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Abstract: Peat is the most representative component for the preparation of growing media used in horticulture. However, environmental issues and increased cost production, related to peat extraction and commercialization, are stimulating the rise of new materials and technologies for suitable alternatives to peat based substrates. Among other locally produced materials, green compost is one of the main alternatives for peat substitution although its variability in chemo physical characteristics represents the main constraint. Therefore, many works focus their attention on compost characteristics while there is a need of studies carried out at whole substrate plant system. In the present work, two composts (selected and mixed green compost), which differed for the initial composting raw materials, were evaluated for peat substitution and their influence on plant growth and quality, nutrient and water uptake, and gaseous exchange activity, was assessed. A bedding plant (geranium), cultivated in intensive growing system, was chosen as test plant and 100 % (pot volume) peat as control substrate. During the greenhouse experiment, plants were grown in five different substrates, i.e. only peat, 30 % and 50 % peat volume replaced by the two composts. Substrate characteristics, plant growth and biometric parameters, water and nutrient uptake, and gaseous exchange activity were evaluated as crop performance indicators. The mixed green compost influenced negatively plant nutrition and photosynthesis thus reducing significantly plant biomass accumulation and quality when replaced at the highest compost/peat ratio. The selected green compost was a more valuable substrate for peat substitution than mixed green compost supporting the diffused and shared opinion that high quality compost can be produced through a careful raw material selection.

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Dear Editor-in-chief,

please find enclosed the manuscript entitled "Evaluation of two green composts for peat substitution in Geranium cultivation: effect on plant growth, quality, nutrition, and photosynthesis." submitted for evaluation.

The paper reports a detailed study regarding the influence that physicochemical characteristics of composts have on plant nutrient and water uptake, and photosynthesis, which in turn mostly determine plant growth and quality of bedding plants. Peat substitution is currently one of the main concerns for nursery and potting plant production due to the cost of substrate and the environmental issues related to peat extraction. The main object of this work was to evaluate two different locally-produced green composts as candidates for the replacement of peat in potted geranium cultivation. The two composts differed for the initial composting raw materials. Peat was used as control treatment while the effects of physicochemical characteristics of four substrate mixtures were evaluated on plant growth and quality, nutrient and water uptake, and gaseous exchange activity.

Kind regards.

Pescia, 27<sup>th</sup> June 2016

Daniele Massa

Jourie Cellossa

- Two locally-produced composts (selected- and mixed-green compost) were used for peat
   substitution
- 9 *Pelargonium zonale* L. was chosen as test plant for the greenhouse experiment
- Plant biomass accumulation and biometric parameters, leaf chlorophyll (SPAD index)
   content and gaseous exchange activity, tissue nutrient concentration, and plant water uptake
   were evaluated
- Selected-green compost performed at the same level of the peat control substrate and better
   than mixed-green compost

1 Title

# 2 Evaluation of two green composts for peat substitution in *Geranium* cultivation:

# 3 effect on plant growth, quality, nutrition, and photosynthesis.

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# 21 Abstract

22 Peat is the most representative component for the preparation of growing media used in horticulture. However, environmental issues and increased cost production, related to peat 23 24 extraction and commercialization, are stimulating the rise of new materials and technologies for 25 suitable alternatives to peat-based substrates. Among other locally-produced materials, green compost is one of the main alternatives for peat substitution although its variability in 26 27 chemo-physical characteristics represents the main constraint. Therefore, many works focus their attention on compost characteristics while there is a need of studies carried out at whole 28 29 substrate-plant system. In the present work, two composts (selected- and mixed-green compost), 30 which differed for the initial composting raw materials, were evaluated for peat substitution and 31 their influence on plant growth and quality, nutrient and water uptake, and gaseous exchange 32 activity, was assessed. A bedding plant (geranium), cultivated in intensive growing system, was 33 chosen as test plant and 100 % (pot volume) peat as control substrate. During the greenhouse experiment, plants were grown in five different substrates, i.e. only peat, 30 % and 50 % peat 34 35 volume replaced by the two composts. Substrate characteristics, plant growth and biometric 36 parameters, water and nutrient uptake, and gaseous exchange activity were evaluated as crop performance indicators. The mixed-green compost influenced negatively plant nutrition and 37 38 photosynthesis thus reducing significantly plant biomass accumulation and quality when replaced at 39 the highest compost/peat ratio. The selected-green compost was a more valuable substrate for peat 40 substitution than mixed-green compost supporting the diffused and shared opinion that high-quality 41 compost can be produced through a careful raw material selection.

42

# 43 Keywords

44 Organic waste reuse, *Pelargonium zonale* L., photosynthesis, plant nutrition, bedding plants,
45 substrate cultivation

# 46 **1** Introduction

47 Notwithstanding the controversial debate on the environmental and economic sustainability of peat harvested from bog wetlands (Altmann, 2008; Bullock et al., 2012; Holmes, 2009), this 48 49 material remains one of the most diffused organic substrates employed in the horticultural industry 50 (Raviv, 2013; Schmilewski, 2009; Sonneveld and Voogt, 2009). Some studies point out how the 51 volume of peat harvested per year is negligible compared with the total natural peat accumulation 52 (Daigle and Gautreau-Daigle, 2001) as well as the area exploited for peat removal is negligible compared with total peatland area (Altmann, 2008). Nevertheless, peat moss is a non-renewable 53 54 resource in the short-medium period and its extraction process and use are highly impacting wetland 55 ecosystems other than producing greenhouse gas emissions (Bullock et al., 2012; Cleary et al., 2005). As matter of fact, in the last ten years, the diffused negative opinion on the criticisms related 56 57 to peat extraction has been contributing to stimulate government regulations more and more 58 restrictive for peat extraction and use in agriculture (Bullock et al., 2012; Holmes, 2009). For 59 example the European Union denies the "Ecolabel" to substrate containing peat. Therefore, it must 60 be taken into account by growers that peat availability in the next years could be limited by regulations looking at the safeguard of ecosystems producing peat. 61

62 A part from environmental issues, it should be also considered that the price of substrates 63 strongly impacts production costs for potted ornamental plants although such crops are highly 64 remunerative among other cultivated species (Brito et al., 2015; De Lucia et al., 2013; Daughtrey 65 and Benson, 2005). This is mainly due to the price of substrate transport and extraction; the latter is also one of the main parameter negatively affecting the environmental sustainability of peat when 66 67 compared with other substrates produced at local level (De Lucia et al., 2013). Moreover, it is 68 reasonable to think that the increasing restrictions for peat extraction will force peat-producing 69 companies to adopt more and more ecofriendly strategies thereby increasing cost production. As 70 matter of fact, the cost peat has been increasing constantly in the last years (Raviv, 2013).

