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Enhancing Soil-Grown Strawberry Fruit Quality through the Synergistic Influence of Beneficial Microorganisms and Digestate

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Abstract:	Purpose The use of livestock manure as agricultural soil amendments is a significant source of ammonia emissions and nitrate leaching. Anaerobic digestion of manure can yield to solid and liquid by-products usable as fertilizers that can limit these negative impacts. They could be further supplemented with plant growth-promoting microorganisms (PGPM) to improve plant growth and yield. This study investigated the impact of PGPMs and anaerobic digestates on strawberry quality and rhizospheric microbial community. Methods Strawberry plants were grown in soils treated with PGPMs (pure culture of Azospirillum brasilense or a commercial product with effective microorganisms) along with either liquid or solid digestate. Effects of digestates and PGPMs were evaluated by measuring plant yield and nutraceutical values, while the rhizospheric microbial community was assessed through an eDNA metabarcoding approach. Results Results suggest using PGPMs combined with digestates enhances plant yield, with increases of up to 40-60% in fruit yield and 9-18% in nutraceutical value, compared to the controls. The rhizospheric microbial community was influenced only by digestates. Nevertheless, these alterations have not led to significant changes in the community, thus ensuring its long-term stability. Moreover, PGPMs were not detected into the rhizospheric community. Conclusions Our data pointed out that both PGPMs and digestates can represent a sustainable approach to increase strawberry plant yield. However, PGPMs require repeated inoculations in long-term projects to achieve and maintain desired outcomes. These findings emphasize the complexity of rhizospheric microbial interactions and underscore the importance of continued research to optimize agricultural practices while maintaining ecosystem stability.
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Enhancing Soil-Grown Strawberry Fruit Quality through the Synergistic Influence of Beneficial Microorganisms and Digestate

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Abstract

Purpose

The use of livestock manure as agricultural soil amendments is a significant source of ammonia emissions and nitrate leaching. Anaerobic digestion of manure can yield to solid and liquid by-products usable as fertilizers that can limit these negative impacts. They could be further supplemented with plant growth-promoting microorganisms (PGPM) to improve plant growth and yield. This study investigated the impact of PGPMs and anaerobic digestates on strawberry quality and rhizospheric microbial community.

Methods

Strawberry plants were grown in soils treated with PGPMs (pure culture of *Azospirillum brasilense* or a commercial product with effective microorganisms) along with either liquid or solid digestate. Effects of digestates and PGPMs were evaluated by measuring plant yield and nutraceutical values, while the rhizospheric microbial community was assessed through an eDNA metabarcoding approach.

Results

Results suggest using PGPMs combined with digestates enhances plant yield, with increases of up to 40-60% in fruit yield and 9-18% in nutraceutical value, compared to the controls. The rhizospheric microbial community was influenced only by digestates. Nevertheless, these alterations have not led to significant changes in the community, thus ensuring its long-term stability. Moreover, PGPMs were not detected into the rhizospheric community.

Conclusions

Our data pointed out that both PGPMs and digestates can represent a sustainable approach to increase strawberry plant yield. However, PGPMs require repeated inoculations in long-term projects to achieve and maintain desired outcomes. These findings emphasize the complexity of rhizospheric microbial interactions and underscore the importance of continued research to optimize agricultural practices while maintaining ecosystem stability.

Keywords: strawberry, digestates, PGPM, fruit quality, plant yield, soil biodiversity

29 **1. Introduction**

30 The increasing demand for sustainable agricultural practices has prompted researchers and farmers to explore
31 alternative methods for improving soil fertility and crop productivity while minimizing environmental impacts
32 (Ferreira et al. 2022; Pe'er et al. 2020; Tahat et al. 2020). One effective approach is utilizing organic waste materials,
33 particularly livestock manure, as agricultural amendments (Abbott et al. 2018; Goldan et al. 2023). Manure-based
34 amendments not only provide essential nutrients to the soil but also contribute to the organic matter content, improving
35 soil structure, water-holding capacity, and nutrient retention (Abbott et al. 2018; Goldan et al. 2023; Indraratne et al.
36 2009). However, the excessive use of manures without appropriate management strategies has led to significant
37 environmental concerns, including the contamination of soil and water bodies (Bijay-Singh & Craswell, 2021;
38 Chadwick et al. 2011; Loyon et al. 2016; Zhang et al. 2017). The spreading of manures on agricultural soils represents
39 one of the major sources of ammonia emission and nitrates leaching (Abbott et al. 2018; Bijay-Singh & Craswell,
40 2021; Holm-Nielsen et al. 2009; Jones et al. 2014). To mitigate these issues, the European Union (EU) has introduced
41 Directives 2001/81/EC and 91/676/EEC, which seek to regulate manure application on agricultural soils and reduce
42 associated environmental impacts (Loyon et al. 2016). Consequently, livestock waste disposal has become an
43 economic problem for farmers since the quantity of waste produced is often higher than the allowed usage (Petersen
44 et al. 2007). To deal with these limitations and to find more sustainable alternatives, the conversion of manure into
45 digestates has gained significant attention. Digestates, obtained through anaerobic digestion of manure, offer several
46 advantages as agricultural fertilizers, including improved nutrient availability and enhanced stability (Doyeni et al.
47 2021; Möller & Müller, 2012; Valentinuzzi et al. 2020). Moreover, integrating plant growth-promoting
48 microorganisms (PGPM) with manure amendments may be a potential strategy to improve plant growth and nutrient
49 uptake efficiency (Omara et al. 2022; Ren et al. 2021) and can significantly affect the rhizosphere microbial
50 community, further enhancing the effects of these fertilizers on plants (Benbrik et al. 2021; Ren et al. 2020, 2021).
51 PGPMs is a group of beneficial microorganisms that colonize the rhizosphere and enhance plant growth through direct
52 or indirect mechanisms (Abbott et al. 2018; Basu et al. 2021; Shah et al. 2021). The indirect action is protection against
53 soil-borne pathogens (mainly fungi), while the direct mechanisms are associated with producing substances that
54 stimulate plants' growth (Abbott et al. 2018; Shah et al. 2021). This effect is achieved by increasing the growth of the
55 root system, allowing plants to explore a higher volume of soil, thus greatly influencing the biogeochemical cycles of
56 elements in the soil (Alegria Terrazas et al. 2016; Pii et al. 2015a). In addition, in recent works, we have also

57 highlighted that PGPMs such as *Azospirillum brasilense (A. brasilense)* can influence the molecular and biochemical
58 mechanisms underlying the acquisition of nutrients (Marastoni et al. 2019; Pii et al. 2016, 2018, 2019). Among the
59 plants that could benefit most from such an integrated approach are berry plants, such as strawberries, because of their
60 economic importance and high phytochemical content. Strawberries are globally one of the most consumed fruits, not
61 only for their excellent taste but also for their high content of bioactive compounds, which are known to have a positive
62 influence on human health because of their antioxidant, anti-inflammatory and anticancer properties (Giampieri et al.
63 2012). Many factors, such as genotype (Tulipani et al. 2011), environment, agriculture, and biofortification practices
64 (Mimmo et al. 2017; Valentinuzzi et al. 2018) can significantly influence these peculiar properties as well as their
65 quality features (e.g., elemental composition, pH, total soluble solids (TSS), total or titratable acidity, organic acids,
66 anthocyanins). In addition, previous works highlighted that nutrient supply could influence strawberries' quality and
67 phytochemical composition (Valentinuzzi et al. 2015a, 2015b). In a hydroponic experiment, was also observed that
68 the inoculation of nutrient solutions with PGPMs could modify the quality of strawberry fruits by enhancing the
69 sweetness index, the concentration of antioxidants, and inducing the accumulation of micronutrients (Pii et al. 2018).
70 In this work, we aimed to assess the effect of liquid and solid digestates, both alone and combined with PGPMs, on
71 the growth of strawberry plants, the quality of strawberry fruits, and the composition of the rhizosphere microbiota.
72 Based on the combined use of PGPMs and manure digestates in the soil of strawberry plants, we hypothesize that
73 treated plants will show a significant increase in yield compared with the untreated control. Furthermore, we
74 hypothesize that the application of PGPMs and manure digestates will affect the composition of the rhizosphere
75 microbial community.

76 **2. Materials and methods**

77 *2.1 Plant growth*

78 Strawberry frigo plants (*Fragaria x ananassa* cv. Elsanta) were purchased from Sant' Orsola Società Cooperativa
79 Agricola (Pergine Valsugana, Trento, Italy), planted in individual 1.5 L plastic pots after one day of thawing, and
80 grown in a climate chamber under the following controlled conditions: 14/10 h day/night ratio, 24°C during the day
81 and 19°C at night, 70% relative humidity, and 250 µmol $m^{-2}s^{-1}$ light intensity. Pots were filled with a 2 cm granulated
82 clay and approximately 900 g of air-dried soil (Table S1) and plants were grown for 78 days, maintaining 60% water-
83 holding capacity during the experiment by watering them twice a week.

84 Plants were then fertilized with different strategies using digestates in solid and liquid forms, *A. brasilense,* and a
85 preparation of effective microorganisms. Both digestates and effective microorganisms were obtained from third-party
86 providers represented by the local Biogas Wipptal plant (Vipiteno, Italy) and the Multikraft manufacturer (Pichl bei
87 Wels, Austria), respectively, while *A. brasilense* Cd (DSM-1843) was grown in LB medium and prepared for plant
88 inoculation as described in Pii et al. (2016). Plants treated with *A. brasilense* were inoculated two weeks after planting
89 in pots, reaching a final concentration of 106 cfu g^{-1} soil, while the inoculation of the effective microorganisms was
90 carried out as specified by the manufacturer's guideline, specifically once a week until flowering, and twice a week
91 thereafter. Each biofertilizer has been applied alone or combined, leading to a total of nine different treatments (7
92 independent pots per treatment) arranged as follows:

93 1. Control: soil without any addition.
94 2. *Azospirillum brasilense* (AZO): soil inoculated with *A. brasilense*.
95 3. Effective microorganisms (EM): soil inoculated with effective microorganisms.
96 4. Pellet: soil mixed with solid digestate at 300 mg N kg^{-1} soil concentration.
97 5. Liquid digestate (LD): soil mixed with liquid digestate at a 75 mg N kg^{-1} soil concentration.
98 6. *A. brasilense* + pellet (AZO+Pellet): soil mixed with solid digestate at 300 mg N kg^{-1} soil concentration and
99 inoculated with *A. brasilense*.
100 7. Effective microorganisms + pellet (EM+Pellet): soil mixed with solid digestate at 300 mg N kg-1 soil
101 concentration and inoculated with effective microorganisms.
102 8. *A. brasilense* + liquid digestate (AZO+LD): soil mixed with liquid digestate at a 75 mg N kg^{-1} soil
103 concentration and inoculated with *A. brasilense*.
104 9. Effective microorganism + liquid digestate (EM+LD): soil mixed with liquid digestate at a 75 mg N kg^{-1} soil
105 concentration and inoculated with effective microorganism.

