste is organic waste originating from urban green spaces such as gardens, parks and wastelands, which together constitute the largest source of municipal solid waste (Reyes-Torres *et al.*, 2018). This green waste is not only composed of pruning from planted species, but is also increasingly derived from invasive plant species, which rapidly dominate invaded ecosystems and threaten native species found in urban landscapes (Alvey, 2006). Removing this biomass can decrease invasive species pressure, while providing an important source of biomass. However, as urban green waste has a low bulk volume and little economic value, it is commercially unappealing and expensive to collect and process, thus most often this waste ends up almost entirely in landfills on former

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2 48 agricultural land (Adhikari *et al.*, 2010), with further consequences for water quality and
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7 51 A possible contribution to solve the issues of waste management and urban food security at
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9 52 the same time is to compost organic urban waste. This process also has great potential in
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11 53 diminishing GHG emissions, as compost can be used as soil amendment, increasing soil
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13 54 organic matter (SOM) concentrations while sequestering carbon (Bong *et al.*, 2017). Increased
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15 55 SOM concentrations additionally help to maintain soil structure and reduce nutrient leaching,
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17 56 and can turn degraded urban sites into productive farmland (Beniston *et al.*, 2016). Thus,
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19 57 municipal waste compost (MC) could help to create a clean, zero-waste system where
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21 58 resources are reused for urban and peri-urban farming (Lim *et al.*, 2016). While MCs often
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23 59 exhibit a high nutrient content (Cerda *et al.*, 2017), other frequently used composts, such as
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25 60 green waste compost (GWC) are poor in macronutrients essential for plant growth (Reyes-
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27 61 Torres *et al.*, 2018). Nevertheless, GWC has many beneficial aspects as an organic soil
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29 62 amendment, such as high recalcitrance, high CN ratios as well as low heavy metal pollution.
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31 63 Due to these characteristics, mixing GWC into contaminated urban soils can decrease heavy
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33 64 metal loads (Fitzstevens *et al.*, 2017). This could be of interest in urban agriculture, as high-
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35 65 nutrient urban composts, such as MC, often also exhibit high levels of contaminants and
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37 66 heavy metals, which accumulate along the food chain (Wei *et al.*, 2017).

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41 68 While there is substantial work available on mixing feedstock for GWC production with
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43 69 nutrient-rich material, such as manure, food waste or inorganic fertilizers in order to decrease
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45 70 composting time or improve compost quality itself (Reyes-Torres *et al.*, 2018), only recently
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47 71 GWC with subsequent fertilizer combinations were assayed for peri-urban food production,
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49 72 with promising results (Eldridge *et al.*, 2018). However, there are still many issues to address,
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51 73 for example, usage of compost with low nutrient content, such as GWC/MC mixes, can lead
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74 to yield reduction if used without chemical fertilizer on very nutrient demanding crops, such
75 as tomato (Ribas-Agustí et al., 2017). In some pedo-climatic conditions, the addition of MC
76 might lead to lower yields compared to conventional farming, at least when considering short
77 term compost application (Forte et al., 2017). On the other hand, after an initial decrease in
78 maize yield in the first year of application, pure GWC/MC treatments can perform better
79 along time than conventional farming or even mixtures of GWC/MC with additional
80 inorganic fertilizer (Bedada et al., 2014). Also, while lower yields in organic agriculture
81 working with compost might be inherent, there is also ample evidence that food produced
82 using only organic amendments is more nutritious (Rahmann et al., 2017). This is of
83 increasing importance for urban populations, as there remain serious deficiency problems for
84 nutritionally essential micronutrients, even in heavily industrialized countries (FAO, 2013).

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86 Even though micronutrient deficiency in urban centres remains a problem, genetic crop
87 selection is still mainly aimed at increasing growth and yield in food crops, with an associated
88 decrease in micronutrient and vitamin concentrations in the edible portion of the plant, the so-
89 called “dilution effect” (Marles, 2017). This effect is long known and most noticeable in high
90 yielding staple crops of global importance, such as maize, which accounts for 20 % of the
91 global food calories (Pixley and Bjarnason, 2002). Also, modern commercial food crops were
92 bred to exploit an abundance of nutrients to produce high yielding growth (van Bueren *et al.*,
93 2011), while in contrast, many of the more traditional, open-pollinated varieties (OPV) were
94 bred to maintain yields and nutrient concentrations also in low-input agricultural systems
95 (Patto *et al.*, 2008). Thus, the biofortification (increase in nutritional value) of staple crops,
96 such as maize, has become a pending issue and recently, OPVs were recognized for being
97 valuable genetic sources for this undertaking (Puglisi et al., 2018). Taking these observations
98 about plant nutrition into account, research into increased recycling of nutrients from society
99 (Röös et al., 2018) and the selection of crop varieties accustomed to low-input farming

100 (Tsvetkov et al., 2018) are research goals of utmost importance to improve sustainability in
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2 101 (urban) agriculture.
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7 103 With these major topics in mind, the work presented here joins nutrient recycling through
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9 104 composting with the prospect of OPVs of maize (*Zea Mays*) as potentially biofortified
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11 105 varieties. In the urban context of constrained space, high growth rates and yields are essential
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13 106 to turn food production relevant, however, to also be sustainable, plant nutrition needs to rely
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15 107 on locally available sources. In a novel approach both of these aspects were assessed
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17 108 simultaneously by growing a commercial hybrid variety and an OPV solely on MC and GWC
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19 109 as nutrient sources and subsequently measuring yield and mineral nutrition. The treatments
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21 110 consisted of either non-fertilized local soil or two types of nutrient applications: either MC
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23 111 alone as a high nutrient input, or mixed with GWC as a lower nutrient input treatment. Apart
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25 112 from exhibiting lower nutrient concentrations, it was also assumed that GWC might have
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27 113 lower heavy metal concentrations. As the GWC employed was derived from a local invasive
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29 114 species (*Acacia longifolia*), it represents, to our knowledge, the first test of compost from this
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31 115 feedstock as an organic soil amendment for maize. Plant vegetative and grain yield as well as
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33 116 soil and grain micro and macronutrients were measured using inductively coupled plasma–
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35 117 optical emission spectroscopy as a state of the art methodology for ionomic profiling (Jaradat
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37 118 and Goldstein, 2018) and the data sets analysed using modern multivariate and univariate
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39 119 techniques. Putting forward the hypothesis that while the compost treatments would in
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41 120 general increase maize yield, it was also postulated that the OPV exhibits higher grain
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43 121 nutrient concentrations than the commercial variety, thereby putatively rendering it a more
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45 122 suitable option for this type of agriculture.
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2. Materials and Methods

2.1. Sample dates, setup and preparation

The study site is located within the Permaculture Living Laboratory (“PermaLab”) on the campus for Faculty of Science at the University of Lisbon (38°45'29.30"N, 9°9'30.40"W), and measures approximately 30 m by 10 m. It was divided into 16 smaller plots, each measuring 4 m². An automatic drip feed system (GREENTEC, AZUD, Spain) regulated equal water supply to each of the sub-plots throughout the growing period. Aside from the control plots, different mixtures of composts were applied superficially to the other plots and the top 20 cm of soil horizon mixed with the respective composts using a gasoline tiller (GM 105FQSTYLE, Chongqing Jiamu Machinery Co. Ltd, China). The MC used in this study was bought from the company ValorSul in Lisbon and is derived from food waste which is anaerobically digested for biogas production and then composted using wood chips as a bulking agent (ValorSul, 2018) (Class IIA from Decreto-Lei n° 103/2015). GWC for the GWC/MC treatments was prepared on site from *Acacia longifolia* plant material (Ramos, 2016). In brief, plant material was harvested and ground mechanically into pieces < 10 cm and then composted in custom made bioreactors. The bioreactors were build from ROOFMATE™ (DOW, 2018) extruded polystyrene foam insulation sheets of 5 cm thickness, which were joined using polyurethane foam adhesive spray and sealed with acrylic to maintain temperature and humidity constant. Compost was cured for 40 days in controlled mesophilic conditions (40 - 53°C), keeping volumetric water content above 40 %. Final mature composted material had: 1.5 N %, 48.0 C % and a CN ratio of 34.3.