71 Indeed, peat represents an ideal substrate in horticulture for its physicochemical characteristics 72 that are optimal for many plant species and management of different cultivation systems; among all, 73 the low electrical conductivity, nutrient and non-nutrient content, and the high porosity and water 74 retention capacity (Raviv, 2013; Sonneveld and Voogt, 2009). However, many other organic 75 materials have been successfully tested for peat replacement (Chong, 2005; Larcher and Scariot, 76 2009). Among these, compost is a valuable candidate (Raviv, 2013). Composting is one of the most 77 effective strategies to convert organic waste - otherwise to be disposed of - in valuable material to 78 be reused for sustainable carbon use (Martínez-Blanco et al., 2013). Therefore, in the last decade, 79 many works have been carried out on different composted materials as candidates for peat 80 substitution, which includes municipal solid waste (Moldes et al., 2007; Ostos et al., 2008), sewage 81 sludge (De Lucia et al., 2013; Ostos et al., 2008), animal manure (Shober et al., 2010; Tittarelli et 82 al., 2009), agro-industrial waste (Bustamante et al., 2008; Kritsotakis et al., 2011), and green waste as well (Brito et al., 2015; López-Cuadrado et al., 2008; Mugnai et al., 2007; Olszewski et al., 2009; 83 84 Tittarelli et al., 2009).

85 The standardization of compost characteristics is seen as one of the major concerns for its 86 operative use (Raviv, 2013; Sonneveld Voogt, 2009). However, protected horticulture is one of the 87 most specialized agricultural sectors, which offers technical solutions to control and modify root 88 zone characteristics very promptly (Chong, 2005). The possibility of selecting local-produced raw 89 materials for composting is an option to achieve high-quality and standardized composts at low cost 90 (Raviv, 2013). This intent cannot be realized for many composted material such as, for example, 91 organic urban refuse. These aspects make the production and use of green compost, for peat 92 substitution, a strategy worthwhile to pursue.

93 Indeed, chemo-physical characteristics of the substrate may influence plant performance at 94 different extents; therefore, most works on compost use in agriculture focus their attention on 95 material characterization. Nevertheless, the effects of peat substitution with compost, on cultivated 96 plants, have been often evaluated by observations limited only to plant growth (e.g. 97 Estévez-Schwarz et al., 2009; Olszewski et al., 2009). Many works report also effects on nutrient
98 and/or non-nutrient element concentrations in plant tissues, and/or on other tissue characteristics
99 (Brito et al., 2015; De Lucia et al., 2013; Larcher and Scariot, 2009; López-Cuadrado et al., 2008;
100 Tittarelli et al., 2009). Very rarely, gaseous exchange activity and/or plant water relations have been
101 assessed for plants cultivate in compost-based substrates (e.g. Mugnai et al., 2007; Bakry et al.,
102 2013). Indeed, there is a lack of works assessing the effects of peat substitution with green compost
103 on plant nutrition and photosynthesis that in turn mostly determine plant growth and quality.

104 The main object of this work was to evaluate two different locally-produced green composts as 105 candidates for the replacement of peat in potted geranium cultivation. The two composts differed 106 for the initial composting raw materials. Peat was used as control treatment and compared with four 107 further substrate mixtures by the evaluation of plant growth and quality, nutrient and water uptake, 108 and gaseous exchange activity.

#### 109 2 Materials and Methods

#### 110 **2.1** Potting substrate mixtures and treatments

111 Two different composts were tested in the experiment. Both composts, provided by private 112 companies, were obtained at local level (Tuscany, Italy) from green refuses. The main difference 113 was in the pristine materials used for the composting process. In one case, only greenhouse and 114 nursery green waste (mostly plant trimmings, prunings and crop residues) was used to obtain the 115 "selected-green compost" (SC). The second compost (mixed-green compost, MC) was instead 116 produced by using green refuse from different cultivation systems, public and private green areas, 117 and heterogeneous environments including urban, peri-urban and costal areas. Composting process 118 was carried out following high quality procedures. In both cases, trapezoidal piles of green organic 119 material were composted for a period of roughly six months until compost maturation. During the 120 composting period, temperature was monitored and the piles were managed to keep a target 121 humidity of 55-65 % with the aim of ensuring optimal conditions for microbial metabolism. The

obtained composts were analysed before starting the experiment according to UNI 10780 (1998).
The main chemical characteristics of both composts are reported in Table 1; none of the analysed
parameters exceeded Italian regulations (D.Lgs. n° 75, 29 April 2010).

The above composts were then combined with peat to obtain the tested substrates. Five different treatments, which corresponded to five different container media mixtures, were tested in the experiment: i.e. i) 100 % peat (PC) chosen as standard (control) substrate following growers' common practices for bedding plants cultivated at local level; ii) 30 % peat volume replaced by selected-green compost (SC30); iii) 50 % peat volume replaced by selected-green compost (SC50); iv) 30 % peat volume replaced by mixed-green compost (MC30); v) 50 % peat volume replaced by mixed-green compost (MC30).

#### 132 2.2 Plant material and growing conditions

The experiment was carried out at the Landscaping Plants and Nursery Research Unit of the Italian Council for Agricultural Research and Economics, Pescia, Tuscany, Italy (lat. 43°54' N, long. 10°42' E), in an unheated plastic greenhouse, under typical Mediterranean climate conditions. The greenhouse was covered with polyethylene film. A 40 % shading net was placed above the canopy to avoid harmful temperatures during sunny days.

Geranium (Pelargonium zonale L.) cuttings with four unfolded leaves were transplanted into 138 1.5-L pots (Ø 14 cm) on 14 March 2014. All plants were fed with the same amount of nutrients 139 supplied through controlled release fertilizer (5 kg m<sup>-3</sup> of Osmocote Pro® 3-4 months) blended with 140 141 the substrate before transplant. Fertilizer was added taking into account plant nutrient requirement, 142 possible nutrient leaching due to water drainage, and chemical composition of the irrigation water. 143 The latter was assessed, on the average of historical laboratory analysis, before experiment initiation. Adjustments of water pH were performed by using sulphuric acids to keep pH value close 144 145 to 6.0.

Plants were moved to the greenhouse and placed in benches for pot cultivation. Eight plants per replicate were spaced to obtain a crop density of 16 pt m<sup>-2</sup> and arranged in a randomized block design with six replicates (48 plants per treatment) for a total of 240 pots. Plants were irrigated drop by drop by means of a pressure-compensated dripper per pot ensuring a flow rate of 2 L h<sup>-1</sup>. The irrigation was trigged using a standard timer that was adjusted weekly on the basis of climate conditions and water leaching fraction (i.e. the ratio between the quantity of water drained out from substrate and the quantity of water supplied during irrigation).