106 *2.2 Assessment of plant growth and fruit yield*

107 During the growing cycle of strawberry plants, leaf chlorophyll content was measured using a portable chlorophyll
108 meter (SPAD-502; Minolta, Osaka, Japan). Measurements were carried out weekly on basal and apical leaves (at least
109 two per plant), and five SPAD measurements per leaf were taken and averaged. The number of produced flowers was
110 also counted during the growing cycle. At the end of the experiment, strawberry plants were harvested, and roots and
111 leaves were separated and weighed to assess the fresh weight (FW). Strawberry fruits were harvested once they

112 showed at least 80% red on their surface. At harvest, the fresh weight (FW), yield per plant (g FW per plant), average
113 yield (g FW), and the average number of fruits per plant were measured.

114 *2.3 Characterization of fruit quality*

115 The colour of all ripened strawberry fruits was determined at harvest using a portable Tristimulus Colorimeter
116 (Chroma Meter CR-400, Konica Minolta Corp., Osaka, Japan). The colour index (CI) was calculated as CI=100 x a /
117 (L x b), where *L* represents luminance (lightness), *a* represents the red/green coordinate, and *b* represents the
118 yellow/blue coordinate, with higher values corresponding to a more intense red colour (Tezotto-Uliana et al. 2014).
119 The total soluble solids (TSS), expressed as Brix degrees (°Bx), were measured using a refractometer (Atago, Tokyo,
120 Japan) on freshly extracted fruit juice, while the titratable acidity (TA) was determined as previously described by
121 Valentinuzzi et al. (2015a). Briefly, TA was assessed by adding 25 mL distilled water to 5 mL of freshly extracted
122 fruit juice, and the mixture was automatically titrated to a final pH of 8.1 (Titration Unit Titro-Line easy; Schott
123 Instruments, Mainz, Germany) with a solution of 0.1 mol L−1 NaOH; the final result was expressed as mmol L^{-1} citric
124 acid. Fresh strawberries' firmness was assessed using a penetrometer (Modell PCE-FM200; PCE Instruments,
125 Southampton, UK) equipped with a 3 mm-diameter cylindrical probe.

126 *2.4 Strawberry extracts and fruit elemental analysis*

127 Freeze-dried strawberry fruits were ball-milled (model MM400; Retsch, Haan, Germany) until a homogeneous
128 powder was obtained; the ground samples were extracted with methanol (HPLC grade, Merck, Darmstadt, Germany)
129 using a 1:10 (m:v) extraction ratio. The mixtures were then sonicated for 30 min in a thermostatic bath, cooled with
130 ice water, and centrifuged at 14000xg for 30 minutes at 0°C. The supernatant was collected, filtered (0.2 µm nylon
131 filter, Phenomenex Inc., USA), and stored at -80°C until the analyses have been carried out.
132 From the ball-milled fruits, approximately 0.3 g of each sample was acid digested with concentrated ultrapure HNO_3
133 (650 ml L^{-1}; Carlo Erba, Milano, Italy) in a single reaction chamber microwave digestion system (UltraWAVE,
134 Milestone, Shelton, CT, USA). The macro- (phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca) and
135 sulphur (S)) and micro-nutrient (iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn)) concentrations were
136 determined by Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) (Arcos Ametek, Spectro,
137 Germany) using tomato leaves (SRM 1573a) and spinach leaves (SRM 1547) as external certified reference material,

138 while total organic carbon (TOC) and total nitrogen (TN) of lyophilized samples were determined using a Flash EA
139 1112 elemental analyzer (Thermo Scientific, Germany).

140 *2.5 Organic acid, sugars, and phenolic compounds analyses*

141 The separation of both organic acids and sugars was performed by high performance liquid chromatography (HPLC)
142 through an isocratic elution using a cation exchange column Aminex 87-H column (300 x 7.8 mm, 9 mm, Bio-Rad)
143 and 10 mM H_2SO_4 as mobile phase, at a flow rate of 0.6 mL min^{-1}. Organic acids were detected at 210 nm with a
144 Waters 2998 photodiode array detector (Waters Spa, Italy), while sugars were detected by a refractive index detector
145 (Waters Spa, Italy). Standard acids and sugars were prepared as individual stock solutions and combined to give
146 diluted reference standards, and then identified by comparing the retention times of the unknown samples to pure
147 compounds with known retention times; finally, the sweetness index (SI) was calculated as in Mahmood et al. (2012)
148 according to the formula:

149 $$SI=1\times[Sucrose]+0.74\times[Glucose]+1.73\times[Fructose]$$

150 The content of total phenols of strawberry extracts was determined following the Folin-Ciocalteu method (Atanassova
151 et al. 2011; Folin & Ciocalteu, 1927), while the concentration of flavonoids and flavonols was determined by a
152 pharmacopeia method, using rutin hydrate as reference compound (Miliauskas et al. 2004).

153 *2.6 Soil elemental analysis*

154 Soil pH was determined in agreement with Sparks et al. (1996). The inorganic nitrogen (N) was extracted with a 1 M
155 KCl (1:10, w:v) solution and determined colorimetrically using a flow analyzer (AA3, Bran Lubbe, Germany). DTPA-
156 extractable fractions of nutrients (Cu, Fe, Mn and Zn) were extracted from approximately 10 g of soil with 20 mL of
157 extracting solution (0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M TEA adjusted to pH 7.3) according to Lindsay and
158 Norvell (1978). Nutrient concentrations were subsequently determined by inductively coupled plasma optical
159 emission spectrometry (ICP-OES) (Arcos Ametek, Spectro, Germany).

160 *2.7 Microbial community assessment - DNA Extraction, Amplification, and Sequencing*

161 At the end of the experiment, soil samples were collected from each pot and molecular analyses were conducted for
162 the taxonomic identification of the rhizospheric microbial communities. The DNA was extracted from 0.25 g (wet
163 weight) of each sample using the DNeasy® PowerSoil® DNA Isolation Kit (Qiagen, Hilden, Germany) according to
164 the manufacturer instructions, and the DNA concentration was assessed with the Qubit (Invitrogen, Milan, Italy).

165 Bacterial and fungal diversity was determined for all samples. The fungal internal transcribed spacer region 2 (ITS2)
166 was amplified using ITS3 and ITS4 primers (Op De Beeck et al. 2014), while the bacterial 16S rRNA gene was
167 amplified using the primer pair 341F/805R (Herlemann et al. 2011; Takahashi et al. 2014). PCR reactions were
168 conducted following the thermocycling conditions reported by Bani et al. (2019), in a final volume of 25 µL. Reaction
169 mix included 12.5 µL of AppTaq RedMix (Appleton Woods Limited, Birmingham, UK), 0.4 µL of each primer (10
170 µM), 2,5 µL of template DNA and 9.2 µL of Invitrogen UltraPure™ DNase/RNase-Free Distilled Water
171 (ThermoFisher Scientific, UK). Amplicons were then purified and multiplexed as reported by Signorini et al. (2021)
172 and sequenced using 300+300 bb paired end reads and an Illumina MiSeq platform at the University of Essex
173 (Colchester, UK). Raw data have been submitted to the National Center for Biotechnology Information (NCBI) under
174 accessions numbers PRJNA1108168.

175 *2.8 Bioinformatics*

176 Fungal and bacterial raw data were checked using FastQC (Andrews, 2010) and then pre-processed, quality-filtered,
177 and trimmed using DADA2 within QIIME2 (Bolyen et al. 2019; Callahan et al. 2016). Chimeras were removed using
178 the "consensus" method (Callahan et al. 2016). Filtered amplicon sequence variants (ASV) were clustered into
179 operational taxonomic units (OTUs) using VSEARCH and applying a cut-off of 97% (Rognes et al. 2016). The
180 taxonomic assignment of the resulting OTUs was performed within QIIME2 by using the Naïve-Bayes classifier
181 trained on SILVA (Quast et al. 2013) for bacteria and on UNITE-INSD (Nilsson et al. 2019) for fungi.

182 *2.9 Statistical Analysis*

183 All datasets were analyzed using statistical software to determine significant differences between the treatments and
184 the control. The results of the chemical measurements are presented as means of at least five replicates ± standard
185 error (SE). Statistical analysis was performed using GraphPad Prism version 10 for Windows (GraphPad Software,
186 San Diego, California, USA), and the Shapiro-Wilk's test was used to check for the normality of the data. For normally
187 distributed data, differences among samples were tested using analysis of variance (ANOVA), followed by Tukey´s
188 post hoc test ($p < 0.05$), while when normality was not met, data were analysed using the non-parametric Kruskal-
189 Wallis test, followed by Dunn's test for pairwise comparisons.
190 For community dissimilarity, the resulting OTUs were filtered, the final datasets were subsequently rarefied with all
191 rarefaction curves reaching the plateau, and statistical analyses were performed using statistical multi-packages

192 implemented in R (R Core Team, 2020). The differential abundance analysis of bacterial and fungal taxa between
193 treatments and control was estimated with MaAsLin2 (Mallick et al. 2021), while to assess bacterial and fungal
194 diversity among different treatments, alpha- and beta-diversity were calculated using 'vegan' (Oksanen et al. 2022),
195 'agricolae' (de Mendiburu & Yaseen, 2020), and 'ggplot2' (Wickham, 2016) packages. Alpha-diversity based on
196 OTUs was calculated using the Chao1 index to characterize the richness of the communities and the Shannon index
197 to characterize their diversity. The normality of the data was checked using Shapiro-Wilk test, and differences were
198 tested using ANOVA or the Kruskal-Wallis test, followed by Tukey's or Dunn's post hoc test, respectively. Canonical
199 Analysis of Principal Coordinates (CAP) based on Bray-Curtis's dissimilarity distance was performed to evaluate
200 bacterial and fungal beta-diversity by applying the forward selection to identify the explanatory variables (Monte
201 Carlo permutation test with 9999 randomizations, $p < 0.05$), which were then fitted on the CAP plots. Variance
202 (ANOVA) was analyzed to establish significant differences between the treatments and the control.