The extrapolated amount of compost applied in all cases was 2,812.5 m³/ha, based on experiments from Brito et al., 2015, who used *Acacia longifolia* compost mixed with other substrates and soil as horticultural growth substrates (50 % v/v). The MC treatments were

147 prepared by adding 2,812.5 m³/ha MC to the plots and the GWC/MC treatments were
148 prepared by mixing in 937.5 m³/ha of mature GWC with 1,875 m³/ha of MC before
149 application. After compost application, the experimental plots were left to settle for one week
150 and maize grain then sown in rows by hand to a depth of 1 cm, at a density of 5.5 plants/m²
151 towards the end of June 2016. No biocides or mechanical clearing were applied during the
152 course of the experiment and all plots received equal amounts of tap water by drip irrigation.
153 At the end of the growing season (first week of October), plant height and amount of ears
154 were determined and aboveground biomass of all plants was harvested and separated out in to
155 stems, leaves, ear husks, cobs and grain. The material from each plant was air-dried at room
156 temperature and weighed. To account for interference from air humidity, subsamples were
157 taken and dried in an oven at 60 °C until constant weight to extrapolate total dry weight per
158 plant. Plant height, number of ears and dry weight of plant and grain mass were treated as
159 pseudo replica, pooled per plot and subsequently their mean was used for statistical analysis.
160 If any plant was damaged by adverse conditions, like wind or animal interference, etc. it was
161 removed from analysis, however, there were never less than 6 plants pooled per plot. This
162 way, the true replicates per plot and variety were: n_{Control} = 5; n_{MC} = 5; n_{GWC/MC} = 3. Nutrient
163 analysis were done on pooled grains per variety per plot and to assess single kernel weight,
164 kernels were grouped by variety and the weight of a single kernel calculated by extrapolating
165 from weighing 36 batches of 10 kernels each.

2.2. Plant variety and phenology

168 The maize varieties used in this experiment were a commercial hybrid variety (SY Sincero,
169 Syngenta, Portugal), termed “commercial variety” later in the text, and a traditional, regional
170 OPV called “pata-de-porco multicolorido”, which describes its multi-coloured kernels and ear
171 fasciation.

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3 173 2.3. Soil collection, preparation and organic matter analysis

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6 174 At the end of September 2016, 3 topsoil subsamples were taken from each plot to a depth of
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9 175 10 cm from the surface and mixed thoroughly to obtain a pooled sample. The collected soil
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11 176 was sifted through a 2 mm sieve and then dried at 60 °C until constant weight before being
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14 177 transferred to a muffle furnace (L3 Nabertherm, Lilienthal, Germany) for 6 hours at 600 °C to
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16 178 determine the soil organic matter. This process was followed by further exposure for 2 h at
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19 179 950 °C to determine the carbonate content on loss of ignition in accordance with the method
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21 180 described in Heiri *et al.* (2001). Finally, each sample was ground to produce a powder using a
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23 181 ball mill (Mixer Mill MM 400, Retsch, Germany) in preparation for isotopic and ionic
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26 182 analysis.

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29 183 2.4. Ionomics, total carbon, nitrogen and stable isotope analysis

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32 184 Ionomics values for each of the plants were determined using inductively coupled plasma
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35 185 optical emission spectrometry (ICP-OES; Thermo Elemental Iris Intrepid II XDL; Franklin,
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37 186 MA, USA) after a microwave assisted digestion with HNO₃:H₂O₂ (4:1, v:v) in the Ionomics
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40 187 Service of the Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC, Spain)
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42 188 following ISO/IEC guidelines (UNE-EN ISO/IEC 17025, 2018).

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45 189 Stable isotope ratio analyses were also measured at the Stable Isotopes and Instrumental
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48 190 Analysis Facility (SIIAF), Centre for Ecology, Evolution and Environmental Changes (cE3c),
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51 191 located in the Faculty of Sciences, University of Lisbon - Portugal. Values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
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53 192 were determined for each sample using continuous flow isotope mass spectrometry (CF-
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55 193 IRMS), on a Sercon Hydra 20-22 (Sercon, UK) stable isotope ratio mass spectrometer,
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58 194 coupled to a EuroEA (EuroVector, Italy) elemental analyser for online sample preparation by
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60 195 Dumas-combustion (Preston and Owens, 1983). A delta Calculation was performed according

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196 to $d = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] * 1000$, where R is the ratio between the heavier and lighter
197 isotopes. In the analysis, $\delta^{15}\text{N}_{\text{Air}}$ values refer to concentrations found in air,
198 and $\delta^{13}\text{C}_{\text{VPDB}}$ values are referred to as PDB (Pee Dee Belemnite). The reference materials
199 used were Sorghum Flour Standard OAS and Wheat Flour Standard OAS (Elemental
200 Microanalysis, UK), for nitrogen and carbon isotope ratio (where $\delta^{15}\text{N}_{\text{Air}}$ (Sorghum Flour
201 OAS) = 1.58 +/- 0.15 ‰, $\delta^{15}\text{N}_{\text{Air}}$ (Wheat Flour OAS) = 2.85 +/- 0.17 ‰, and $\delta^{13}\text{C}_{\text{VPDB}}$
202 (Sorghum Flour OAS) = -13.68 +/- 0.19 ‰, $\delta^{13}\text{C}_{\text{VPDB}}$ (Wheat Flour OAS) = -27.21 +/- 0.13
203 ‰) (Coleman and Meier-Augenstein, 2014). The level of predicted uncertainty in the
204 observed value for the isotope ratio analysis was ≤ 0.1 ‰ and calculated using the results
205 from 6 to 9 replicates of secondary isotopic reference material (wheat flour, $\delta^{15}\text{N}_{\text{Air}} = 2.88$ +/-
206 0.19 ‰, $\delta^{13}\text{C}_{\text{PDB}} = -27.27$ +/- 0.19 ‰), which were interspersed among samples in every batch
207 analysis. The major mass signals for N and C were used to calculate total N and C
208 abundances, using Sorghum Flour Standard OAS and Wheat Flour Standard OAS, with 1.47
209 % N, 46.26 % C and 1.47 % N, 39.53 % C respectively, as elemental composition reference
210 materials (Elemental Microanalysis, UK).

211 2.5. Statistical analysis

212 Plant traits (Height, Plant Weight, Grain Weight, Ears) were measured for each plant to
213 provide a mean value calculated for all plants per plot. Stable isotope ratio and Ionic
214 analyses were performed on aggregated sample data for each plot. Statistical analyses were
215 performed with the package “stats” using version R 3.3.2 (R Core Team, 2016) and executed
216 on RStudio (IDE version 1.0.136). Additional packages used were: “Hmisc”, “lawstat”,
217 “lmtest” and “FactoMineR” (Lê *et al.*, 2008). Pairwise comparisons between groups were
218 calculated using Pairwise Welch’s t-test with Bonferroni - Holm correction. If only two
219 groups were compared, Welch’s t-test was used. Normality assumptions for group wise
220 comparisons were verified using the Shapiro-Wilk Normality Test. If the normality
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222 assumption was violated, data was transformed to log or square root values. Linear
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2 223 regressions were performed after verifying assumptions using the Breusch-Pagan Test for
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4 224 homoscedasticity and the Shapiro-Wilk Normality Test on the regression model residuals.
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7 225 Figure 3 is a network plot created from a spearman correlation matrix. Figure 4 is based on an
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9 226 RV coefficient matrix, generated by using the package FactoMineR to highlight relationships
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12 227 between groups of variables. For multivariate explorative data analysis of grain and plant
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14 228 traits, a multiple factor analysis (MFA) was employed, using plant traits, grain nutrients and
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17 229 the two factors treatment type and plant variety as input (Figure 5).

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3. Results and Discussion

3.1. Soil changes

The topsoil conditions in all the study plots were significantly altered by the compost addition (Table 1). Apart from SOM, the quantities of total C and N were both ca. 10-fold higher in the treatment plots than in the control. For most macro- and micronutrients, soil amended with MC exhibited higher nutrient levels than with GWC/MC, whilst the control soil plots recorded the lowest values for all nutrients except for K and Mn, where no significant difference was found. The original soil in the study plot had lower than average levels of N and S for the city (0.31 % and 0.05 %, respectively) (Costa *et al.*, 2012), while after the compost treatment macro- and micronutrient concentrations far exceeded the mean concentrations reported for most Lisbon soils (Costa *et al.*, 2012). Urban environments are often viewed as hostile to food crop production because of unnaturally high levels of heavy metals; particularly bulk lead (Fitzstevens *et al.*, 2017). However, readings for heavy metals were unaltered by the treatments with the exception of Cd, which in the MC treatment showed levels twice as high as in the control plots, while for the plots treated with GWC/MC, there was a moderate increase in Cd. In any case, heavy metal concentrations well below the lower guideline values considered to be of risk to the ecology of soils and even for Cd, where soil with MC additions showed increased values, ca. half of the threshold value of 1 µg Cd per g soil (Tóth *et al.*, 2016).

Table 1: Topsoil parameters (< 20 cm) of untreated soil (control), soil with municipal compost (MC) and soil with a mix of green waste compost and municipal compost (GWC/MC). Numbers depict means ($n_{\text{Control}} = 5$; $n_{\text{MC}} = 5$; $n_{\text{GWC/MC}} = 3$) with standard errors in parentheses. Letters depict significant differences (pairwise Welch's t-test with holm correction; $p < 0.05$, values were square root transformed to conform with normality assumption).