Radiation, relative humidity, and air temperature were monitored over the whole experimental period by a portable data logger (Decagon Em50; Decagon Devices Inc., Pullman, WA 99163 -USA). Minimum, mean and maximum daily averaged photosynthetic active radiation were 32.5, 142.3, and 323.3  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, respectively. Mean daily cumulated global radiation was 6.1 MJ m<sup>-2</sup> d<sup>-1</sup>. Average of minimum, mean and maximum daily air temperature was 11.8, 17.7 and 26.6 °C, respectively. Air mean daily relative humidity averaged 66.5 %.

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# 2.3 Substrate and plant analyses

The physicochemical characteristics of the peat control substrate and each mixture obtained by peat and composts were determined before the addition of chemical fertilizer. Total N and C content were assessed on a dry matter basis (EN 13654-1, 2001 and UNI 10780, 1998, respectively) while other chemical parameters were analysed in the 1:5 (V:V) substrate:water extract: i.e. pH (EN 13037, 1999), EC (EN 13038, 1999), N-NO<sub>3</sub>, P-PO<sub>4</sub>, K, Ca, Mg, Fe, Na, and Cl (EN 13652, 2001). In addition, physical parameters were determined according to De Boodt et al. (1974).

During the experiment, crop water balance was calculated on three over six replicate per treatment. Leaching fraction, for the operative irrigation management, was calculated as the ratio between the water drained out from the substrate and the total water supplied during irrigation. The first quantity was determined weekly by measuring the water volume drained out from eight pots (per replicate) in a drainage tank; the tank was covered with plastic film thereby making negligible 171 water evaporation. The latter quantity was measured by a flow meter placed on the irrigation pipe.

172 Finally, the difference between irrigation and water drainage represented crop evapotranspiration.

Plants were monitored continuously for general status while standard agricultural practices were adopted for pest and disease management. A non-destructive analysis was performed at 55 days after transplant (DAT). Plant height (H, cm pt<sup>-1</sup>), mean diameter (of the canopy projected to the soil), number of flowers (n pt<sup>-1</sup>), and leaf chlorophyll (SPAD index) were measured for all plants. Plant volume (V, cm<sup>3</sup>) of an ellipsoid was calculated by combining the two former parameters. Chlorophyll content was assessed by averaging SPAD index of six leaves pinched from the bottom to the canopy of each plant (for a total of 288 measurements per treatment).

180 Between the first non-destructive analysis and the end of the experimentation, leaf gaseous 181 exchange analysis was performed at 65 DAT. A portable gas analyzer (Portable Photosynthesis 182 System Ciras-2, PPSystems, Amesbury, MA 01913 USA) was used to measure the photosynthetic 183 light response curve of two plants per replicate in three replicates, over six, per treatment (i.e. six 184 plant per treatment). Measurements were performed between 9.00 and 12.00 am on the first mature 185 and healthy leaf of the main stem. During measurements, to maintain comparable analytical conditions, the chamber was set at a constant value of CO<sub>2</sub> (400 g m<sup>-3</sup>), temperature (27.7  $\pm$ 5.2 %) 186 187 and vapour pressure deficit (VPD =  $1.1 \pm 0.3$  kPa); the last two quantities were calculated as the 188 average of climate data recorded with a datalogger, in the same daily period of measurements, 189 during three days before measurement initiation. Photosynthetic active radiation (PAR) was 190 varying, during the analysis, from saturating light to zero, using the following steps: 0, 50, 150, 300, 800, 1400, 2000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> During the analysis, leaf transpiration rate (E, mmol m<sup>-2</sup> s<sup>-1</sup>) and 191 stomatal conductance (Gs, mmol  $m^{-2} s^{-1}$ ) were also monitored. Finally, data obtained from the same 192 193 replicate was averaged, and then three photosynthetic light response curves per treatment were fitted with a modified non-rectangular hyperbola as reported by Thornley and Johnson (1990). 194 Non-linear statistic model procedure was used to fit Eq. 1 where Pn ( $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) is the leaf net 195 photosynthetic rate,  $\alpha$  (µmol CO<sub>2</sub> µmol PAR m<sup>-2</sup> s<sup>-1</sup>) is the quantum yield, I<sub>leaf</sub> (µmol PAR m<sup>-2</sup> s<sup>-1</sup>) is 196

197 the incident leaf radiation,  $Pn_{max}$  (µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) is the maximum photosynthetic rate for I<sub>leaf</sub>  $\rightarrow \infty$ , 198  $\theta$  (dimensionless) is the equation curvature (sharpness), R<sub>d</sub> (µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) is the leaf dark 199 respiration. Finally, the light compensation point (I<sub>Pn0</sub>, µmol PAR m<sup>-2</sup> s<sup>-1</sup>) was calculated by 200 rearranging Eq. 1.

201

$$Pn = \frac{\alpha \cdot I_{leaf} + Pn_{max} \cdot \sqrt{(\alpha \cdot I_{leaf} + Pn_{max})^2 \cdot 4 \cdot \theta \cdot \alpha \cdot I_{leaf}}}{2 \cdot \theta} \cdot Rd$$
Eq. 1

202

203 The experiment lasted 85 DAT and ended with the final plant destructive analysis. In this 204 case, all measurements already determined with the first non-destructive analysis (55 DAT) were 205 repeated. Furthermore, all plants were separated into flowers, stems and leaves, and the respective 206 biomass of eight plants per replicate was weighted to assess fresh and dry weight (dried in a 207 forced-air oven at 80 °C for 72 h), and organ partitioning. Leaf area was measured through a leaf 208 area meter (WinDIAS Image Analysis System, Delta-T Devices, UK). Afterward, a quantity of dry 209 matter from all organs was stored and analysed for mineral content. Reduced N was determined by 210 Kjeldahl distillation after dry matter digestion with sulphuric acid. A portion of dry matter was 211 subjected to nitric-perchloric acid digestion to be analysed for: i) P content through colorimetric 212 method using a spectrophotometer (Evolution<sup>™</sup> 300 UV-Vis Spectrophotometer, Thermo Fisher 213 Scientific Inc., MA USA); ii) K, Ca, Mg, Fe and Na content through atomic absorption 214 spectrophotometry (AAS240 FS Varian, Australia). Finally, Cl was determined through titration 215 with mercury nitrate after dry matter water extraction.