203 3. Results

204 *3.1 Plant growing parameters and yield*

205 The growing parameters evaluated to assess the effect of the different treatments on strawberry plants are shown in
206 Table 1. Shoot biomass measured at the end of the experiment displayed significant differences between treatments,
207 with the highest values in treatment AZO+LD (35.67 ± 1.24 g), and the lowest measured in Control plants (21.53 ±
208 1.67 g). To evaluate the effect of the applied treatments on the chlorophyll content of strawberry leaves, the SPAD
209 index was measured weekly (data not shown). At the end of the experiment, the observed SPAD values did not differ
210 among the treatments.
211 Plant productivity was assessed by measuring the number of flowers per plant, the number of fruits per plant, and the
212 average yield per plant (Table 1). Plants inoculated with PGPMs only did not differ from the control while all the
213 other treatments were statistically different. Similarly, the number of fruits per plant was the highest in all plants
214 treated with LD (alone and combined) and with AZO+Pellet, while all the other treatments showed values like those
215 from Control plants. Finally, the average yield per plant was significantly enhanced in all treatments (except for EM
216 plants), with the highest productivity obtained in plants amended with LD and subsequently inoculated with PGPMs.

217 *3.2 Fruit quality parameters*

218 Strawberry quality parameters such as color index, firmness, total soluble solids, and titratable acidity were determined
219 in fresh fruits at harvest (Fig. 1). Concerning color index, the fruits with the most intense red coloration were those
220 produced by AZO (Fig. 1a), while treatments did not affect fruit firmness (Fig. 1b). In contrast, significant differences
221 were measured in the total soluble solids concentration (expressed as °Brix): the highest concentration was detected
222 in not combined treatments, while the lowest values were determined in strawberry juices of fruits collected from
223 plants inoculated with PGPMs and amended with LD (Fig. 1c). Regarding titratable acidity, the treatments did not
224 significantly modify this parameter, being only slightly higher in EM+Pellet fruits (Fig. 1d).

225 *3.3 Organic acids, sugars concentration, and phenolic compounds*

226 The concentration of sugars, organic acids, and the sweetness index of strawberry fruits are shown in Fig. 2, while
227 Fig. 3 presents the data on bioactive compounds. Whereas the concentration of citric acids was unaffected by the
228 treatments (Fig. 2a), the concentration of malic acid (Fig. 2b) presented some differences, being the highest in
229 AZO+LD plants. Among sugars, the highest sucrose concentration was measured in strawberries inoculated with each
230 PGPM (Fig. 2c), while fruits harvested from all the other treatments were not significantly different from Control
231 plants. The concentration of both glucose (Fig. 2d) and fructose (Fig. 2e) showed a similar trend, with the lowest
232 concentration of both sugars found in Control, Pellet, and LD plants, which also had the lowest sweetness index values
233 (Fig. 2f). In comparison, bioactive compounds were less affected by the treatments, with only minor changes observed
234 in total phenols (Fig. 3a), where a significant decrease was noted only in plants inoculated with AZO; no differences
235 were found in flavonoids and flavonols (Fig. 3b and 3c) among the treatments.

236 *3.4 Strawberry nutrient concentration*

237 The concentration of nitrogen (N), carbon (C), and macro- and micronutrients in strawberry fruits is shown in Table
238 2. N concentration was significantly reduced only in fruits harvested from AZO and EM samples and in the
239 combinations of PGPMs with LD. Strawberry P concentration was the highest in Control, Pellet, and LD, while it
240 significantly decreased in all the other treatments in which PGPMs were inoculated. The concentration of cations such
241 as K, Mg, and Ca was only slightly affected by the different treatments, with K and Ca being the highest in AZO+LD
242 plants and Mg in Pellet plants. In contrast, S concentration was significantly affected, with the highest concentration
243 detected in AZO+LD fruits and the lowest in Control and all EM plants (combined or not with digestates). Concerning
244 micronutrient concentration, similar trends could be observed. In all plants treated with digestates, both in the form of

245 pellet and liquid digestate, the concentration of Fe, Cu, Mn, and Zn was significantly higher than in Control plants or
246 plants treated with PGPMs only.

247 *3.5 Soil analyses*

248 The measurement of extractable soil metals (Table 3) revealed significant variations only for copper and manganese,
249 both of which showed significant reductions in the treatments compared to the Control plants. Specifically, the
250 concentration of Cu slightly decreased in soils amended with both digestates and inoculated with *A. brasilense*, and
251 Mn concentration decreased in the same treatments as well as in Pellet and LD samples. Regarding the other
252 parameters, soil pH remained relatively stable across all treatment groups by the end of the experiment (Fig. 4a), while
253 nitrate and ammonium exhibited different trends: nitrate was highest in liquid-amended plants but remaining
254 comparable to the Control plants (Fig. 4b), whereas inoculated and Pellet-amended soils had similarly low nitrate
255 levels. For ammonium levels (Fig. 4c), the only significant difference was observed in the Pellet-treated samples,
256 where ammonium concentrations were significantly higher.

257 *3.6 Rhizosphere microbial community diversity*

258 After bioinformatic analysis, 218240 and 306354 raw reads were generated for the 16S rRNA and the ITS2, resulting
259 in 538 bacterial and 371 fungal OTUs. The alpha diversity of both fungal and bacterial communities (Table S2),
260 assessed using the Chao1 richness and the Shannon Diversity Index, indicated no significant differences between
261 Control and treated plants. The community structure of bacteria (Fig. 5) and fungi (Fig. 6) in relation to treatments
262 and environmental parameters was investigated using the Canonical Analysis of Principal Coordinates (CAP). The
263 envfit function showed that both bacterial ($p < 0.001$) and fungal ($p < 0.001$) communities diversified accordingly to
264 the amendments, while only for the fungal community we observed a diversification of the structure following the
265 inoculation of PGPMs ($p < 0.05$). No correlation was highlighted between the communities and the environmental
266 parameters.

267 *3.7 Soil Microbial community composition*

268 The analysis of the 16S rRNA gene showed that the dominant phyla (Fig. 7a) in the bacterial community were
269 Proteobacteria (35.11%), Acidobacteriota (24.47%), Bacteroidota (10.27%), and Actinobacteriota (9.50%), while only
270 the 1.60% of taxa remained unclassified. Among the identified genera (Fig. 7b) belonging to Proteobacteria, the most
271 abundant were *Bradyrhizobium* (3.28%) and *Acidobacter* (3.04%). However, the vast majority of Proteobacteria's

272 genera remained unclassified, and *A. brasilense* was detected in only one sample, with a total abundance of 14 reads.
273 Among the Acidobacteriota, the most abundant genera were represented by *RB41* (Acidobacteria bacterium; 5.23%),
274 *Vicinamibacter* (4.96%), and the aerobic taxa *Gaiella* (2.67%).
275 In all samples, the fungal community was dominated by Ascomycota (50.32%), followed by Mortierellomycota
276 (29.53%), and Basidiomycota (9.66%) (Fig. 7c). Only 5.87% of taxa remained unclassified at the phylum level.
277 However, as for bacteria, the most abundant Ascomycota genera remained unclassified, while the identified ones were
278 represented by *Exophiala* (1.60%), *Fusarium* (1.11%), and *Tetracladium* (1.06%) (Fig. 7d). On the other hand, the
279 results showed that almost all taxa belonging to Mortierellomycota were classified as *Mortierella* (27.12%).
280 The Masslin2 analysis revealed significant differences in three bacterial phyla and one fungal phylum (Fig. 8).
281 Bacteroidota was significantly increased in samples treated with Pellet and LD, while Firmicutes increased only in
282 samples fertilized with LD (Fig. 8a). In contrast, Proteobacteria decreased in treated samples, with a higher decrease
283 in LD samples (Fig. 8a). At the genus level, *Acidibacter* showed trends similar to Bacteroidota, whereas *RB41*
284 exhibited pattern similar to Firmicutes (Fig. 8b). For the fungal community, only Basidiomycota were affected by
285 treatments, showing a decrease in samples treated with Pellet (Fig. 8c).