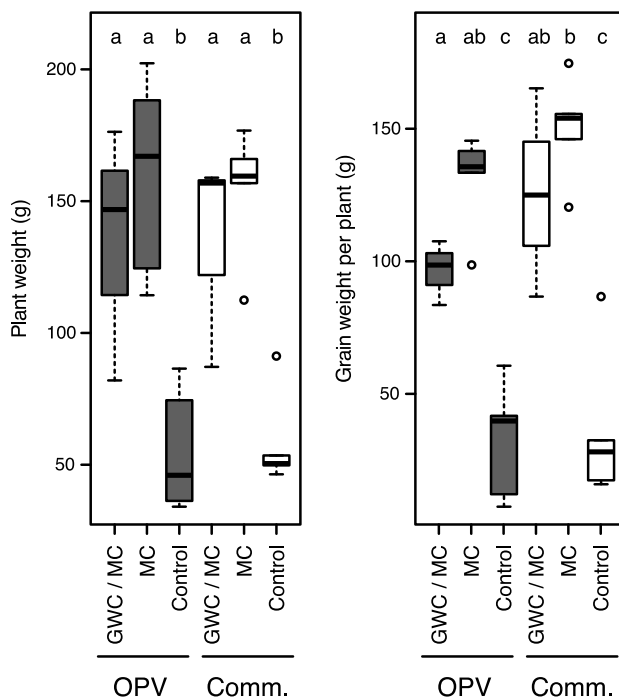
		GWC/MC	MC	Control
General	SOM (%)	16.99 (3.44) ^a	23.05 (2.07) ^a	5.69 (0.21) ^b

characteristics	Carb (%)	3.55 (0.43) ^a	4.41 (0.34) ^a	2.06 (0.16) ^b
	$\delta^{13}\text{C}$ (‰)	-24.13 (0.09) ^a	-23.64 (0.2) ^a	-20.14 (1.01) ^b
	$\delta^{15}\text{N}$ (‰)	10.7 (0.31) ^a	9.98 (0.35) ^a	5.52 (0.11) ^b
	C (%)	9.27 (1.7) ^a	11.6 (0.72) ^a	1.64 (0.18) ^b
	N (%)	0.9 (0.15) ^a	1 (0.08) ^a	0.12 (0.02) ^b
Macronutrient concentrations (mg/g)	P	5.86 (1.48) ^b	9.72 (1.17) ^a	0.31 (0.02) ^c
	K	3.79 (0.73)	4.22 (0.48)	4.07 (0.28)
	Ca	34.77 (6.75) ^b	49.57 (4.94) ^a	12.22 (0.76) ^c
	S	1.64 (0.37) ^b	2.43 (0.25) ^a	0.3 (0.01) ^c
	Mg	3.57 (0.8) ^{ab}	4.57 (0.29) ^a	2.67 (0.26) ^b
	Na	0.71 (0.17) ^{ab}	1.16 (0.22) ^a	0.26 (0.03) ^b
Micronutrient and heavy metal concentrations ($\mu\text{g/g}$)	B	11.15 (2.41) ^{ab}	14.35 (1.05) ^a	7.33 (0.48) ^b
	Mn	145.96 (25.79)	174.48 (5.6)	180.61 (12.55)
	Zn	119.09 (27.06) ^b	172.91 (14.9) ^a	42.33 (1.41) ^c
	Fe	10,895.99 (1381.13) ^a	9,965.15 (725.88)	15,749.67 (685.98) ^b
	Cu	29.66 (5.91) ^b	41.62 (2.76) ^a	16.33 (1.39) ^c
	Mo	0.44 (0.09) ^a	0.53 (0.15) ^a	0.08 (0.05) ^b
	Ni	10.19 (1.33)	11.08 (0.42)	12.76 (0.81)
	Cd	0.37 (0.1) ^{ab}	0.55 (0.05) ^a	0.28 (0.02) ^b
	Pb	19.32 (2.4)	19.59 (0.69)	22.21 (0.91)
	Cr	44.31 (2.68)	44.12 (3.02)	43.47 (3.48)
	As	0.33 (0.11)	0.95 (0.34)	0.16 (0.14)

In the case of macronutrients, the largest differences between treated and control plots were observed for P levels, which were 32 times higher under MC treatment, and 20 times higher under GWC/MC treatment. Most of the readings for macro- and micronutrient levels registered between 1.5 and 3 times higher in the MC treatment, with the exception of Mo, which was 7 times higher in the MC plots, and 6-fold higher in the plots subjected to GWC/MC treatment. However, even though GWC/MC and MC treatments were effective in increasing Mo concentrations, the values recorded here were nevertheless lower than Lisbon average values of 1.67 $\mu\text{g/g}$ (Costa et al., 2012) and at the plant growth limiting end of soil Mo concentrations (Marschner, 2012).

3.2. Effects on plant biomass and nitrogen

268 In all experimental plots treated with compost the growth of maize plants and the
 269 accumulation of biomass were significantly higher than they were in the control sites,
 270 irrespective of the variety used (**Figure 1, left**). Similarly, grain yield was significantly higher
 271 in treated plots (**Figure 1, right**), although, the commercial variety grown in MC plots
 272 yielded higher grain counts than the OPV grown in GWC/MC plots. Based on the values
 273 displayed in Figure 1 (grain yield per plant), the average grain yield for the OPV of maize was
 274 extrapolated to be 1.8 t/ha in the control plots; 5.3 t/ha in the GWC/MC plots; and 7.2 t/ha for
 275 soil treated with MC. For the commercial variety, the average grain yield was calculated at 2
 276 t/ha in the control plots; 6.9 t/ha in the GWC/MC plots; and 8.3 t/ha in the MC plots. Thus,
 277 both maize varieties grown with compost compete favourably in grain yield with industrially
 278 produced transgenic or non-transgenic commercial hybrid varieties in Portugal (Skevas *et al.*,
 279 2010). By extrapolating from the yield data, ca. 22 m² of compost-fed urban ground would be
 280 sufficient to produce enough maize in the period of 3 months to support one individual for the
 281 year, leaving the rest of the year to grow other crops.



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 283 **Figure 1:** Boxplots describing the differences in vegetative plant weight (left) and grain weight (right)
 284 of both corn varieties (open-pollinated and commercial) in all treatments (per variety: $n_{\text{Control}} = 5$; $n_{\text{MC}} =$
 285 5 ; $n_{\text{GWC/MC}} = 3$). Letters depict significant differences (pairwise Welch's t-test with holm correction; $p <$

286 0.05). GWC = Green Waste Compost; MC = Municipal Compost. OPV = open-pollinated variety,
1 287 Comm. = commercial variety.

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5 289 Interestingly, while there were marked differences in plant weight and grain yield between
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8 290 treatments ($F = 26.71$, $p < 0.001$ for GWC/MC plots; and $F = 50.58$, $p < 0.001$ for the MC
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10 291 plots), there was no clear evidence of significant differences in N concentrations in the grain
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13 292 between treated plots ($F = 2.86$, $p = 0.081$), but there was a significant difference in levels of
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15 293 N between the two varieties ($F = 29.58$, $p < 0.001$). Grain from the OPV yielded higher N
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17 294 concentrations in both control as well as all treated plots (**Table 2**), indicating higher protein
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20 295 content in this variety. The mean difference observed here (1.4 times more N in the OPV vs.
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22 296 the commercial variety) is in accordance with results found for other Portuguese OPVs, which
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25 297 reported up to 1.3 times higher protein content in OPVs, compared to hybrid varieties (Brites
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27 298 *et al.*, 2010). This effect might be due to higher N use efficiency inherent to this variety, as
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30 299 OPVs of maize might be more resource efficient under low nutrient conditions (Omondi *et al.*,
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32 300 2014) and accumulate more protein (Flint-Garcia, 2017). Similar to the pattern observed for N
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35 301 concentrations, K and P concentrations did not change in response to the treatments ($F =$
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37 302 0.0258 , $p = 0.974$ and $F = 0.5191$, $p = 0.603$, respectively), but were significantly higher in
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39
40 303 the OPV kernels in all treatments ($F = 100.64$, $p < 0.001$ and $F = 116.1389$, $p < 0.001$,
41
42 304 respectively). While mean K concentrations found in the commercial variety (3645 $\mu\text{g/g}$) are
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44 305 below values found in OPVs in another study (3745 $\mu\text{g/g}$; Jaradat and Goldstein, 2018), the
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47 306 OPV observed here exhibits substantially higher K concentrations (5332 $\mu\text{g/g}$). On the
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49 307 contrary, the P values observed in the commercial variety are lower (2685 $\mu\text{g/g}$) than what
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52 308 was observed in OPVs in the study mentioned above (3362 $\mu\text{g/g}$; Jaradat and Goldstein,
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54 309 2018), while they are higher than in the OPV observed in this study (4298 $\mu\text{g/g}$). In contrast
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56
57 310 to K, where an increased concentration in OPVs kernels is a beneficial trait, higher P values
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59 311 can be potentially problematic if P is stored mainly as phytic acid. The ratio of free P in
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61 312 comparison to phytic acid was not measured, however, it is known from other OPVs that they
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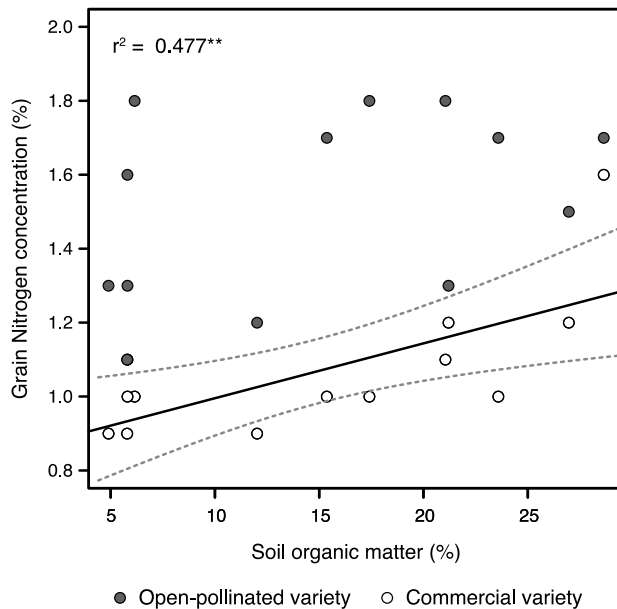
313 can potentially exhibit high free P and low phytic acid levels (Puglisi et al., 2018), rendering
 314 them more suitable as P source for human consumption.