#### 216 **2.4** Statistics

Collected data were analysed by one-way ANOVA, to assess significant ( $P \le 0.05$ , 0.01 and 0.001) differences among treatments. Mean values were then separated by Tukey's (HSD) multiple-range test (P = 0.05). Data analysis included also non-linear models for fitting Eq. 1 to photosynthetic light response curves. Statistics and graphics were supported by the programs Statgraphics Centurion XV (Stat Point, Inc., Herndon, VA, USA) and Prism 5 (GraphPad Software,
Inc., La Jolla, California USA).

#### 223 **3 Results**

#### 224 3.1 Substrate mixture characteristics

Table 2 shows the main physicochemical characteristics of the substrate mixtures obtained by the combination of selected-green compost or mixed-green compost with peat at the different substitution ratios. Total N was significantly increased by the addition of both composts while C content showed an opposite trend with the exception of MC30 that did not cause significant reductions. The combination of both parameters shows a significant decrease in C/N ratio when both composts were applied at increasing volume.

Determinations performed on the 1:5 water extract (see section 2.3) showed a significant 231 232 increase in pH for the selected-green composts only when it was used to replace peat volume by 233 50 %. The mixed-green compost caused the same significant increase but in both 30 and 50 % 234 substitution. At the end of the cultivation, pH values of all treatments were levelled around pH 6.1 ranging within pH 5.6-6.7; only the treatment SC30 was significantly lower than the peat control 235 236 treatment (Fig. 1). Electrical conductivity was increased by the addition of both composts thereby 237 causing the 84 % increase, on average, compared with the peat control treatment. Among compost 238 treatments, SC50 caused the highest EC value, however such an increase was significantly higher 239 only with respect to MC30 (Table 2). The same initial trend was observed at the end of the 240 cultivation in the analysed substrates for EC; in this case, the former treatment and the MC30 241 treatment performed at the highest level compared with the other substrates while the peat control 242 treatment showed the lowest EC value (Fig. 1). Nitrate and Mg concentrations were both increased 243 only by the use of selected-green compost that caused the highest values when replaced at 50 % of 244 peat volume (Table 2). Similar results were observed for soluble P with the exception of MC50 that 245 performed at the same level of SC30 but at lower level than SC50 treatment. Selected-green

246 compost at 50 % peat substitution showed also a higher concentration in Ca, which was instead 247 significantly lower in MC30 and MC50 substrates compared with the peat control treatment (Table 248 2). Both composts enhanced the presence in the root zone of other macro and micronutrients in 249 comparison with peat substrate. These were K and Fe that were increased by 177 and 75 % (on the 250 average of compost treatments), respectively. However, the presence of composts in the substrates 251 also increased the concentration of saline ions. In spite of the different content in the pristine 252 materials (Table 1), Na concentration in the water extract was increased at the same extent in all 253 compost treatments compared with the peat control treatment. On the contrary, Cl concentration in 254 water extract (Table 2) reflected data shown in Table 1. In this case, the addition of mixed-green 255 compost caused a remarkable increase (+404 %) compared with the peat control substrate. Chloride 256 in the selected-green compost was significantly higher than the in the control substrate only for 257 SC50 treatment; however, Cl concentration observed in the latter was significantly lower than those 258 observed in both treatments obtained by mixed-green compost (i.e. MC30 and MC50).

Among physical parameters, bulk density was significantly increased by the presents of both composts compared with 100 % peat treatment while opposite trends were observed for water container capacity and total porosity. On the contrary, no significant difference could be observed for both easily available water and water buffering capacity (Table 2).

# 263 **3.2** Plant biomass accumulation and quality

Table 3 summarizes all destructive measurements performed on the biomass accumulated at the end of the experiment (85 DAT). The highest fresh weight values for leaves resulted by compost treatments SC30, SC50 and MC30 although no significant difference was found compared with the peat control treatment. Similar results were obtained for leaf dry weight and plant leaf area. Only the mixed-green compost MC50 treatment decreased significantly fresh and dry weight, in comparison with the other compost treatments, and plant leaf area that showed the lowest value among all treatments. Finally, the above treatment caused the highest leaf to total weight ratio. No 271 significant variation was instead observed for leaf dry weight percentage among all treatments. 272 Stem biomass accumulation showed significant higher values for both composts when applied at 273 30 % peat substitution ratio compared with the peat control treatment whereas no difference was 274 observed between the latter and both composts replaced at 50 % of peat volume. A different 275 scenario is shown in Table 3 for stem dry weight that was significantly lower for MC50 compared 276 with the other treatments; differences between fresh and dry weight reflected the pattern of the dry 277 matter percentage that was consequently lower in the latter treatment as compared with the other 278 ones (-14 % on average). The presence of compost at 50 % peat substitution ratio affected severely 279 flower fresh and dry weight that were both significantly reduced as compared with peat control 280 treatment; the other compost treatments performed at the same level of the latter treatment (Table 281 3). On the other hand, the presence of compost reduced flower dry matter percentage compared 282 with the peat control treatment (-24 % on average). Finally, the total (shoot) fresh weight, measured at the end of the experiment, resulted significantly higher in the compost treatments SC30 and 283 284 MC30 compared with the peat control treatment, which performed at the same level of SC50. Only 285 MC50 reduced significantly the total shoot fresh and dry weight of geranium compared with the 286 other four treatments (Table 3).

Plant aesthetic characteristics, which varied significantly among the different treatments, are 287 288 reported in Fig. 2. All plant performance indicators were measured in two different periods, i.e. at 289 55 DAT by non-destructive analysis and at the end of the experiment by destructive analysis. Plant 290 height and flower number were not influenced by any treatments at 55 DAT whereas a significant 291 reduction was observed for treatment MC50 at the end of the experiment (-21 % and -14 % on 292 average, respectively). On the contrary, the same treatment reduced significantly plant volume as 293 determined both at 55 and 85 DAT. Smaller variations were found for SPAD index that showed an 294 opposite tendency considering the two measurement periods; this indicator was reduced by the 295 presence of 50 % mixed-green compost measured at 55 DAT whereas all compost treatments

performed at the same level of the peat control treatment at 85 DAT, or even better as in the case of
SC50 (Fig. 2). The former data were in agreement with Fig. 3.