286 **4. Discussion**

287 This study explored the impact of various fertilizers and plant growth-promoting microorganisms (PGPMs) on
288 strawberry plant performance, soil properties, and the microbial rhizospheric community. Our investigation covered
289 the impact on plant growth and fruit quality, changes in soil nutrient dynamics, and variations in bacterial and fungal
290 community diversity to gain a comprehensive understanding of how these treatments affect the overall plant grow and
291 yield. Our results showed that using PGPMs and fertilizers promoted overall plant performance by increasing shoot
292 biomass, flowers, and number of fruits. LD performed better than Pellet alone and combined, and the subsequent
293 inoculation of PGPMs also improved the results. The reason for such performance could be attributable to the
294 enhanced activity of PGPMs in increased N availability (Fan et al. 2017; Lovaisa et al. 2015; Sangakkara & Higa,
295 1994). Indeed, LD contains higher amounts of N immediately available (Valentinuzzi et al. 2020), while N is bound
296 to organic matter in the Pellet. Concerning PGPMs, their improved performance in the presence of greater N
297 availability has already been observed in previous experiments. For instance, the inoculation of *A. brasilense* in tomato
298 plants grown in soil fertilized with high amounts of N resulted in higher tomato yields (Fan et al. 2017). Similarly, a

299 significant improvement in crop yields was obtained by combining EM with organic fertilizers (Sangakkara & Higa,
300 1994). The increased strawberry growth and yield can be related to the ability of these bacteria to produce auxin and
301 cytokine, fix N_2, solubilize phosphates, and produce antimicrobial substances (Aslantaş et al. 2007; Esitken et al. 2010;
302 Karlidag et al. 2007; Pirlak et al. 2007). These improvements have been emphasized by the combined use of EM with
303 digestates, most likely due to the increased availability of N.
304 Among commercially essential parameters, we evaluated fruit color, TSS, titratable acidity, and fruit firmness, all of
305 which showed similar or greater values than Control plants. A more detailed analysis of organic acids (citric and
306 malic) and sugars (glucose, fructose, and sucrose) was carried out, and the fruit sweetness index was calculated. In
307 general, it was seen that the use of digestates alone significantly reduced glucose and fructose, and consequently the
308 sweetness index, while no significant differences were shown for the other treatments. A different trend was observed
309 for sucrose, for which no significant differences were shown in any of the treatments, except for AZO and EM,
310 characterized by a significant increase in sugars. This trend in sugar content could be related again to the amount of
311 available N. In previous works, it has been observed that higher N availability can lead to higher mobilization of
312 sugars (Lemoine et al. 2013); indeed, our results showed higher N concentrations mainly in plants (Control, Pellet,
313 LD and, EM+Pellet) showing the lowest sugar concentrations.
314 Among other elements, bioactive compounds recognized as beneficial to human health (Giampieri et al. 2012; Tulipani
315 et al. 2008), macronutrients, and micronutrients were considered in this work. Several studies already pointed out that
316 inoculation of PGPMs increases the bioactive compounds of strawberries (Aaby et al. 2007; Pesakovie et al. 2016; Pii
317 et al. 2018). However, no significant differences were shown in this experiment for phenolic compounds, except for
318 a decrease in total phenols in plants inoculated with *A. brasilense*. Regarding micro- and macronutrients, it was shown
319 that plants inoculated with AZO and EM were among those with a significant reduction in most nutrients. This could
320 be explained by the ability of some PGPMs to induce changes in root exudate release (Pii et al. 2015b), which is
321 relevant for nutrient mobilization and in the molecular and biochemical activities underlying nutrient acquisition (Pii
322 et al. 2016).
323 Moving on to soil analysis, we measured soil parameters such as pH, nitrate, ammonium, and elements like Fe, Cu,
324 Mn, and Zn. Our results showed that pH did not change significantly following treatments. Although both digestates
325 presented pH values of 9 or higher (data not shown), the soil maintained a pH of around 6.2 until the end of the

326 experiment, ensuring high nutrient availability. This phenomenon may be related to the high buffering capacity of the
327 soil, attributed to the high content of organic matter (3%) (Curtin & Trolove, 2013; Zheng et al. 2022).
328 There were also no significant changes for ammonium, except for Pellet-amended plants. In contrast, for nitrate,
329 significant reductions were shown in almost all treatments, except for all plants amended with LD. These trends can
330 be explained by the different availability of N in soils Pellet- or LD-treated. Indeed, it is known that soils fertilized
331 with solid digestates have higher rates of immobilized N when compared with the positive mineralization balance
332 observed for those fertilized with liquid digestate (Laboski et al. 2010; Möller & Müller, 2012). Regarding metals, Fe
333 and Zn did not change significantly, while slight changes were observed in Cu and Mn, particularly in samples treated
334 with AZO+Pellet and AZO+LD. However, these differences did not affect the availability of micronutrients in the
335 soil, thus not representing a problem for plant performance.
336 Lastly, we investigated the effects of PGPMs and fertilizers on the microbial rhizospheric community. Beta-diversity
337 showed significant differences after using fertilizers, while alpha-diversity remained constant across the different
338 samples. This is not surprising, as microbial communities display remarkable resilience to environmental changes,
339 often outperforming individual species in complex environments (Mejias Carpio et al. 2018; Shade et al. 2012).
340 Consequently, while beta-diversity may change in response to environmental changes, microbial alpha-diversity tends
341 to remain relatively stable, thanks to the adaptation of both fungal and bacterial communities to the new environmental
342 conditions (Signorini et al. 2021, 2023; Tian et al. 2015).
343 The use of fertilizers had the most significant impact on the structure of the bacterial community, leading to its division
344 into three distinct groups, while no effect was observed from the use of PGPMs. This subdivision can be directly
345 linked to the different availability of nutrients in the treated and untreated soils. Nutrient availability is indeed known
346 to be one of the major drivers of soil microbial community structure and assembly (Fierer et al. 2007; Leff et al. 2015).
347 In our study, this correlation is reflected by alterations in taxa that exhibit either copiotrophic or oligotrophic nature
348 (adapted to nutrient-rich or nutrient-poor soils respectively).
349 Being copiotrophic phyla (Fierer et al. 2012; Guo et al. 2019; Ling et al. 2022), significant variations were observed
350 among Bacteroidota, Proteobacteria, and Firmicutes. Bacteroidota exhibited higher abundances in soils treated with
351 Pellet and LD, in which soil nutrients (e.g., N) were more abundant than Control. Conversely, Proteobacteria and
352 Firmicutes displayed contrasting abundance patterns. The highest abundance of Firmicutes was observed in LD-
353 treated soils, while Proteobacteria increased in Control and Pellet-treated pots. This unusual behavior is in line with

354 the results of Li et al. (2020), which suggest a complementary relationship between Firmicutes and Proteobacteria
355 based on soil nutrient availability. LD is known to be richer in nutrients (N, P, Ca, K, Mg) than pellets (Valentinuzzi
356 et al. 2020), which are also rapidly released into the environment. This phenomenon could, therefore, make more
357 nutrients available for the entire bacterial community, facilitating the proliferation of taxa that, in the case of lower
358 nutrient availability, would have difficulty competing. In the pellet, especially N is often found in an immobilized
359 form, making release times longer and nutrient availability low (Valentinuzzi et al. 2020). These findings could imply
360 higher soil fertility with LD treatment. Yet, its rapid nutrient release poses long-term inefficiency and substance
361 volatilization risks. In contrast, pellets offer nutrient stability and long-term availability to plants and microorganisms
362 due to their slow release (Valentinuzzi et al. 2020). Similar trends in nutrient availability were observed at the genus
363 level, with changes seen in the bacterial genera *RB41* and *Acidibacter*. Given their copiotrophic nature, they were
364 more abundant in samples treated with Pellet and LD due to the enrichment of organic matter and N provided by these
365 amendments (Ai et al. 2018; Tang et al. 2023). As for bacteria, the fungal community exhibited differences only in
366 beta-diversity, with Basidiomycota being less abundant in Pellet-treated samples. This may be linked to their
367 oligotrophic nature (Guo et al. 2019; H. Zhang et al. 2021), indicating adaptation to substrates with low or limited
368 nutrients. In contrast to bacterial observations, the use of PGPMs affected fungal beta diversity, suggesting their
369 influence on fungal phyla without, however, causing significant alterations in the main taxa.
370 Despite the changes in the communities, no alterations in the development and yield of the plants were highlighted.
371 The genera and phyla in question are widespread throughout agricultural soils and play a fundamental role in
372 promoting good crop yields, including effects on organic matter turnover, compound degradation and biocontrol
373 (Fierer et al. 2007; Hashmi et al. 2020; Mhete et al. 2020; al., 2020; Spagna et al. 2009; Wieczorek et al. 2019).
374 A final important aspect is the non-detection of PGPMs in these communities. None of the PGPM taxa used were
375 identified in the inoculated soils. Despite their positive effects on plants and fruits, it can be hypothesized that these
376 organisms, once inoculated, performed their function but could not compete with the pre-existing microbial
377 community, leading to their disappearance at the end of the experiment. This aspect is crucial in such treatments, as it
378 underlines the temporary nature of PGPMs inoculations. It is essential to consider the costs and benefits of treatment,
379 as it will not permanently alter soil communities and will need to be periodically repeated.

380 **5. Conclusions**

381 Our results have shown how biofertilizers based on *Azospirillum brasilense (A. brasilense)* and effective
382 microorganisms, or deriving from anaerobic digestion of manures, can represent a sustainable approach to improve
383 the growth and yield of strawberry plants. In general, all treatments showed an enhanced growth and yield of
384 strawberry plants, improving or maintaining fruit nutraceutical values such as macro- and micro-nutrients, while the
385 phenolic compounds concentration remained mostly unchanged. Combinations between plant-growth promoting
386 microorganism (PGPMs) and liquid digestate were the best performing, presumably due to a higher amount of
387 nutrients directly available to plants and soil organisms. Regarding the rhizospheric microbial community, its structure
388 changed following the use of the fertilizer and the supply of nutrients in different quantities and forms, while an effect
389 following the inoculation of *A. brasilense* or effective microorganisms was highlighted only for the fungal community.
390 However, these alterations did not drastically affect the microbial community, which was only slightly altered by the
391 treatments, and maintained stable phyla of fundamental importance for plant development, such as Proteobacteria,
392 Firmicutes and Bacteroidota, and Basidiomycota. Therefore, we can conclude that both PGPMs and digestates can
393 represent a sustainable alternative for the fertilization of horticultural crops, given that no negative effects on microbial
394 community or plants have been highlighted and that their combination can lead to a further improvement of growth
395 and yields of strawberry plants. However, no trace of the presence of PGPMs was found. This emphasizes the need to
396 carefully weigh the costs and benefits of employing PGPMs, as their application does not lead to permanent changes
397 in the microbial community and necessitates repeated treatments.