315
 316 **Table 2:** Nutrient concentrations in maize grain of plants growing in soil without treatment (Control),
 317 soil with municipal compost (MC) and soil with a compost-mix (GWC/MC) of green waste compost
 318 (GWC) and MC. All values are given in $\mu\text{g/g}$, values in bold show significant differences between
 319 open-pollinated and commercial variety. Numbers depict means (per variety: $n_{\text{Control}} = 5$; $n_{\text{MC}} = 5$;
 320 $n_{\text{GWC/MC}} = 3$) with standard errors in parentheses. Asterisks depict p-values (* = $p < 0.05$, ** = $p < 0.01$,
 321 *** = $p < 0.001$; Welch's t-test, values were square root transformed to conform with normality
 322 assumption). OPV = open-pollinated variety, Comm = commercial variety

	GWC/MC		MC		Control	
	OPV	Comm.	OPV	Comm.	OPV	Comm.
N	15,333.33 (1,666.67)	9,666.67 (333.33)	16,200 (969.54)	12,200 (1,019.80)*	14,200 (1,240.97)	9,800 (374.17)*
P	4,411.80 (78.59)	2,740.47 (202.43)*	4,455.05 (99.36)	2,567.32 (72.15)***	4,028.44 (305.09)	2,747.81 (147.45)**
K	5,396.02 (192.22)	3,566.51 (189.06)**	5,345.69 (111.04)	3,679.58 (208.68)**	5,253.05 (248.11)	3,688.55 (199.39)**
S	1,105.02 (89.76)	713.64 (39.16)*	1,174.91 (20.41)	758.48 (30.82)***	1,002.36 (71.97)	804.44 (46.53)
Mg	1,846.34 (97.23)	1,015.90 (101.56)**	1,907.84 (60.22)	939.79 (39.24)***	1,582.74 (160.11)	1,046.24 (54.63)*
Ca	75.81 (6.41)	45.76 (8.68)	79.71 (6.07)	59.78 (7.05)	97.79 (9.29)	90.07 (9.80)
B	2.47 (0.14)	1.76 (0.04)*	2.49 (0.22)	1.67 (0.19)*	2.39 (0.18)	1.89 (0.08)*
Mn	19.93 (1.31)	10.89 (0.80)**	22.26 (1.02)	10.84 (0.41)***	19.16 (1.58)	12.34 (0.45)**
Zn	53.36 (3.77)	23.28 (2.15)**	54.64 (1.75)	23.22 (1.14)***	50.81 (5.16)	26.47 (1.27)**
Fe	30.90 (4.89)	23.74 (2.51)	39.68 (2.79)	29.67 (7.00)	31.88 (2.15)	21.94 (3.20)*
Cu	3.00 (0.04)	1.57 (0.07)**	3.45 (0.08)	1.66 (0.11)***	3.20 (0.32)	1.76 (0.16)**
Mo	0.24 (0.11)	0.01 (0.01)	0.25 (0.08)	0.01 (0.01)*	0.23 (0.08)	0.00 (0.00)**
Ni	0.07 (0.05)	0.24 (0.14)	0.02 (0.01)	0.20 (0.07)*	0.20 (0.05)	0.51 (0.06)*

323
 324 While the OPV was apparently more efficient in nutrient acquisition, resource efficiency was
 325 not reflected in grain weights, with significantly lower weights (Welch's t-test, $p < 0.001$), for
 326 the OPV with 0.312 g (± 0.004 SE), compared to the commercial variety, which yielded 0.347
 327 g (± 0.005 SE) per grain. This is contrary to earlier reports indicating higher kernel weights
 328 for OPVs compared to hybrid varieties, however kernel weights were in general high in
 329 comparison to values observed by other authors, ranging around 0.26 – 0.28 g (Flint-Garcia,
 330 2017). On the other hand, the increased grain weight in the commercial variety could be

331 related to the dilution effect, which is known to occur in maize (Marles, 2017). While in both
 332 varieties grain yield was clearly connected with the compost treatments (**Figure 1**), only the
 333 grain of the commercial maize variety exhibited a linear, positive correlation between grain N
 334 concentrations with SOM levels (**Figure 2**). Thus, both varieties respond to fertilization with
 335 increased grain yield, however, the commercial variety also increases nutritional value with
 336 increased fertilization, while the OPV accumulates N largely independent of fertilization.
 337 From a nutritional standpoint, the OPV is therefore indicated as a better food source also in
 338 nutrient-poor agricultural regimes.



339
 340 **Figure 2:** Pearson correlation between grain nitrogen (N) content and soil organic matter (OM). Dark
 341 grey dots depict the open-pollinated variety, white dots the commercial variety. Only the commercial
 342 variety shows a significant correlation between soil OM and grain N concentrations (black line, 95 %
 343 confidence interval and r^2 shown).

344 3.3. Grain micronutrient concentrations in commercial and open-pollinated varieties

345 The status of other nutrients recorded in grain samples was variable between treatments and
 346 maize varieties (**Table 2**). Apart from Ca, Ni and S, all other recorded nutrients were
 347 observed to be higher in the OPV growing under control conditions and also in the MC plots,
 348 with the exception of Ca and Fe. The results for the GWC/MC plots indicated the main