298

#### 3.3 Leaf gaseous exchange activity

299 The effects of the different treatments on leaf gaseous exchange activity are summarized in 300 Fig. 3. Equation 1 was successful in fitting photosynthetic rates, measured at different light intensity, explaining 99 % of the measurement variability. All photosynthesis parameters were 301 estimated at high level of significance (P < 0.001). However, only Pn<sub>max</sub> was significantly affected 302 by the different treatments; the other parameters averaged as follow:  $\alpha = 0.046$  (µmol CO<sub>2</sub> µmol 303 PAR<sup>-1</sup>),  $\theta = 0.824$  (dimensionless),  $R_d = 1.940$  (µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>),  $I_{Pn0} = 42.395$ 304 (µmol PAR m<sup>-2</sup> s<sup>-1</sup>). The 30 % compost treatment of both selected- and mixed-green compost gave 305 the highest  $Pn_{max}$  values (23.68 and 23.06  $\mu$ mol  $CO_2$  m<sup>-2</sup> s<sup>-1</sup>, respectively) that resulted significantly 306 increased compared with the peat control treatment and SC50 (20.87 and 20.89  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, 307 308 respectively); the latter treatments performed both at the same level of significance. On the 309 contrary, the 50 % peat substitution with mixed-green compost gave poorer results (17.19  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) thereby reducing significantly the maximum leaf photosynthetic capacity 310 311 compared with both the peat control treatment and the other compost treatments (-19% on average). The observed trends were already clear starting from 800  $\mu$ mol PAR m<sup>-2</sup> s<sup>-1</sup> as showed by 312 the photosynthetic response curves in Fig. 3. Finally, a significant (P < 0.0001) relationship was 313 314 found between total plant dry weight and Pn<sub>max</sub>; the linear regression reported in Fig. 3 was suitable 315 to explain 80 % of the experimental variability found between the two observed parameters.

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Leaf stomatal conductance and transpiration followed the same trend showing a significant reduced activity by the application of composts compared with the peat control treatment (Fig. 3).

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## 3.4 Plant nutrient and water uptake

Table 4 shows nutrient and non-nutrient ion concentrations in the different organ of plant shoots. Nitrogen concentration in all investigated plant tissues was significantly increased by the 321 presence of the selected-green composts compared with the peat control treatment (+25 % on 322 average); a similar trend was observed also for mixed-green composts but only for stem dry matter. 323 The presence of compost enhanced significantly the accumulation of P only when it was replaced at 324 the maximum ratio of peat volume (50 %) and only for leaves. Potassium concentration showed the 325 major remarkable effects among all nutrient ions; in this case the presence of compost generally 326 increased ion concentration in all investigated organs with the exception of stems in the SC30 327 treatment that performed at the same level of the peat control substrate. However, the highest values 328 were recorded for mixed-green compost treatments that showed up to 184 % increase in stems (i.e. 329 stems of MC50). The presence of composts did not affect much the concentration of Ca in plant 330 tissues with the exception of MC50 treatment that reduced significantly Ca content in two over the 331 three investigated organs compared with the peat control substrate. On the contrary, all compost 332 treatments enhanced the accumulation of Mg in stems and flowers while no difference was 333 observed for leaves. Iron concentration was enhanced by the presence of the selected-green 334 compost; however, significant differences could be found only in comparison with MC50.

335 Table 4 also show the concentration of Na and Cl in plant tissues as they represent the most 336 important saline ions in Mediterranean cultivation areas. In general, the presence of composts 337 reduced significantly the concentration of Na in leaves and flowers with the exception of the SC30 338 treatment that produced results similar to the peat control substrate. A different trend was observed 339 for stems where only the replacement of peat at the maximum investigated volume, with the 340 mixed-green compost, allowed a significant reduction in Na compared with the peat control 341 treatment. Conversely, the addition of mixed-green compost caused a significant increase in Cl 342 tissue content compared with the peat control and the selected-green compost treatments; the most 343 remarkable increase (+40 %) was detected in leaf tissue for MC50 compared with the peat control 344 substrate.

Data collected on the cumulated water uptake (Fig. 4) showed reduced values for the plants treated with 50 % mixed-green compost compared with the other treatments. However, such a reduction was statistically significant only starting from 68 DAT. At the end of the experiment, the
total amount of water taken up by plants in the above treatment was, on average, 18 % lower than in
the other treatments.

350 **4 Discussion** 

351 Peat substitution with selected- or mixed-green compost altered significantly the chemical 352 composition of the root zone compared with the peat control substrate (Table 2). However, as 353 expected by the composition reported in Table 1, N and C concentration, and C/N ratio 354 remained within the limits imposed by Italian regulations. Substrates obtained by mixing peat and 355 green composts exhibited pH values higher than the optimal range (i.e. roughly 5.5-6.5) expected in 356 the root zone to ensure high nutrient availability and plant nutrient uptake (De Lucia et al., 2013), 357 especially in presence of mixed-green composts (Table 2). The increase in pH by using compost in 358 growing media is common (Mugnai et al., 2007; Tittarelli et al., 2009) and represents one of the 359 main concerns for its wide application in different agricultural sectors (Brito et al., 2015; Sonneveld 360 and Voogt, 2009). Nevertheless, it should be underlined that, in substrate-grown crops, the 361 adjustment of pH is one of the simplest technical practices especially if drip irrigation is adopted for 362 watering plants (Chong, 2005); as shown in Fig. 1, the expedient of adjusting pH in the irrigation 363 water with sulphuric acid was sufficient to level pH of all treatments within optimal range (Sonneveld and Voogt, 2009). 364

The higher EC exhibited by compost mixtures related to an increased potential capacity in nutrient ion availability (Table 2). Cation concentration is significantly correlated with EC in the root zone and it can be used to estimate nutrient availability for substrate cultures (Sonneveld and Voogt, 2009). To this purpose, K was found to be significantly higher in all compost treatments compared with the peat control treatment. The presence of selected-green compost enhanced also Ca and Mg concentration. Together with macro-cations, the presence of both the two composts enhanced P and Fe availability in the root zone while the selected-green compost showed also 372 remarkable concentrations in N-NO<sub>3</sub>. This nutrient budget could be likely used by plants thereby
373 reducing the supply of chemical fertilizers (Martínez-Blanco et al., 2013); in other works, composts
374 mixed with peat have been found improving growth and quality of bedding plants compared with
375 low-fertilized substrates (Estévez-Schwarz et al., 2009).