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Author contributions

Conceptualization: Tanja Mimmo, Stefano Cesco; *Methodology*: Hannes Graf, Fabio Valentinuzzi, Luigimaria Borruso; *Formal analysis and investigation*: Ilaria Fracasso, Fabio Valentinuzzi, Hannes Graf, Luigimaria Borruso, Tanja Mimmo, Youry Pii, Luciano Cavani, Alex Dumbrell, Alessia Bani, Stefano Cesco; *Writing - original draft preparation*: Ilaria Fracasso, Fabio Valentinuzzi, Youry Pii, Tanja Mimmo, Luigimaria Borruso; *Writing - review and editing*: Luigimaria Borruso, Stefano Cesco, Alessia Bani, Alex Dumbrell; *Supervision*: Tanja Mimmo, Luigimaria Borruso.

Data availability

Sequencing data supporting the findings of this study have been submitted to the National Center for Biotechnology Information (NCBI) under accessions number PRJNA1108168.

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Declarations

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

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Table 1 Shoot biomass, SPAD index, number of flowers per plant, number of fruits per plant and yield per plant of strawberries harvested from plants grown in soils either without treatment (Control), either inoculated with *Azospirillum brasilense* (AZO), either inoculated with effective microorganisms (EM), either amended with pellet (300 mg N kg^{-1} soil) (Pellet), either amended with liquid digestate (75 mg N kg^{-1} soil) (LD), either combining AZO and pellet (AZO+Pellet), either combining AZO and LD (AZO+LD), either combining EM and pellet (EM+Pellet) or combining EM and LD (EM+LD). Data are reported as means and SE (n=5). The statistical significance was evaluated by means of ANOVA with Tukey post hoc-test. Different letters indicate statistically different values ($p<0.05$)

	Control	**AZO**	**EM**	**Pellet**	**LD**	**AZO+Pellet**	**AZO+LD**	**EM+Pellet**	**EM+LD**
Shoot biomass	21.53±1.67^{d}	27.56±2.65^{bc}	29.40±2.51^{bc}	23.32±3.76^{d}	24.71±2.64^{cd}	28.26±1.62^{bc}	35.67±1.24^{a}	25.91±2.55^{c}	31.48±2.05^{b}
SPAD index	39.33±2.73	39.08±0.61	38.25±1.35	38.15±0.83	37.58±0.92	38.23±2.64	39.30±2.31	37.93±1.51	37.48±2.76^{ns}
N° flowers plant^{-1}	16.83±4.07^{c}	18.50±4.72^{c}	20.33±6.77^{bc}	24.60±5.02^{bc}	28.40±3.57^{a}	30.25±4.13^{a}	26.33±5.12^{ab}	18.00±3.11^{bc}	31.17±4.07^{a}
N° fruits plant^{-1}	11.33±1.44^{c}	10.40±1.95^{c}	12.70±2.24^{bc}	12.60±1.95^{bc}	14.75±5.91^{bc}	15.00±1.16^{ab}	16.14±2.82^{ab}	12.80±1.09^{c}	18.00±1.30^{a}
Yield plant^{-1} (g)	24.65±5.25^{e}	37.92±6.36^{cd}	30.27±4.05^{de}	38.65±5.82^{cd}	51.95±4.66^{b}	55.74±6.07^{b}	67.23±8.71^{a}	44.77±8.71^{c}	62.06±5.07^{ab}

701 **Table 2** Macro- and micronutrients of freeze-dried strawberry fruits harvested from plants grown in soils either without treatment (Control), either inoculated
702 with *Azospirillum brasilense* (AZO), either inoculated with effective microorganisms (EM), either amended with pellet (300 mg N kg^{-1} soil) (Pellet), either
703 amended with liquid digestate (75 mg N kg^{-1} soil) (LD), either combining AZO and pellet (AZO+Pellet), either combining AZO and LD (AZO+LD), either
704 combining EM and pellet (EM+Pellet) or combining EM and LD (EM+LD). Data are reported as means and SE (n=5). The statistical significance was
705 evaluated by means of ANOVA with Tukey post hoc-test. Different letters indicate statistically different values ($p<0.05$)

	Control	**AZO**	**EM**	**Pellet**	**LD**	**AZO+Pellet**	**AZO+LD**	**EM+Pellet**	**EM+LD**
C (%)	40.45±1.66	39.02±0.26	38.89±0.62	40.57±0.59	40.40±0.97	39.86±0.38	39.93±0.36	39.97±0.81	40.93±0.77^{ns}
N (%)	1.77±0.25^{a}	0.77±0.11^{bc}	0.70±0.09^{c}	1.51±0.29^{ab}	1.47±0.46^{ab}	1.11±0.16^{b}	1.03±0.30^{bc}	1.43±0.57^{ab}	0.91±0.21^{bc}
P (mg g^{-1})	4.84±0.24^{a}	2.28±0.24^{c}	2.66±0.23^{bc}	4.59±0.31^{a}	5.19±0.42^{a}	3.22±0.28^{b}	3.71±0.37^{b}	3.06±0.44^{b}	2.61±0.24^{bc}
K (mg g^{-1})	8.96±0.16^{ab}	8.56±0.36^{b}	8.82±0.45^{b}	8.94±0.33^{ab}	8.37±0.66^{b}	8.70±0.54^{b}	9.84±0.40^{a}	8.44±0.50^{b}	8.48±0.09^{b}
Mg (mg g^{-1})	2.60±0.05^{b}	1.91±0.20^{d}	2.27±0.11^{c}	3.03±0.15^{a}	2.75±0.15^{ab}	2.09±0.12^{cd}	2.50±0.13^{bc}	2.41±0.16^{bc}	1.86±0.10^{cd}
Ca (mg g^{-1})	7.10±0.26^{b}	5.54±0.39^{c}	6.39±0.53^{bc}	7.25±0.38^{b}	7.23±0.38^{b}	7.00±0.23^{b}	8.49±0.62^{a}	6.65±0.64^{b}	6.36±0.25^{bc}
S (mg g^{-1})	1.85±0.14^{d}	3.22±0.15^{cd}	5.82±0.23^{c}	9.83±0.90^{b}	9.12±0.13^{b}	4.75±0.22^{c}	18.26±1.83^{a}	1.01±0.19^{d}	0.71±0.08^{d}
Fe (µg g^{-1})	42.50±1.71^{b}	28.79±0.51^{d}	35.88±0.85^{c}	47.82±1.53^{a}	46.08±0.69^{ab}	42.45±2.12^{b}	39.90±2.10^{bc}	50.30±0.75^{a}	37.12±3.66^{c}
Cu (µg g^{-1})	7.62±0.49^{b}	5.35±0.49^{c}	5.10±0.07^{c}	8.54±0.12^{ab}	8.68±0.53^{a}	5.97±0.35^{c}	6.20±0.12^{c}	7.18±0.61^{b}	5.05±0.39^{c}
Zn (µg g^{-1})	15.35±1.94^{c}	15.60±1.78^{c}	15.70±1.99^{c}	23.18±1.90^{b}	22.06±1.10^{b}	18.18±1.16^{bc}	29.43±2.09^{a}	22.77±1.22^{b}	20.78±1.47^{b}
Mn (µg g^{-1})	30.08±1.43^{b}	21.97±2.35^{c}	23.32±1.75^{c}	39.97±1.59^{a}	27.22±0.27^{b}	22.99±1.18^{c}	23.53±1.13^{c}	29.59±1.22^{b}	19.85±1.18^{cd}

706

707 **Table 3** Extractable concentration of metals in soils collected after 78 days of cultivation of strawberry fruits either without treatment (Control), either
708 inoculated with *Azospirillum brasilense* (AZO), either inoculated with effective microorganisms (EM), either amended with pellet (300 mg N kg^{-1} soil)
709 (Pellet), either amended with liquid digestate (75 mg N kg^{-1} soil) (LD), either combining AZO and pellet (AZO+Pellet), either combining AZO and LD
710 (AZO+LD), either combining EM and pellet (EM+Pellet) or combining EM and LD (EM+LD). Data are reported as means and SE (n=5). The statistical
711 significance was evaluated by means of ANOVA with Tukey post hoc-test. Different letters indicate statistically different values ($p<0.05$)

	Control	**AZO**	**EM**	**Pellet**	**LD**	**AZO+Pellet**	**AZO+LD**	**EM+Pellet**	**EM+LD**
Cu (mg g^{-1})	2.99 ± 0.18^{a}	2.57 ± 0.24^{ab}	2.57 ± 0.23^{ab}	2.65 ± 0.34^{ab}	2.52 ± 0.21^{ab}	2.47 ± 0.11^{b}	2.46 ± 0.23^{b}	2.64 ± 0.23^{ab}	2.54 ± 0.19^{ab}
Fe (mg g^{-1})	84.61±4.25	83.36±7.13	88.08±10.29	86.45±26.98	76.01±8.19	71.42±4.50	74.48±11.08	75.87±7.89	79.51 ± 10.69^{ns}
Mn (mg g^{-1})	16.64 ± 1.15^{ab}	19.99 ± 2.74^{a}	19.99 ± 2.75^{a}	15.76 ± 2.31^{b}	15.51 ± 1.78^{b}	15.58 ± 0.40^{b}	12.81 ± 1.20^{b}	17.27 ± 1.54^{ab}	16.79 ± 1.42^{ab}
Zn (mg g^{-1})	7.78±0.53	7.15±0.81	6.84±0.74	7.31±0.34	6.94±0.63	8.04±0.68	7.02±0.68	7.80±0.66	7.33 ± 0.62^{ns}