350 nutrients with the exception of Ca, Fe, Mo and Ni were higher in the OPV of maize. As maize
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2 351 is also a source of essential minerals and the average consumption of maize per capita in
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4 352 Portugal is 48 g/day (FAOSTAT, 2013), this portion would provide 52 % of females and 36
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7 353 % of males with their daily requirements of Zn if open-pollinated varieties were selected for
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9 354 (FAO and WHO, 2005). Current biofortification goals in breeding programs set the target
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11 355 goals for Zn and Fe concentrations in kernels at 33 and 52 µg/g, respectively (Hindu et al.,
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13 356 2018), thus, kernels of the OPV even under unfertilized conditions achieve about 155 % and
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15 357 62 %, respectively, of these targets. In terms of trace elements, the average consumption of
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17 358 OPV maize would furthermore provide 35 % of daily needs of Mo (34 µg) (Food and
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19 359 Nutrition Board, Institute of Medicine, 2000). In contrast, the commercial variety would yield
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22 360 less than 2 % of daily requirements. What is more, heavy metals did not accumulate in the
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24 361 grain of the OPV, and levels for Ni recorded in samples were around 0.51 µg/g, which would
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27 362 equate to 13.5 % of the recommended daily threshold limit for Ni of 2.8 µg Ni/kg body
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29 363 weight (ESFA, 2015) in an average diet of maize. An analysis using a network plot and
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32 364 Spearman's correlation matrix revealed a relationship between various macro- and
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34 365 micronutrients (**Figure 3**). For instance, in the kernels of the OPV, macronutrients such as N,
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37 366 P, S and Mg were positively correlated with the micronutrients Mn and Zn, while Ni was
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39 367 negatively correlated with Fe, Mg and S. On the contrary, in the commercial variety, Ni was
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41 368 positively correlated with other nutrients, such as S, Mg, Na, Mn and Zn. Interestingly, in
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44 369 both varieties, no correlation was found between Zn and Fe, which is in accordance with
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47 370 Indian varieties, but in contrast to observations from African cultivars (Akinwale et al., 2016).
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50 371 The positive correlation between P and various micronutrients in the OPV was also found in
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53 372 several maize varieties selected for biofortified crops, potentially indicating a co-selection of
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56 373 traits relevant for higher kernel nutrient accumulation (Gu et al., 2015). Contrary to these
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58 374 positive correlations, the correlations between S and Mg with Ni are negative in the OPV,
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375 while being highly positive in the commercial variety. This is potentially problematic, as any
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 2 376 accumulation of micronutrients in this variety would be accompanied by an increase in Ni.
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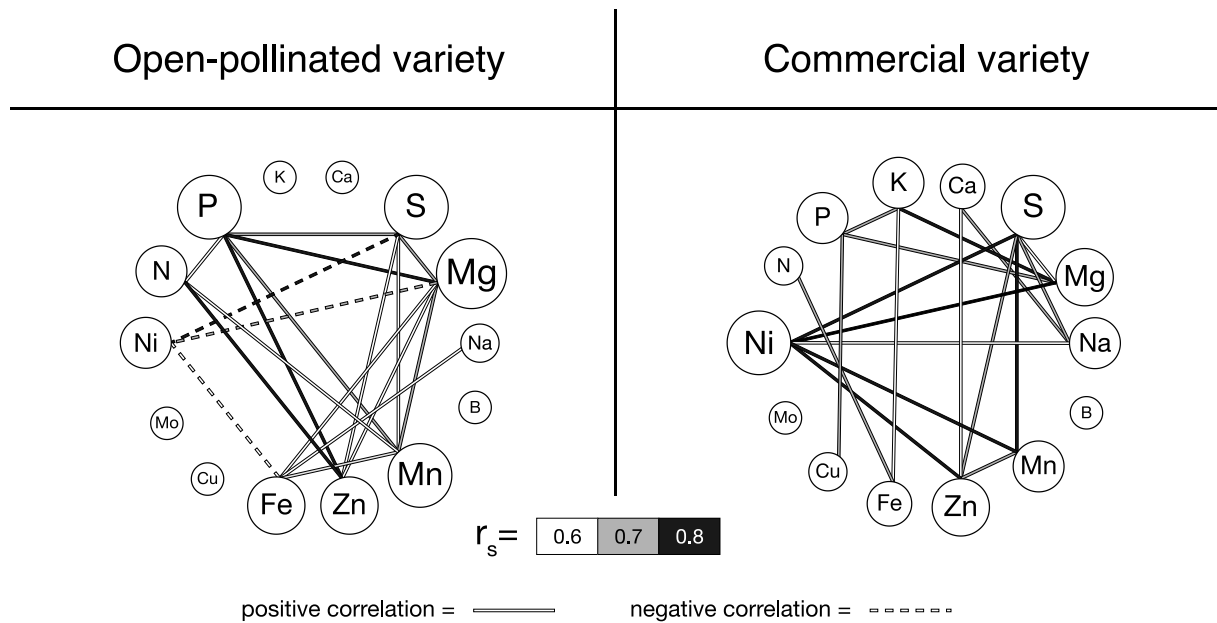


Figure 3: Nutrient Network, based on a matrix of spearman correlations (r_s) between all macro- and micronutrients found in the maize kernels (dotted lines indicate negative correlation, full lines indicate positive correlations). White, grey or black lines indicate the strength of the relationship (0.6, 0.7 and 0.8, respectively), while the sphere sizes indicate the amount of existing relationships (min = 0; max = 6), basis for the nutrient network was $n = 13$ for each variety.

384 While RV coefficients (**Figure 4**) suggest a strong relationship between soil micro- and
 385 macronutrient concentrations and grain production in both varieties, they also suggest that in
 386 the OPV, the micronutrient concentrations appear to be linked to plant traits, whereas, in the
 387 commercial variety these concentrations were more closely associated with soil micronutrient
 388 concentrations. These results could suggest the selective breeding strategies for commercial
 389 maize favour nutrient up-take and grain yield at the expense of other traits (van Bueren *et al.*,
 390 2011). Thus, when growing these varieties under conditions of slow nutrient release, the
 391 genetic traits of the plant that dictate its eco-physiology are important considerations in
 392 strategies for sustainable urban agriculture. For example, the differences observed in our trials
 393 could be due to higher arbuscular mycorrhizal fungi (AMF) colonization rates in the OPV,
 394 which has been shown in other OPVs grown alongside commercial hybrids (Hess *et al.*,

2005). If this were the case, it would also explain the observed differences between the two varieties in the take-up of Ni. The commercial variety accumulated larger concentrations of this metal unlike its open-pollinated counter-part (Table 2, Figure 3). While AMF colonization was not measured, this would be an interesting field for further research, as changes in Ni accumulation has been reported after AMF colonization (Ramírez-Flores et al., 2017).

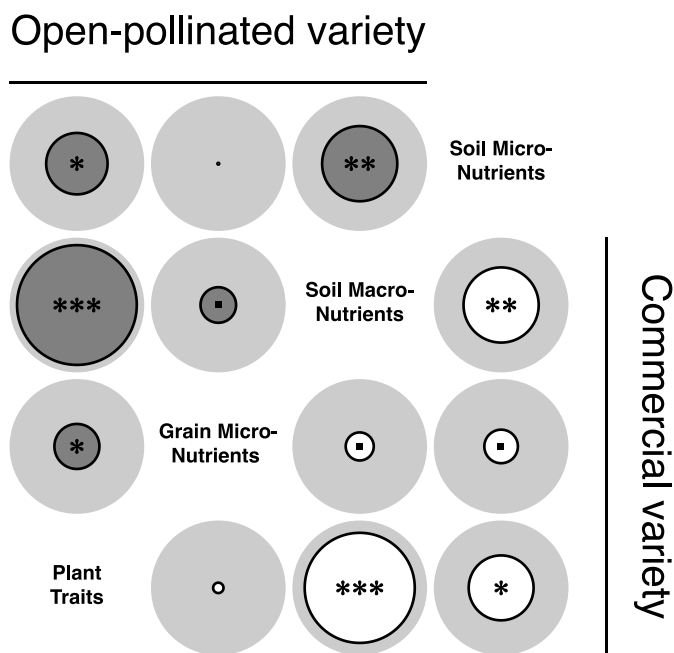


Figure 4: Schematic describing the relationships between two data matrices each, sphere size shows similarity (RV-coefficients), with the light grey spheres indicating a similarity of 100%. Dark grey spheres indicate open-pollinated variety, white spheres commercial variety. Three asterisks: $p < 0.001$, two asterisks: $p < 0.01$, one asterisk: $p < 0.05$, square: $p < 0.1$, $n = 13$ per variety.

Plant Traits = Plant Height; Number of Ears; Grain weight; Vegetative Plant Weight; total grain C, N and P. **Soil Macronutrients** = Soil organic matter (SOM), soil carbonates, total C, N, P, K, Ca, S, Mg. **Soil Micronutrients** = Na, B, Mn, Zn, Fe, Cu, Mo, Ni, Cd, Pb, Cr. **Grain Micronutrients** = Concentration of B, Mn, Zn, Fe, Cu, Mo, Ni, Mg, Ca, S, Na, K, Al in grain.

A multiple factor analysis (MFA) using data on grain nutrient concentrations and plant traits (Figure 5, left) revealed again a strong distinction between the OPV and commercial variety. The first dimension (X axis), explaining 41 % of the total variance separated OPV and commercial variety, while the second dimension (Y axis), explaining 28 % of the total variance, separated the different experimental treatments, in which the control plots were