376 Indeed, the different substrates influenced plant nutrient content (Table 4); the presence of 377 selected-green composts enhanced N, P, K and Mg concentration (basing on tissue average) 378 compared with peat control treatment while only K content was higher in the mixed-green compost 379 treatments. The presence of composts in growing media is often associated with enhanced nutrient 380 tissue content in ornamental crops (e.g. De Lucia et al., 2013). The higher nutrient concentration in 381 the root zone can boost plant nutrient uptake (Marshner at al., 2011; Massa et al., 2009), even 382 determining nutrient luxury accumulation (Richard-Molard et al., 2008). Furthermore, green 383 composts are reach in organic substances, bearing similarities with humic and fulvic acids (Table 384 1), which have been found improving plant nutrition processes (Calvo et al., 2014), such as N 385 accumulation in ornamental plants (Massa et al., 2016). The presence of humic-like substances in 386 the root zone induces higher P availability for cultivated plants since they prevent calcium 387 phosphate precipitation (Calvo et al., 2014). Increases in K or Mg tissue content were found for 388 bedding plants cultivated in green compost compared with peat substrates (Grigatti et al., 2007; 389 López-Cuadrado et al., 2008). All these effects can be recognized in Table 4. However, the most 390 remarkable increase was observed for K tissue concentration in all composts compared with the 391 peat control treatment; the highest value in K was caused, on tissue average, by MC50 treatment 392 (Table 4). A significant negative correlation was found between K and Na concentrations in plant 393 tissues (R = -0.70, P < 0.0001, n = 45); the latter was generally reduced by the presence of compost. 394 Calcium was the only macronutrient significantly reduced, on tissue average, by MC50 compared 395 with the peat control substrate. The above results were in agreement with the hypotheses that: i) 396 increased K concentration in the root zone may help ornamental plants to counteract Na passive 397 intake (Massa et al., 2009); ii) higher K intake into the symplast is generally coupled to depletion in

Ca (Li et al., 2013). The lower Ca accumulation could be also due to the lower water uptake (Fig. 4)
caused by MC50 treatment (Marshner, 2011).

400 However, the different macronutrient contents observed among the different treatments (Table 401 4) did not seem to influence plant growth (Table 3). In the present experiment all plants were fed 402 with an optimal dose of chemical fertilizers (see section 2.2) that probably mitigated the effects of 403 possible nutrient contributions by composts to plant biomass accumulation and quality. This is due to the fact that plant nutrient uptake rates increase hyperbolically by increasing nutrient 404 405 concentration in the root zone thus approaching a constant value after an optimal threshold is 406 reached (Massa et al., 2009), afterward no increase in yield and quality can be detected by 407 increasing nutrient concentration in the root zone (Marshner, 2011). As matter of fact, most of the 408 investigated growth parameters for the selected-green compost and MC30 treatments were at the 409 same level of the peat control treatment (Table 3). The only significant increase, by comparison 410 between compost and peat treatment, was observed for the total fresh weight in the 30 % peat 411 replacement of both selected- and mixed-green compost; such an increase was related to the higher 412 stem fresh weight. To this purpose, heterogeneous results were obtained in other works with 413 bedding plants where the application of green waste, for partial peat substitution, produced 414 enhanced macronutrient concentration in plant tissues with and without significant effects on plant 415 growth and quality depending on species (Grigatti et al., 2007).

416 Conversely, mixed-green compost at the higher 50 % rate of peat substitution reduced 417 significantly plant leaf area and dry weight in all organs (Table 3). The negative influence of this 418 treatment was also observed on aesthetic and biometric parameters (Fig. 2), which are strongly 419 related to product marketability in ornamental crop productions; among all SPAD index that has 420 been proposed as quality parameter due to its correlation with leaf greenness (Loh et al., 2002). We 421 suspect that such a poor performance was mainly due to direct and indirect consequences of the 422 high Cl concentration caused by mixed-green compost in the substrate (Table 2) and its consequent 423 accumulation in plant tissues (Table 4). Geranium and other ornamental plants typically show

424 positive correlation between Cl concentration in the root zone and Cl tissue content, which in turn 425 may cause detrimental effects on plant performance (Breś et al., 2016; Cai et al., 2014). Excess Cl 426 in the root zone limits N uptake of ornamental plants (Massa et al., 2009) thus increasing energy 427 consumption for active N intake. High Cl concentration in green composts has been found reducing 428 plant growth and quality of bedding plants depending on species (Garcia-Gomez et al., 2002).

429 Data collected on photosynthesis (Fig. 3) were in strong agreement with biomass accumulation 430 (Table 3). Photosynthetic response curves showed a significant reduction in Pn<sub>max</sub> while no other 431 curve parameter resulted significantly influenced. The reduction in net photosynthesis, caused by 432 the mixed-green compost at 50 % peat substitution, was probably the main driving variable 433 affecting plant growth. The above results were consistent with leaf chlorophyll (SPAD index) 434 content (Fig. 2) and the highest value in leaf/shoot dry weight ratio, which is a typical plant 435 adaptation response to lower carbon intake per leaf unit area (Table 3). Reduced chlorophyll content and photosynthesis efficiency have been correlated to higher Cl accumulation in leaf tissue of 436 437 geranium (Breś et al., 2016) as also observed in this work (Table 4). Conversely, no relationship 438 could be found between net photosynthesis and substrate physical characteristics as instead reported 439 by other authors (Bakry et al., 2013). Since physical parameter changes were comparable among 440 compost treatments with respect to 100 % peat treatment, we can conclude that the physical 441 characteristics of the substrates played a minor role in influencing plant performance in the present 442 experiment. Furthermore, no significant difference was found among compost treatments for leaf 443 stomatal conductance and evapotranspiration rate measured at 65 DAT (Fig. 3). Therefore, the 444 lower water uptake observed in the MC50, compared with the other treatments, was mainly related 445 to the lower plant leaf area (Table 3).

In general, the poor performance of the mixed-green compost arose only at 50 % of peat substitution. This evidence is compatible with the hypothesis that the high Cl concentration in the mixed-green compost had a major role in the reduction of plant growth and quality compared with the other treatments. In fact, as observed from the early studies by Maas and Hoffman (1977), plant 450 response to the presence of saline ions in the root zone can be described by a segmented linear 451 model, in which plant growth and quality are kept constant up to a specie-specific tolerance 452 threshold; afterward, a linear decrease can be observed. This model has been widely validated on 453 ornamental (Villarino and Mattson, 2011) and other horticultural crops (Magán et al., 2008).

The above results suggested that the selected-green compost was a more valuable and production-safely product for peat substitution in geranium cultivation while the mixed-green compost determined major uncertainty. These evidences are in agreement with the hypothesis that high-quality compost can be produced through a careful raw material selection (Raviv, 2013).

# 458 **5** Conclusions

Data reported in this paper show how compost characteristics may influence plant growth and 459 460 quality in relationship with plant physiology. The selected-green compost used in the test trial, on container-grown geranium, was more valuable than the mixed-green compost for peat substitution. 461 462 The former compost ensured high plant quality since all investigated plant indicators performed at 463 the same level (not significantly different) of the standard peat substrate, or even better as in the 464 case of nutrient tissue content (i.e. N, P, K and Mg, on tissue average). The latter compost affected 465 negatively plant biomass accumulation, biometric parameters, SPAD index, net photosynthesis, Ca 466 tissue content and water uptake when replaced at 50 % peat substitution rate. The collected data show how i) compost characteristics can influence plant growth, quality, nutrition and 467 photosynthesis, and ii) the use of selected composting raw material can contribute to produce 468 469 high-quality compost for the substitution of peat in substrate-grown ornamental crops.