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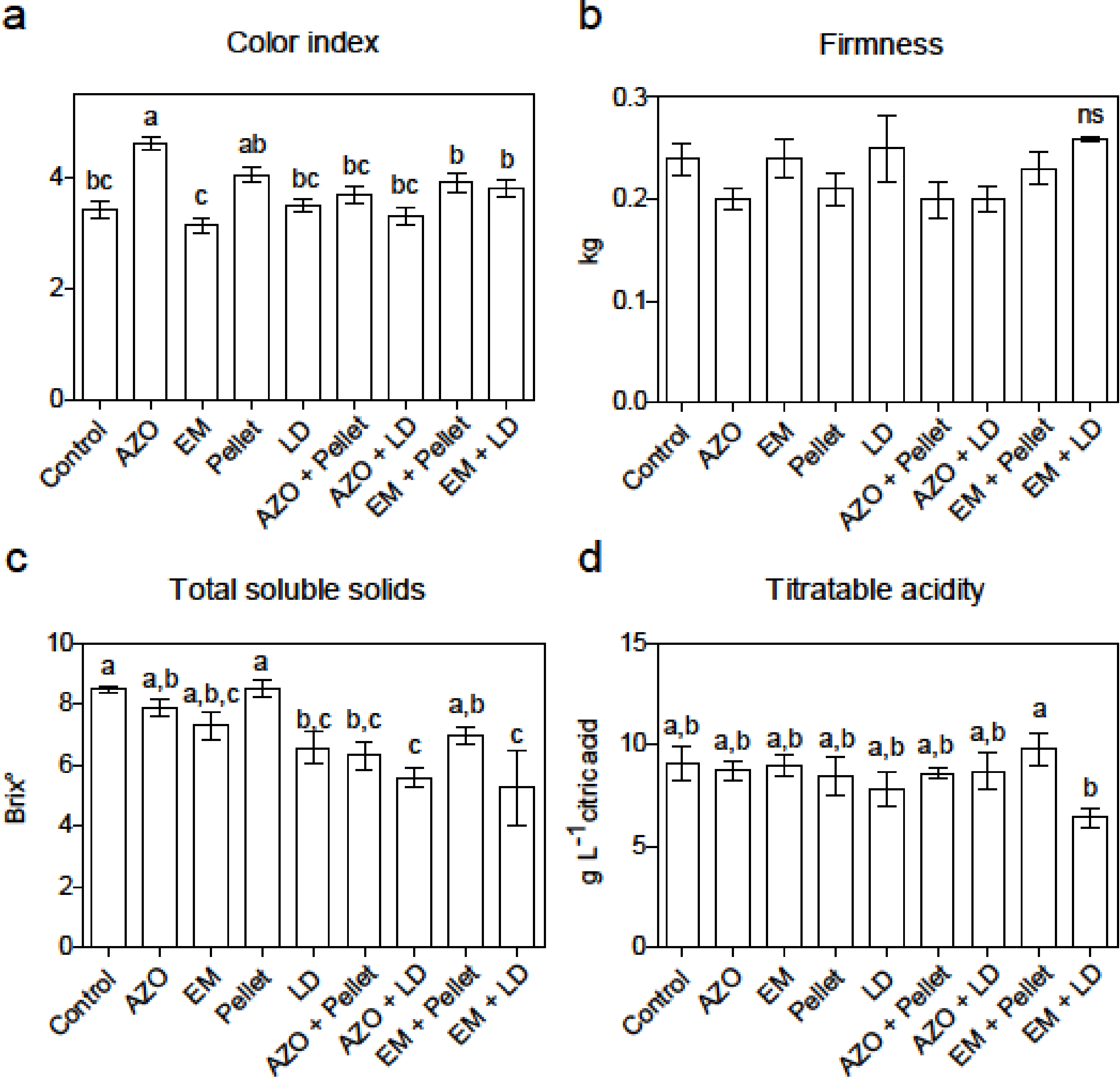

713

714 **Fig. 1** Color index (A), firmness (B), total soluble solids (C) and titratable acidity (D) of strawberry fruits
715 harvested from plants grown in soils either without treatment (Control), either inoculated with *Azospirillum*
716 *brasilense* (AZO), either inoculated with effective microorganisms (EM), either amended with pellet (300 mg
717 N kg-1 soil) (Pellet), either amended with liquid digestate (75 mg N kg-1 soil) (LD), either combining AZO and
718 pellet (AZO+Pellet), either combining AZO and LD (AZO+LD), either combining EM and pellet (EM+Pellet)
719 or combining EM and LD (EM+LD). Data are reported as means and SE (n=5). The statistical significance was
720 tested by means of ANOVA with Tukey post-test. Different letters indicate statistically different values ($p<0.05$)

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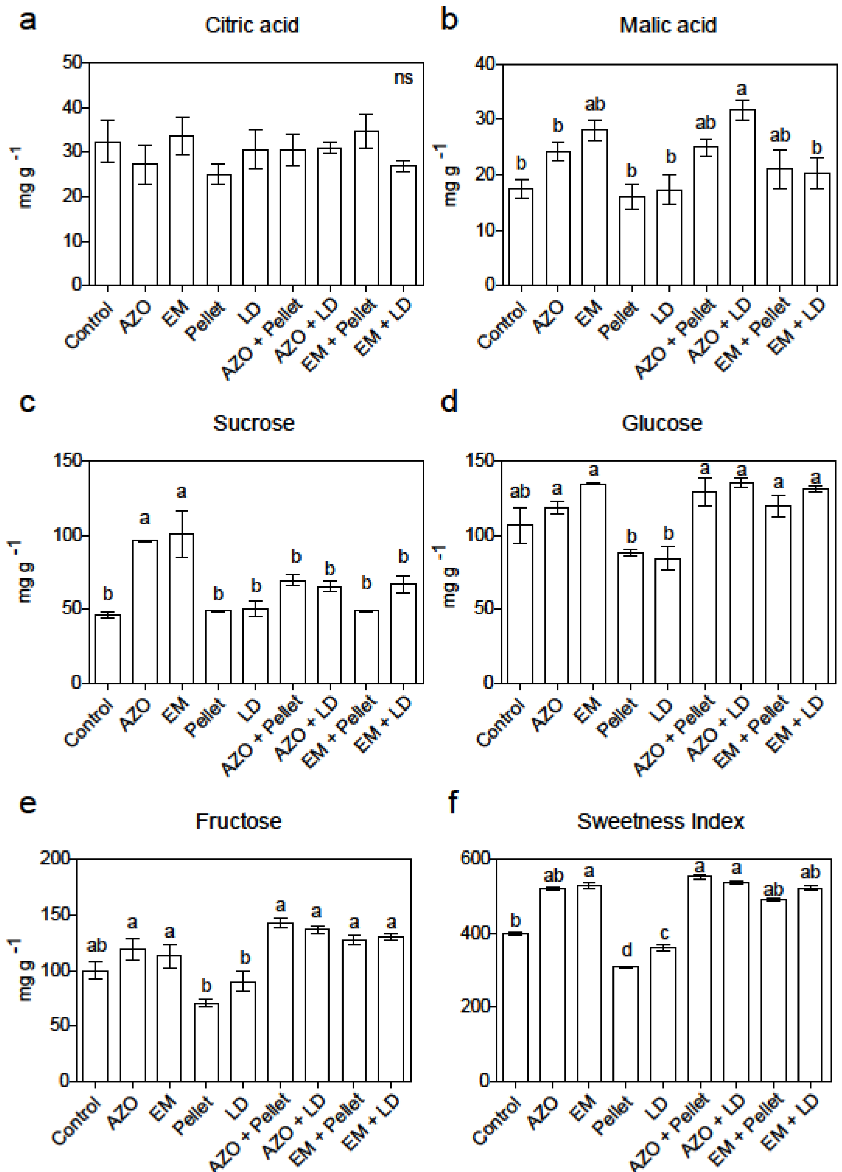
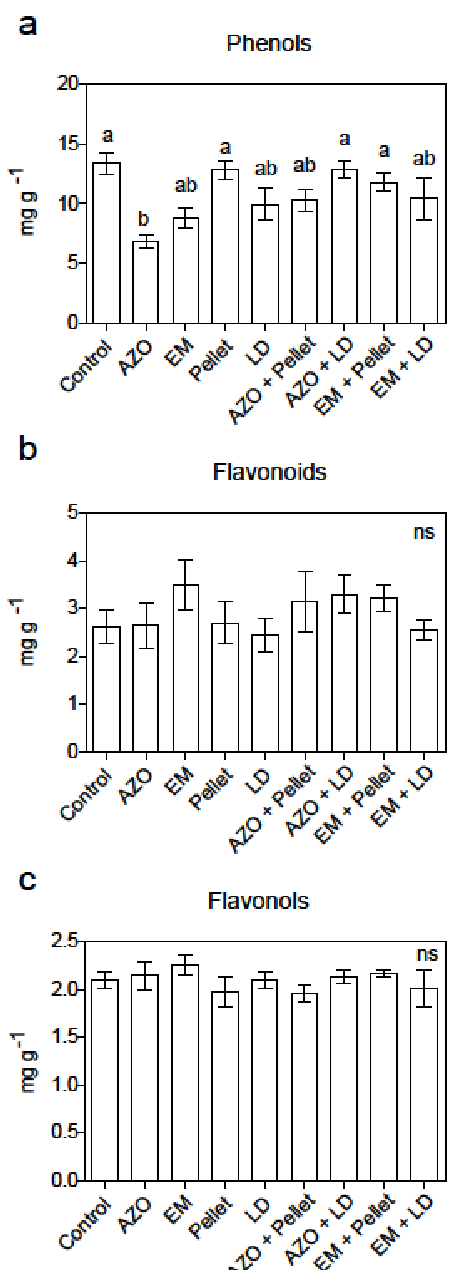
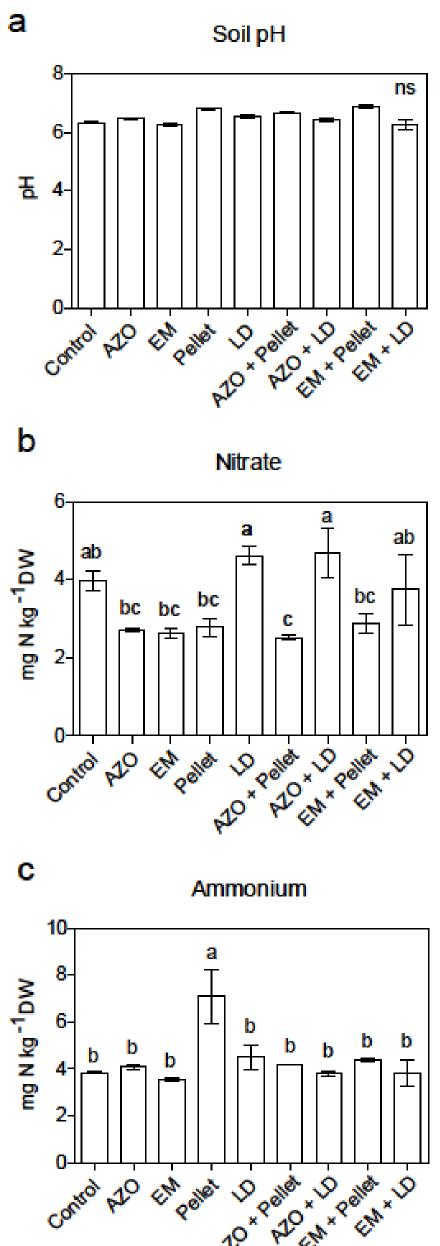