416 distinct from the two treatments. This orthogonal statistical response further underlines that
1
2 417 variety effect and treatment effect were independent, with varieties being clearly separated by
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4 418 grain nutrient content (52 %) as well as C, N and P concentration and the number of ears per
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7 419 plant (46 %) (**Figure 5, right**). Accordingly, the nutrient status of the grain is linked primarily
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9 420 to plant variety, while indicators of plant productivity, such as plant height and weight as well
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11 421 as grain weight per plant, are mainly related to the treatments applied. As development of
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13 422 multi-nutrient rich strains of maize is of utmost importance for the sustainable nutrition of the
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15 423 global population (Jaradat et al., 2018), this pattern underlines the recently proposed selection
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17 424 of OPVs as a good way forward to achieve this feat (Puglisi et al., 2018). Also, the clear,
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19 425 positive effect of both compost types on the yield of both maize varieties measured here,
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21 426 contributes to evidence from recently published work on the feasibility of using GWC in
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23 427 periurban farming (Eldrige et al., 2018). Lastly, as the GWC used in this project was derived
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25 428 from a locally invasive plant (*A. longifolia*) and had no adverse effects on maize growth, e.g.
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27 429 similar responses than MC, these results add to recent work about its possible usage in
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29 430 agriculture or horticulture (Brito et al., 2015).
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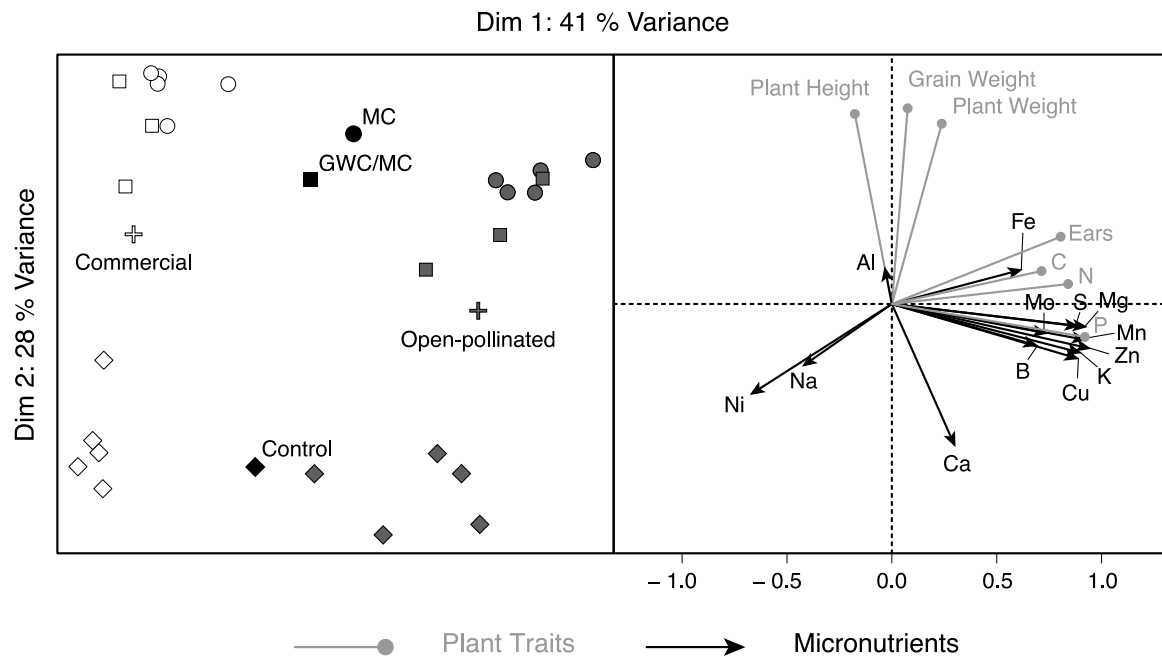


Figure 5: Multiple factor analysis (MFA) of variables related with the maize plants (n = 13 per plant variety). The three groups used in this MFA were:

Grain micronutrient content = B, Mn, Zn, Fe, Cu, Mo, Ni, Mg, Ca, S, Na, K, Al

Plant traits = plant height, plant weight, grain weight, number of ears, total C, N, P

Factors = treatments, varieties

Left: Individual factor map, including the centroids of the varieties (crosses), the individual data points of open-pollinated (grey) and commercial (white) maize varieties and the treatment centroids in black (sphere = municipal compost, square = municipal compost and green waste mix, diamond = untreated soil)

Right: Correlation cycle of all variables used.

4.4. Conclusions

This study demonstrated that it is possible to cultivate a staple food crop (*Zea Mays*) with adequate yields (up to 8.3 t/ha) in an urban setting, using only MC and GWC from nearby sources. As fertilization was based solely on soil amendments from composted food and green waste, this helps to achieve sustainable urban consumption patterns by recycling organic waste while increasing soil organic matter (up to 4 fold), thus mitigating GHG emissions. Furthermore, comparing an OPV and a commercial maize variety, it was found that the former exhibits higher nutrient use efficiency and increased macro- and micronutrient concentrations without accumulating heavy metals. It was found that this effect was variety and not treatment dependent, which points to OPVs as potentially interesting candidates for

454 biofortified staple crops. In conclusion, this work contributes to a growing body of scientific
1
2 455 evidence supporting alternative methods of intensive sustainable farming that can be adapted
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5 456 to urban landscapes.
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7 457

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Highlights

- Open-pollinated and commercial maize were grown using compost in an urban setting.
- Municipal compost and a mix of green waste compost were tested.
- Maize grain production and vegetative growth was similar on both compost treatments.
- Nitrogen and micronutrient concentrations were higher in open-pollinated maize grain.
- Grain micronutrient levels were high in open-pollinated maize, independent of treatment.