470

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#### 597 **Figures captions**

Fig. 1 Effect of treatments on electrical conductivity (EC) and pH as determined in the 1:5 (V:V) water extract at the end of the experiment (85 DAT). Columns represent the average of three replicates  $\pm$  standard deviation. Statistical analysis was performed through one-way ANOVA; different letters for the same period/date indicate significant differences according to Tukey's (HSD) multiple-range test (*P* = 0.05).

603

Fig. 2 Effect of treatments on plant height (H), number of flowers, plant volume (V), and leaf chlorophyll (SPAD index) content. Measurements were performed at 55 (grey columns) and 85 (black columns) DAT through non-destructive or destructive analysis, respectively. Columns represent the average of six replicates  $\pm$  standard deviation. Statistical analysis was performed through one-way ANOVA; different letters for the same date indicate significant differences according to Tukey's (HSD) multiple-range test (*P* = 0.05).

610

611 Fig. 3 Effect of treatments on leaf gaseous exchange activity. 3A reports net photosynthesis (Pn) 612 response to PAR; points represent the average of three replicates ( $\pm$  mean standard error is reported 613 on the upper left side to avoid line overlaps) while lines represent non-linear regressions performed 614 by fitting a non-rectangular hyperbola (Eq. 1) to measured data; the presence of an asterisk indicates significant differences at the corresponding PAR intensity assessed through one-way ANOVA ( $P \leq$ 615 0.05). 3B reports total (shoot dry weight) versus maximum Pn rate (Pn<sub>max</sub>) as determined by the 616 non-linear regression analysis performed through Eq. 1 (± mean standard error is reported on the 617 618 upper right side to avoid line overlaps). 3C and 2D report the mean leaf stomatal conductance (Gs) 619 and transpiration (E), respectively, measured during light-response curves; columns represents the 620 average of three replicates  $\pm$  standard deviation. Statistical analysis was performed through one-way

621 ANOVA; the presence of different letters indicates significant differences according to Tukey's 622 (HSD) multiple-range test (P = 0.05).

624	Fig. 4 Effect of treatments on water uptake (WU) cumulated over the whole period of observation.
625	Points represent the average of three replicates $\pm$ standard error. Statistical analysis was performed
626	through one-way ANOVA; the presence of an asterisk indicates significant differences at the
627	corresponding date for $P \le 0.05$ .
628	
629	
630	
631	

# 1 Tables

2 Table 1 Chemical and microbial characterization of the composts (compost SC and MC) used for

Parameter	Units	SC	МС	Reference values <sup>a</sup>
Humidity	g 100g <sup>-1</sup>	25.6	17.3	<50
Total N	g 100g <sup>-1</sup>	1.8	2.6	
Total C	g 100g <sup>-1</sup>	22.2	28.3	>20
C/N ratio		12.6	10.9	<50
Humic and fulvic acids	g 100g <sup>-1</sup>	14.2	7.6	>2.5
pH (1:10)		7.6	7.5	6.0-8.5
EC (1:10)	µS cm⁻¹	264	322	
Na	g kg <sup>-1</sup>	1.05	2.49	
Cl	g kg <sup>-1</sup>	1,21	3.47	
Cd	mg kg <sup>-1</sup>	< 0.25	< 0.25	<1.5
Cr	mg kg <sup>-1</sup>	< 0.25	< 0.25	<0.5
Hg	mg kg⁻¹	< 0.10	0.11	<1.5
Ni	mg kg <sup>-1</sup>	36.9	44.6	<100
Pb	mg kg <sup>-1</sup>	48.1	15.9	<140
Cu	mg kg <sup>-1</sup>	128.3	62.0	<230
Zn	mg kg <sup>-1</sup>	177.2	144.9	<500
Salmonella	MPN 25g <sup>-1</sup>	absent	absent	absent
Escherichia coli	CFU g <sup>-1</sup>	<10	<10	<m=1000 m="5000&lt;/td" or=""></m=1000>

3 the preparation substrate mixtures.

<sup>a</sup>Limit values imposed by the Italian law D.Lgs. n° 75, 29 April 2010.

6	Table 2. Physicochemical characterization of the control substrate (PC) and compost mixtures
7	(SC30, SC50, MC30, MC50). Total nitrogen (N) and carbon (C) are expressed as concentration in
8	the dry weight (g $100g^{-1}$ ) whereas pH, electrical conductivity (EC, $\mu$ S cm <sup>-1</sup> ), and nutrient (N-NO <sub>3</sub> ,
9	P-PO <sub>4</sub> , K, Ca, Mg and Fe) and saline ions (Na and Cl) concentrations (mg L <sup>-1</sup> ) were measured in the
10	1:5 (V:V) water extract. Bulk density (BD, g cm <sup>-3</sup> ), water container capacity (CC, % V/V), total
11	porosity (TP, % V/V), easily available water (EAW, % V/V), and water buffering capacity (WBC,
12	% V/V) are reported for substrate physical characteristics. All parameters were determined before
13	the addition of controlled release chemical fertilizers.

Parameter	PC	SC30	SC50	MC30	MC50	ANOVA <sup>a</sup>
Chemical cha	aracteristics					
Ν	0.98 b	1.35 a	1.52 a	1.39 a	1.52 a	**
С	37.85 a	28.12 bc	25.37 с	35.00 a	30.57 b	***
C/N	38.55 a	21.15 c	16.82 d	25.28 b	20.09 cd	***
pН	5.99 b	6.68 ab	6.96 a	7.43 a	7.47 a	**
EC	177.07 c	273.47 ab	453.67 a	241.33 b	332.67 ab	***
N-NO3	1.43 c	17.24 b	34.32 a	2.26 c	2.48 c	***
P-PO4	1.82 c	2.72 b	4.43 a	2.45 bc	2.69 b	***
Κ	21.27 c	50.81 b	69.09 a	47.24 b	68.40 a	***
Ca	16.01 bc	19.49 b	26.96 a	12.47 c	11.63 c	***
Mg	1.63 c	5.13 b	8.32 a	1.96 c	2.25 c	***
Fe	0.88 b	0.96 a	0.98 a	1.96 a	2.25 a	**
Na	16.91 b	22.30 a	25.60 a	22.35 a	25.51 a	***
Cl	9.17 d	15.50 cd	20.83 c	38.50 b	54.00 a	***
Physical char	racteristics					
BD	0.08 c	0.19 b	0.28 a	0.18 b	0.20 b	***
CC	85.60 a	70.40 bc	65.31 c	73.71 b	72.26 b	***
TP	94.77 a	87.16 b	80.97 c	87.47 b	86.25 b	***
EAW	27.81	23.70	31.20	30.80	29.71	n.s.
WBC	7.55	6.11	7.49	8.85	6.92	n.s.