722 **Fig. 2** Citric acid (A), malic acid (B), sucrose (C), glucose (D), fructose (E) and sweetness index (F) of
723 strawberry fruits harvested from plants grown in soils either without treatment (Control), either inoculated with
724 *Azospirillum brasilense* (AZO), either inoculated with effective microorganisms (EM), either amended with
725 pellet (300 mg N kg-1 soil) (Pellet), either amended with liquid digestate (75 mg N kg-1 soil) (LD), either
726 combining AZO and pellet (AZO+Pellet), either combining AZO and LD (AZO+LD), either combining EM
727 and pellet (EM+Pellet) or combining EM and LD (EM+LD). Data are reported as means and SE (n=5). The
728 statistical significance was tested by means of ANOVA with Tukey post-test. Different letters indicate
729 statistically different values ($p<0.05$)



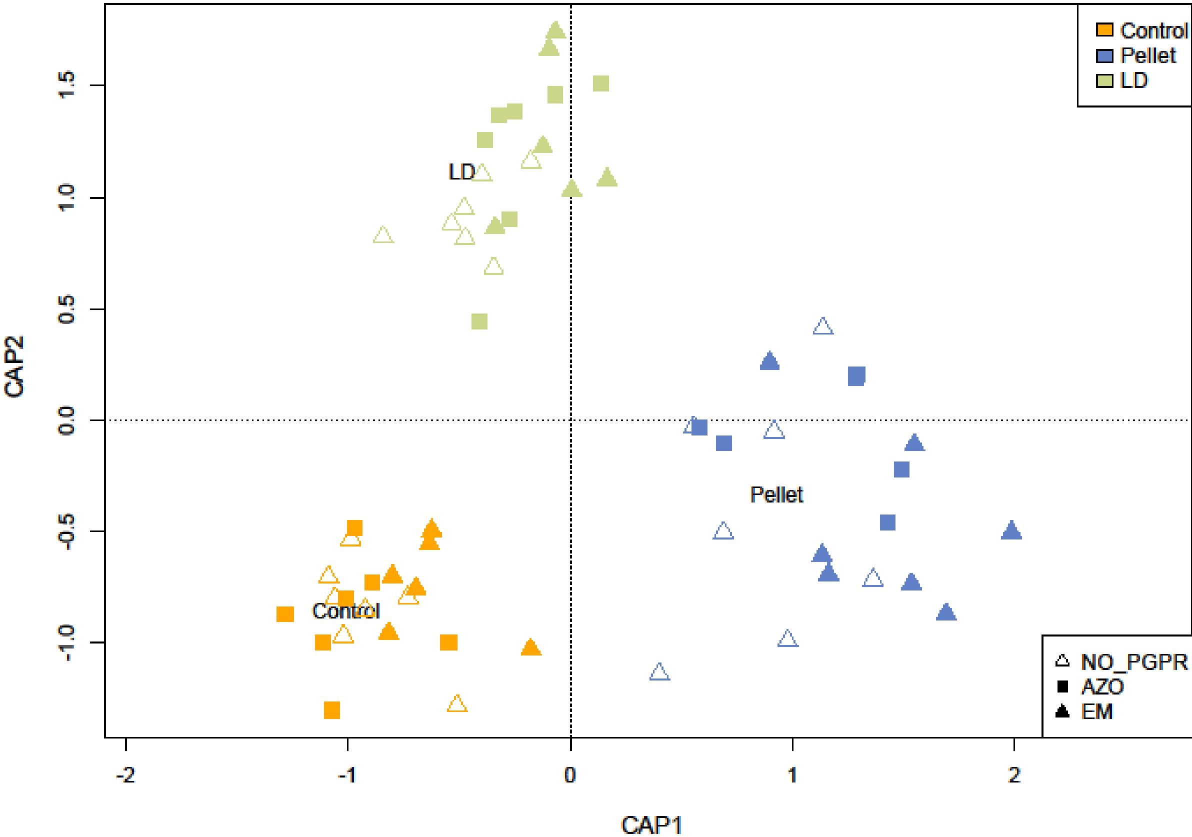
730

731 **Fig. 3** Total phenols (A), total flavonoids (B) and total flavonols (C) content of strawberry fruits harvested from
732 plants grown in soils either without treatment (Control), either inoculated with *Azospirillum brasilense* (AZO),
733 either inoculated with effective microorganisms (EM), either amended with pellet (300 mg N kg-1 soil) (Pellet),
734 either amended with liquid digestate (75 mg N kg-1 soil) (LD), either combining AZO and pellet (AZO+Pellet),
735 either combining AZO and LD (AZO+LD), either combining EM and pellet (EM+Pellet) or combining EM and
736 LD (EM+LD). Data are reported as means and SE (n=5). The statistical significance was tested by means of
737 ANOVA with Tukey post-test. Different letters indicate statistically different values ($p<0.05$)



738

739 **Fig. 4** pH (A), nitrate concentration (B) and ammonium concentration (C) of soils collected after 78 days of
740 cultivation of strawberry fruits either without treatment (Control), either inoculated with *Azospirillum brasilense*
741 (AZO), either inoculated with effective microorganisms (EM), either amended with pellet (300 mg N kg-1 soil)
742 (Pellet), either amended with liquid digestate (75 mg N kg-1 soil) (LD), either combining AZO and pellet
743 (AZO+Pellet), either combining AZO and LD (AZO+LD), either combining EM and pellet (EM+Pellet) or
744 combining EM and LD (EM+LD). Data are reported as means and SE (n=5). The statistical significance was
745 tested by means of ANOVA with Tukey post-test. Different letters indicate statistically different values ($p<0.05$)



746

747 **Fig. 5** Cap-scale analysis of soil bacterial community computed by fitting soil composition with microbial
748 operational taxonomic unit (OTU) tables. Colors represent untreated or amended samples, while shapes indicate
749 whether plant growth-promoting microorganisms (PGPMs) were inoculated. Control = control plants; Pellet =
750 plants amended with pellet; LD = plants amended with liquid digestate; NO_PGPR = not inoculated plants;
751 AZO = plants inoculated with *Azospirillum brasilense*; EM = plants inoculated with effective microorganisms

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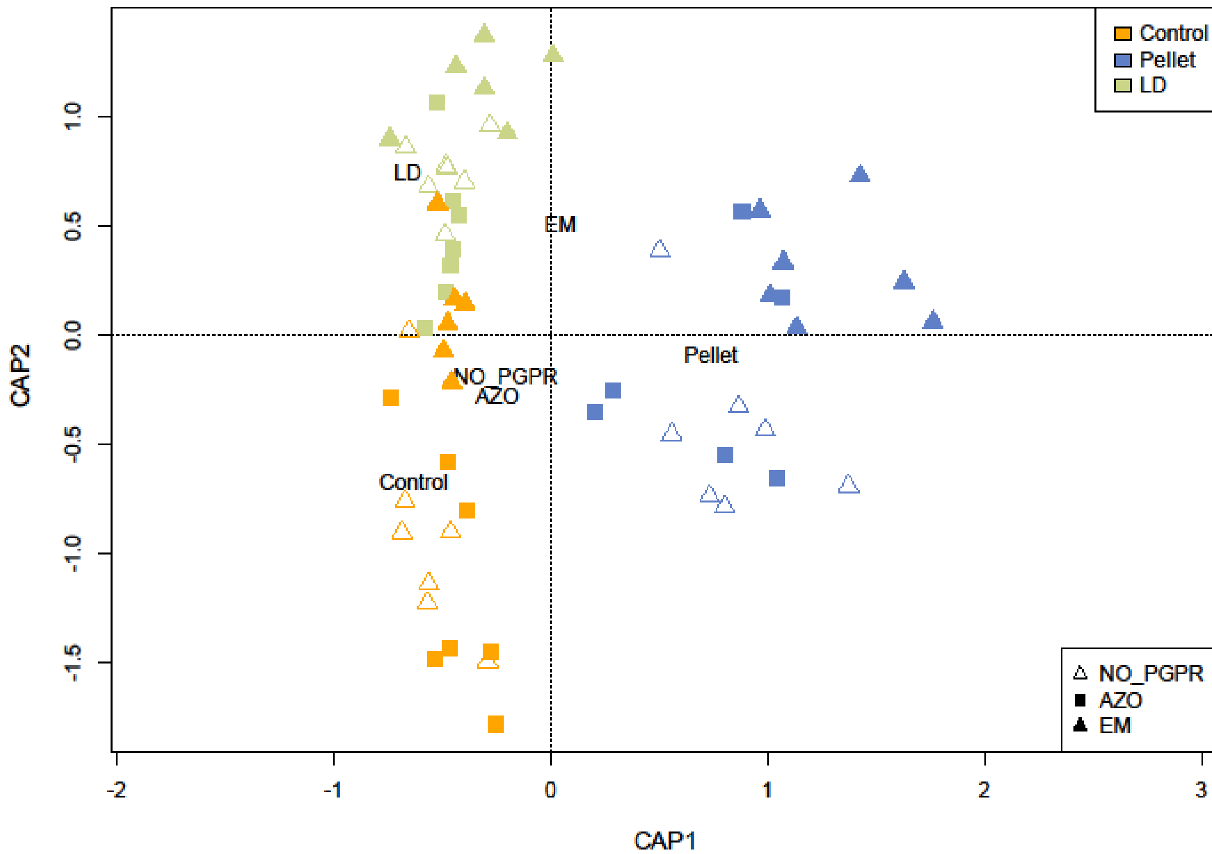