Figure 1

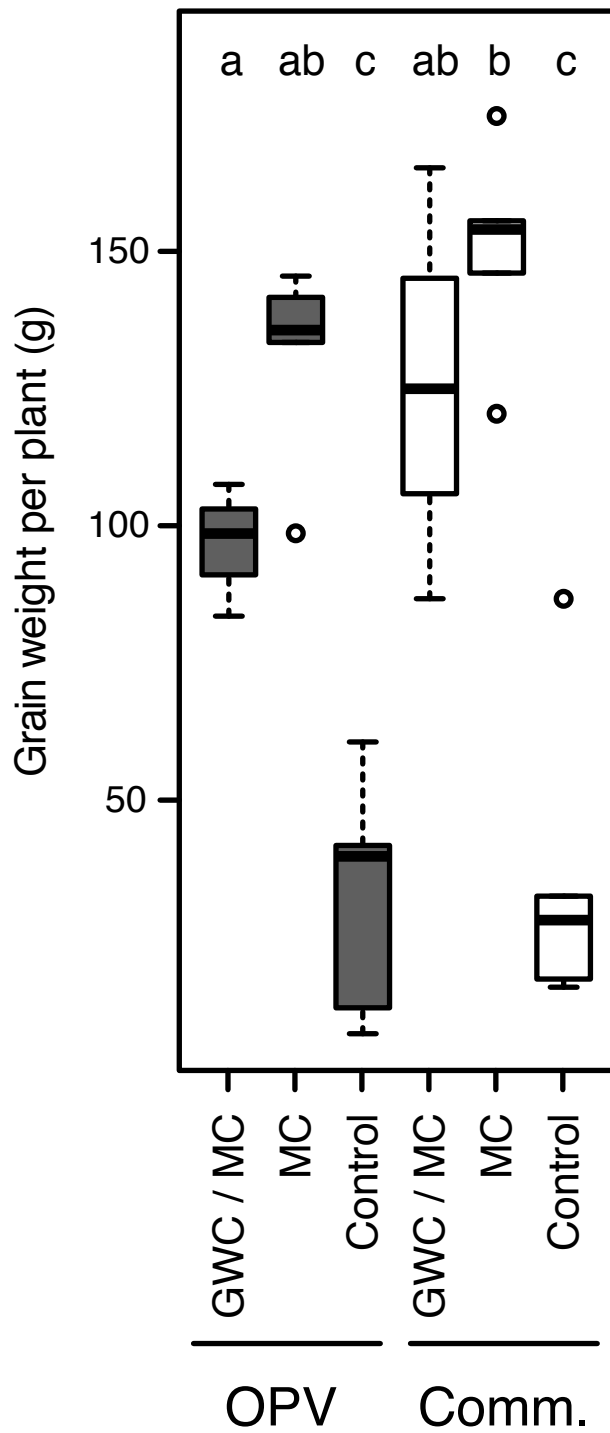
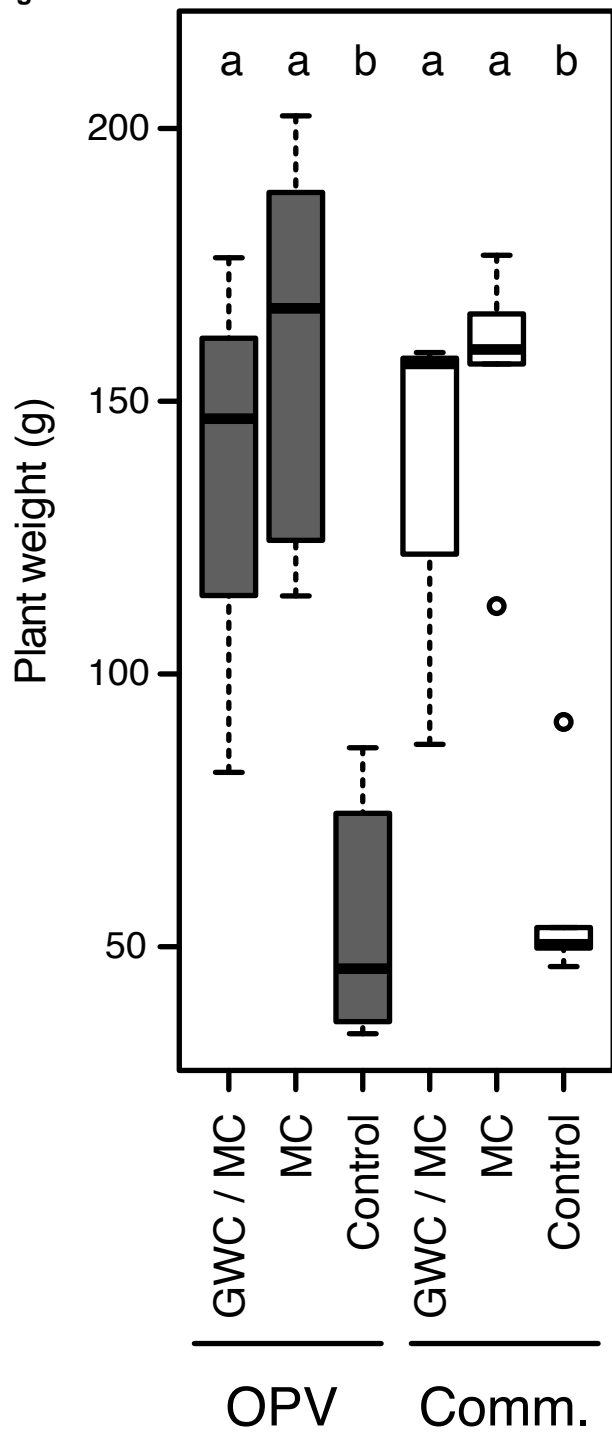
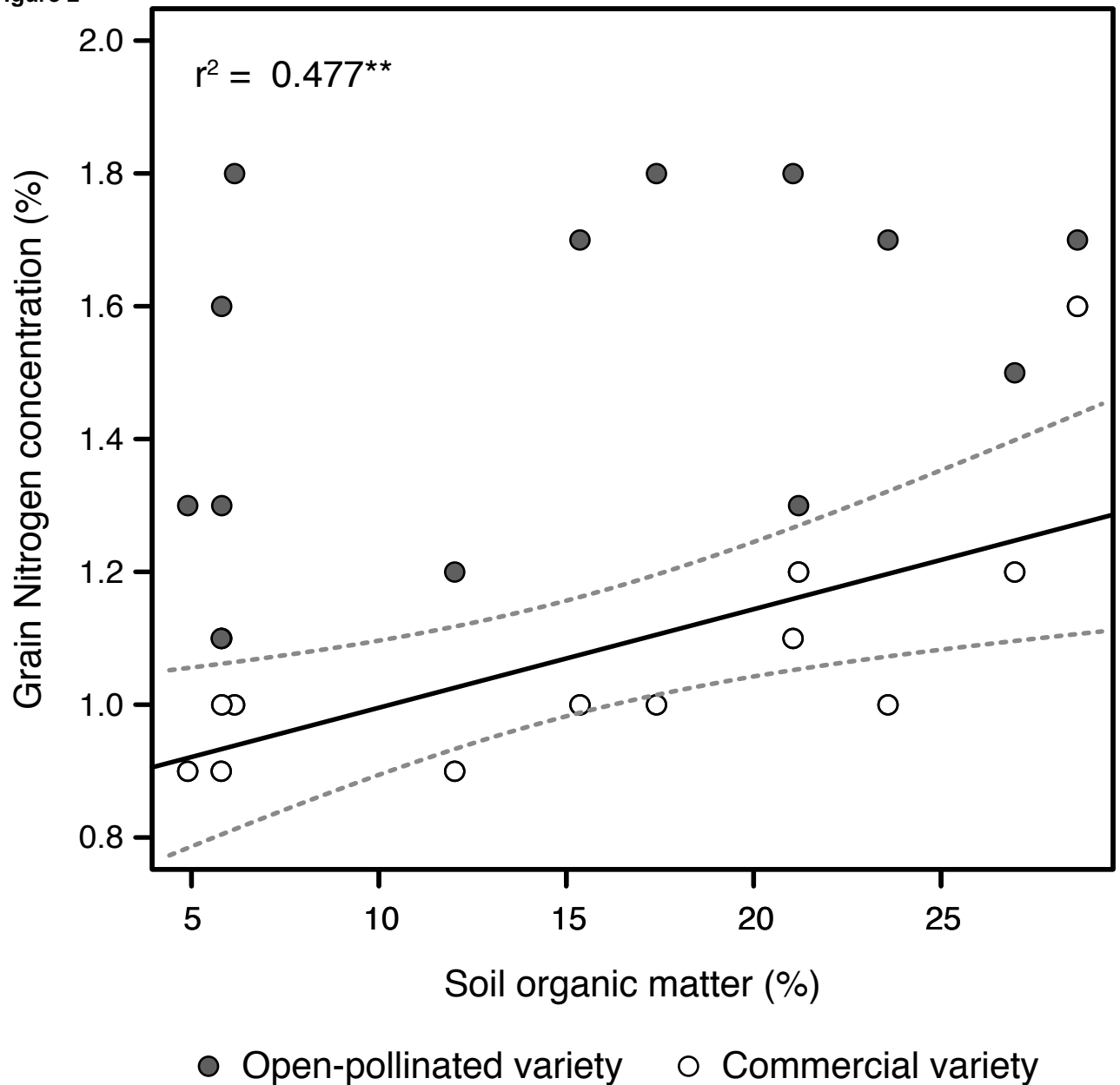
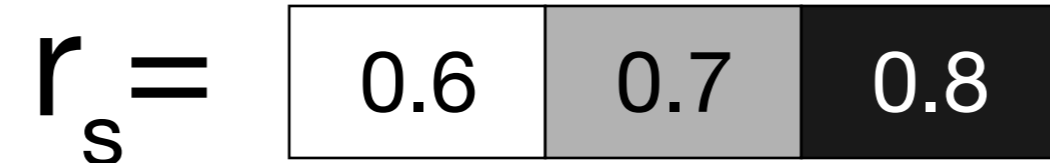
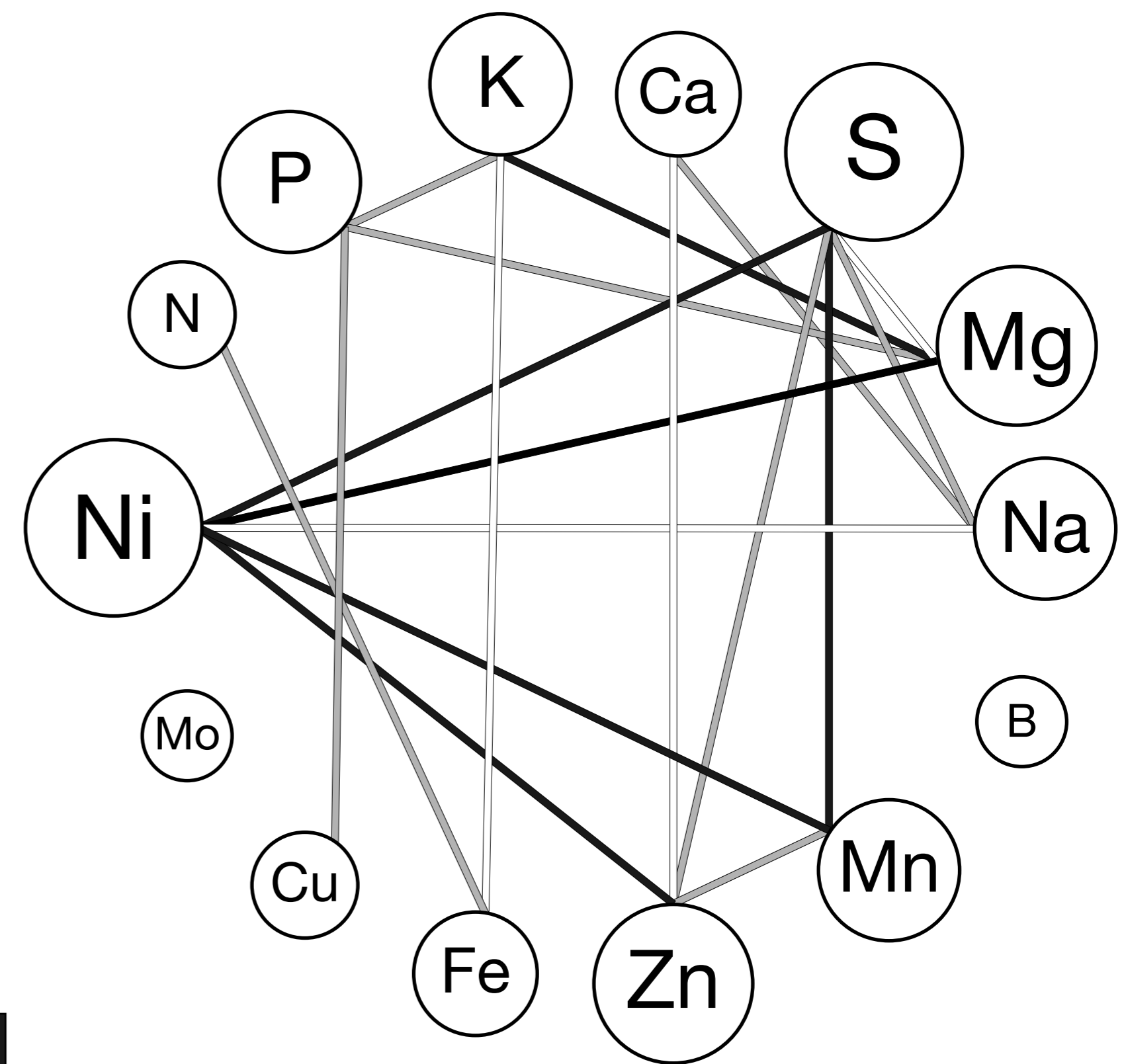
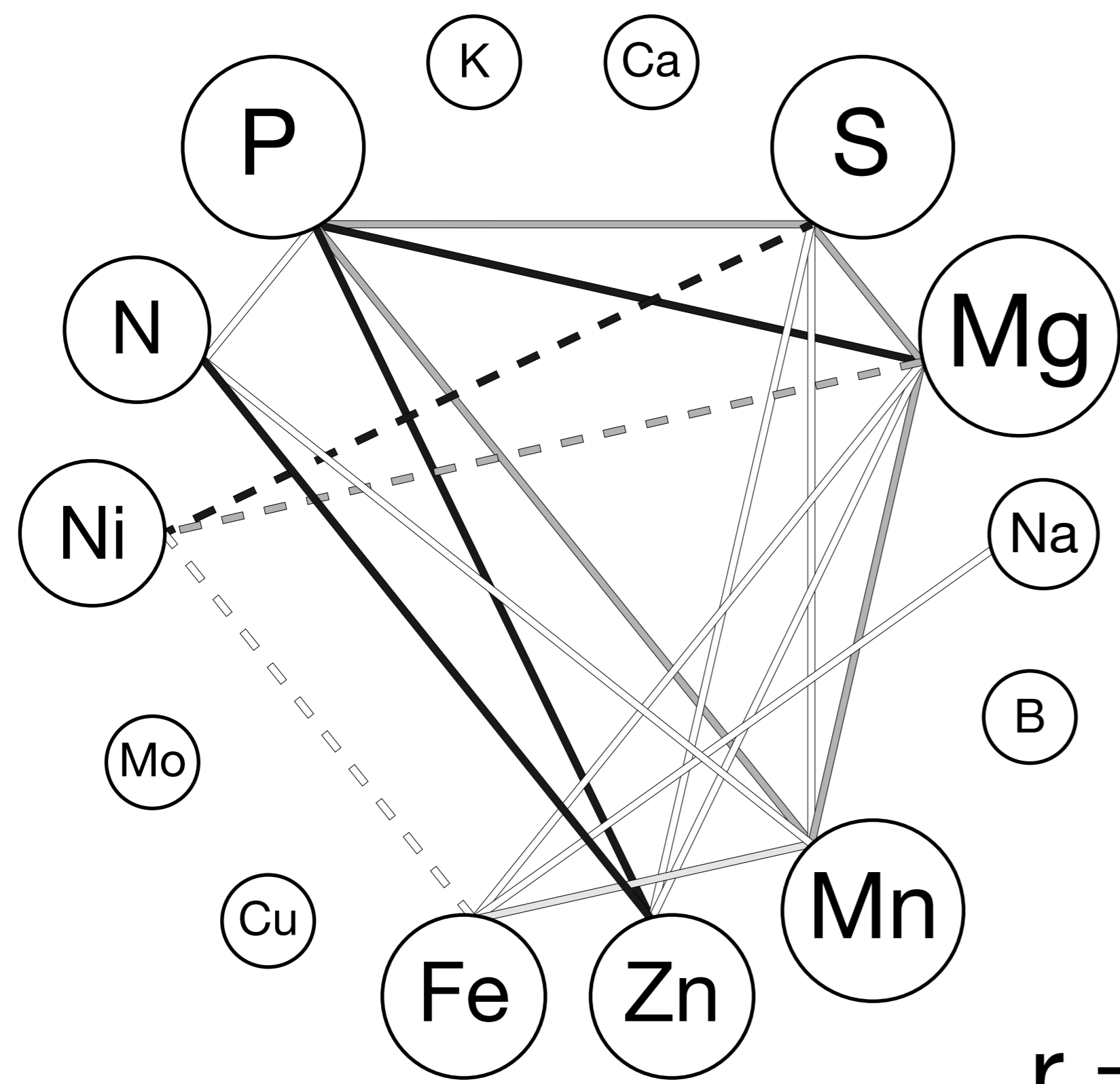