<sup>a</sup>Statistical analysis performed through one-way ANOVA; n.s. = non significant or \*,\*\*,\*\*\* = significant at  $P \le 0.05$ , 0.01 and 0.001, respectively. Different letters for the same parameter

16 indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).

18	Table 3. Biomass accumulated in the different organs is reported as fresh weight (FW, g pt <sup>-1</sup> ), dry
19	weight (DW, g pt <sup>-1</sup> ), percentage of DW (%DW, g 100g <sup>-1</sup> ), leaf area (LA, cm <sup>2</sup> pt <sup>-1</sup> ); biomass
20	partitioning is reported as leaves, stems, and flower DW percentage of total - shoots - (Tot) DW
21	(%Lv/Tot DW, %St/Tot DW, %Fl/Tot DW, respectively). Measurements were performed during the
22	final destructive analysis (85 DAT). Values represent the average of six replicates.

Organ	Parameter	PC	SC30	SC50	MC30	MC50	ANOVA <sup>a</sup>
Leaves	FW	69.06 ab	78.39 a	77.13 a	80.08 a	65.04 b	**
	DW	6.16 ab	6.79 ab	6.54 ab	7.01 a	5.92 b	*
	%DW	8.94	8.73	8.49	8.83	9.08	n.s.
	%Lv/Tot DW	37.57 b	39.70 ab	38.86 ab	39.92 ab	41.89 a	*
	LA	1208.89 ab	1390.24 a	1265.67 ab	1329.99 a	1121.15 c	**
	FW	24.20 c	30.97 a	28.18 abc	30.45 ab	24.59 bc	**
Stome	DW	2.80 a	2.88 a	2.78 a	2.93 a	2.12 b	**
Stems	%DW	11.61 a	9.33 ab	9.88 ab	9.84 ab	8.59 b	**
	%St/Tot DW	17.22	16.77	16.51	16.74	14.95	n.s.
	FW	54.01 a	52.00 a	50.32 ab	53.84 a	41.89 b	**
Flower	DW	7.44 a	7.44 a	7.53 a	7.56 a	6.07 b	*
Flowers	%DW	13.75 a	10.38 b	10.62 b	10.36 b	10.57 b	***
	%Fl/Tot DW	45.21	43.53	44.63	43.34	43.15	n.s.
	FW	147.27 b	161.36 a	155.64 ab	164.38 a	131.52 c	**
Total	DW	16.41 a	17.11 a	16.85 a	17.49 a	14.11 b	**
	%DW	11.14	10.66	10.83	10.78	10.72	n.s.

<sup>a</sup>Statistical analysis performed through one-way ANOVA; n.s. = non significant or \*,\*\*,\*\*\* = significant at  $P \le 0.05$ , 0.01 and 0.001, respectively. Different letters for the same parameter

25 indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).

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Organ	Element	PC	SC30	SC50	MC30	MC50	ANOVA
·	Ν	23.01 b	28.42 a	28.56 a	24.92 b	24.69 b	**
	Р	2.35 b	4.02 ab	6.05 a	4.95 ab	5.78 a	**
	Κ	35.35 c	40.28 c	59.74 a	49.85 b	62.89 a	***
Leaves	Ca	24.77 a	22.63 ab	20.27 ab	21.55 ab	18.85 b	*
Leaves	Mg	4.11	5.28	4.73	4.97	4.85	n.s.
	Fe	0.31	0.43	0.17	0.47	0.43	n.s.
	Na	9.19 a	9.28 ab	3.47 c	5.39 bc	3.32 c	***
	Cl	23.90 b	21.54 b	20.20 b	24.73 b	33.33 a	**
	Ν	11.29 c	18.06 a	16.19 b	14.93 b	16.19 b	***
	Р	3.31	6.22	7.14	4.87	7.14	n.s.
	Κ	23.00 c	34.63 bc	43.73 b	43.92 b	65.43 a	***
C.	Ca	34.36 b	39.38 a	35.43 ab	31.92 bc	27.97 с	***
Stems	Mg	3.45 c	6.34 a	6.53 a	4.85 b	5.34 b	***
	Fe	0.10 ab	0.14 a	0.15 a	0.07 ab	0.06 b	*
	Na	6.05 b	9.18 a	7.03 ab	4.23 bc	1.64 c	***
	Cl	19.26 b	21.90 b	19.69 b	27.91 a	29.58 a	***
	Ν	20.67 b	23.15 a	22.73 a	21.70 ab	20.49 b	**
	Р	5.75	6.54	6.72	6.86	7.01	n.s.
	Κ	28.53 d	33.94 c	42.58 b	40.44 b	46.94 a	***
Florens	Ca	9.76	9.71	9.67	9.58	9.36	n.s.
Flowers	Mg	2.29 b	2.77 a	2.72 a	2.78 a	2.74 a	*
	Fe	0.21	0.29	0.29	0.25	0.36	n.s.
	Na	7.30 a	6.03 ab	4.37 c	4.70 bc	3.55 c	***
	Cl	13.81 b	13.33 b	15.74 ab	16.32 a	16.45 a	*
	Ν	18.32 c	23.21 a	22.49 a	20.52 b	20.02 bc	***
	Р	3.80 b	5.59 a	6.64 a	5.56 ab	6.36 a	***
	Κ	28.96 d	36.29 c	48.68 b	44.74 b	58.42 a	***
т.	Ca	22.96 a	23.91 a	21.79 a	21.02 ab	18.73 b	**
1 issue average	Mg	3.28 b	4.80 a	4.66 a	4.20 ab	4.31 a	**
	Fe	0.21	0.29	0.20	0.26	0.28	n.s.
	Na	7.51 a	8.16 a	4.96 b	4.77 bc	2.84 c	***
	Cl	18.99 c	18.92 c	18.54 c	22.99 b	26.45 a	***

Table 4. Mineral concentration (g kg<sup>-1</sup> of DW) in the different organs and their average as determined at the final destructive analysis (85 DAT). Values represent the average of three replicates.

<sup>a</sup>Statistical analysis performed through one-way ANOVA; n.s. = non significant or \*,\*\*,\*\*\* = significant at  $P \le 0.05$ , 0.01 and 0.001, respectively. Different letters for the same parameter indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05).

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# Figures