Figure 2



Open-pollinated variety

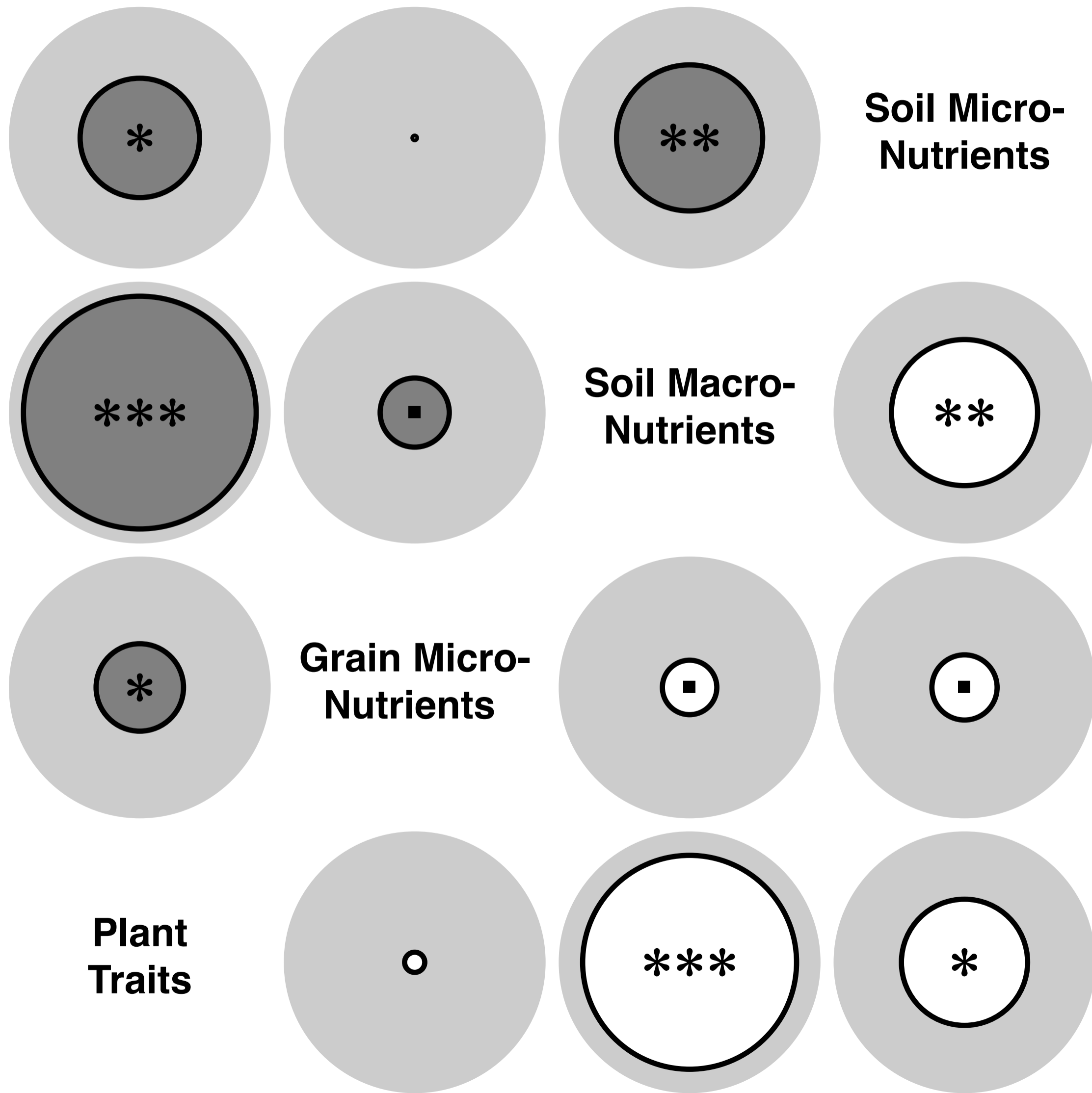
Commercial variety



positive correlation =

negative correlation =

Open-pollinated variety



Commercial variety

Figure 5