758

759 **Fig. 6** Cap-scale analysis of soil fungal community computed by fitting soil composition with fungal operational

760 taxonomic unit (OTU) tables. Colors represent untreated or amended samples, while shapes indicate whether

761 plant growth-promoting microorganisms (PGPMs) were inoculated. Control = control plants; Pellet = plants

762 amended with pellet; LD = plants amended with liquid digestate; NO_PGPR = not inoculated plants; AZO =

763 plants inoculated with *Azospirillum brasilense*; EM = plants inoculated with effective microorganisms

764

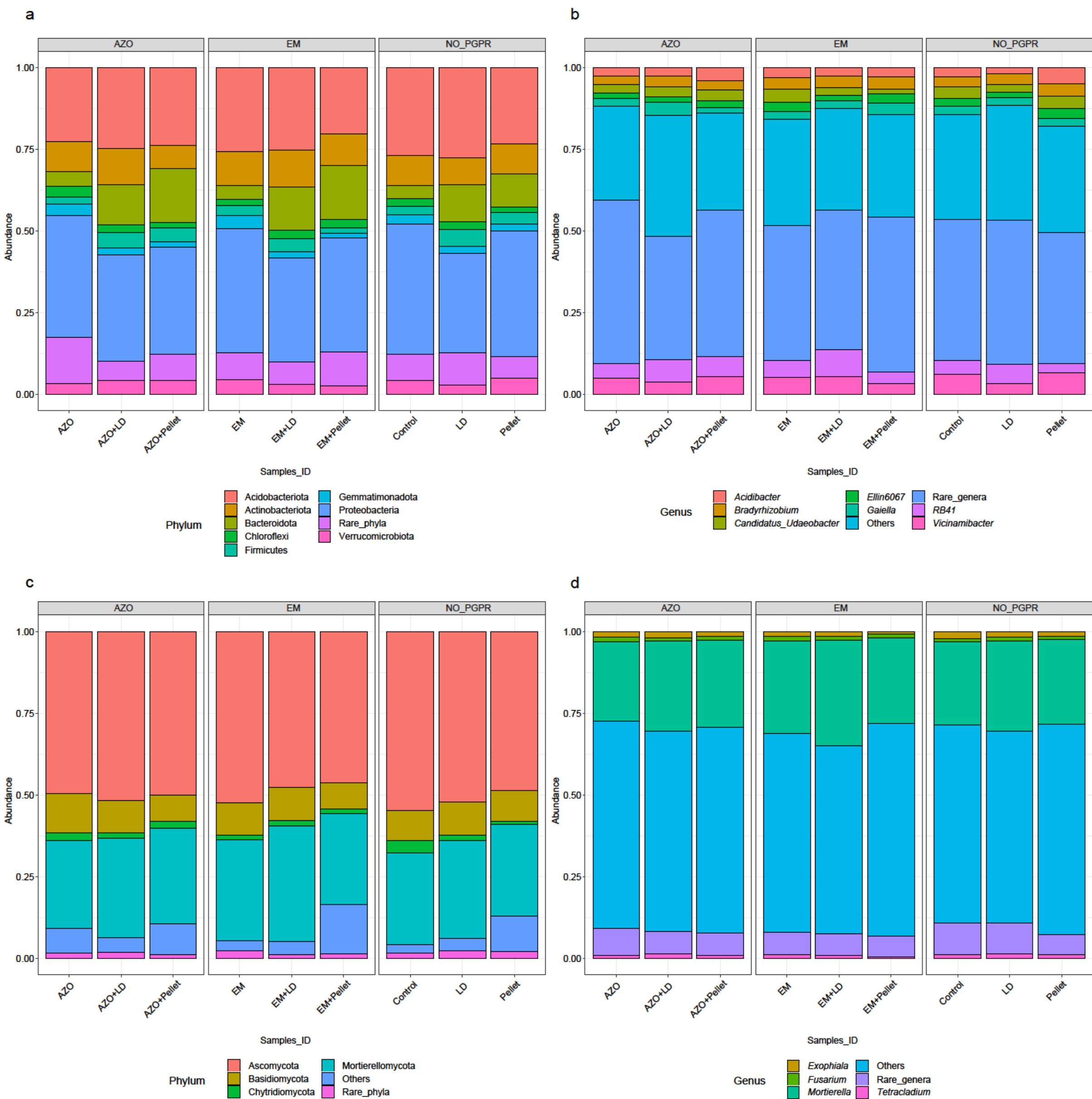
765

766

767

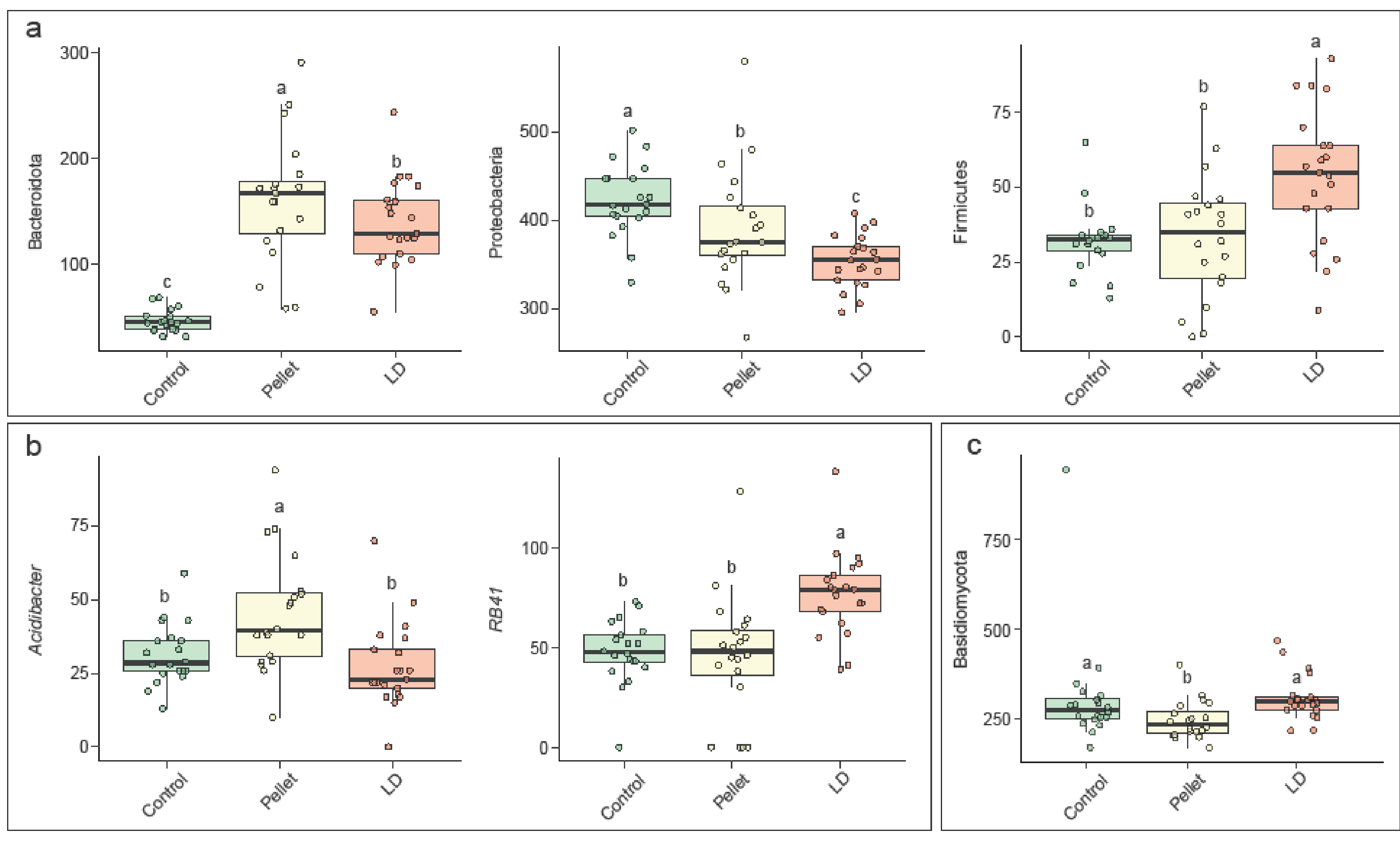
768

769



770

771 **Fig. 7 a.** Bacterial taxonomy at the phylum level. **b.** Bacterial taxonomy at the genus level. **c.** Fungal taxonomy
772 at the phylum level. **d.** Fungal taxonomy at the genus level. NO_PGPR = not inoculated plants; AZO = plants
773 inoculated with *Azospirillum brasilense*; AZO+LD = plants amended with liquid digestate and inoculated with
774 *A. brasilense*; AZO+Pellet = plants amended with pellet and inoculated with *A. brasilense*; EM = plants
775 inoculated with effective microorganisms; EM+LD = plants amended with liquid digestate and inoculated with
776 effective microorganisms; EM+Pellet = plants amended with pellet and inoculated with effective
777 microorganisms; Control = control plants; LD = plants amended with liquid digestate; Pellet = plants amended
778 with pellet



779

780 **Fig. 8** Significant Multivariable Association between amendments and taxa. Only taxa with significant differences were reported; letters indicate statistical

781 significance ($p<0.01$) obtained through Maaslin2 test. **a.** Bacterial taxa at the phylum level. **b.** Bacterial taxa at the genus level. **c.** Fungal taxon at the phylum

782 level. Control = control plants; Pellet = plants amended with pellet; LD = plants amended with liquid digestate

Figure 1

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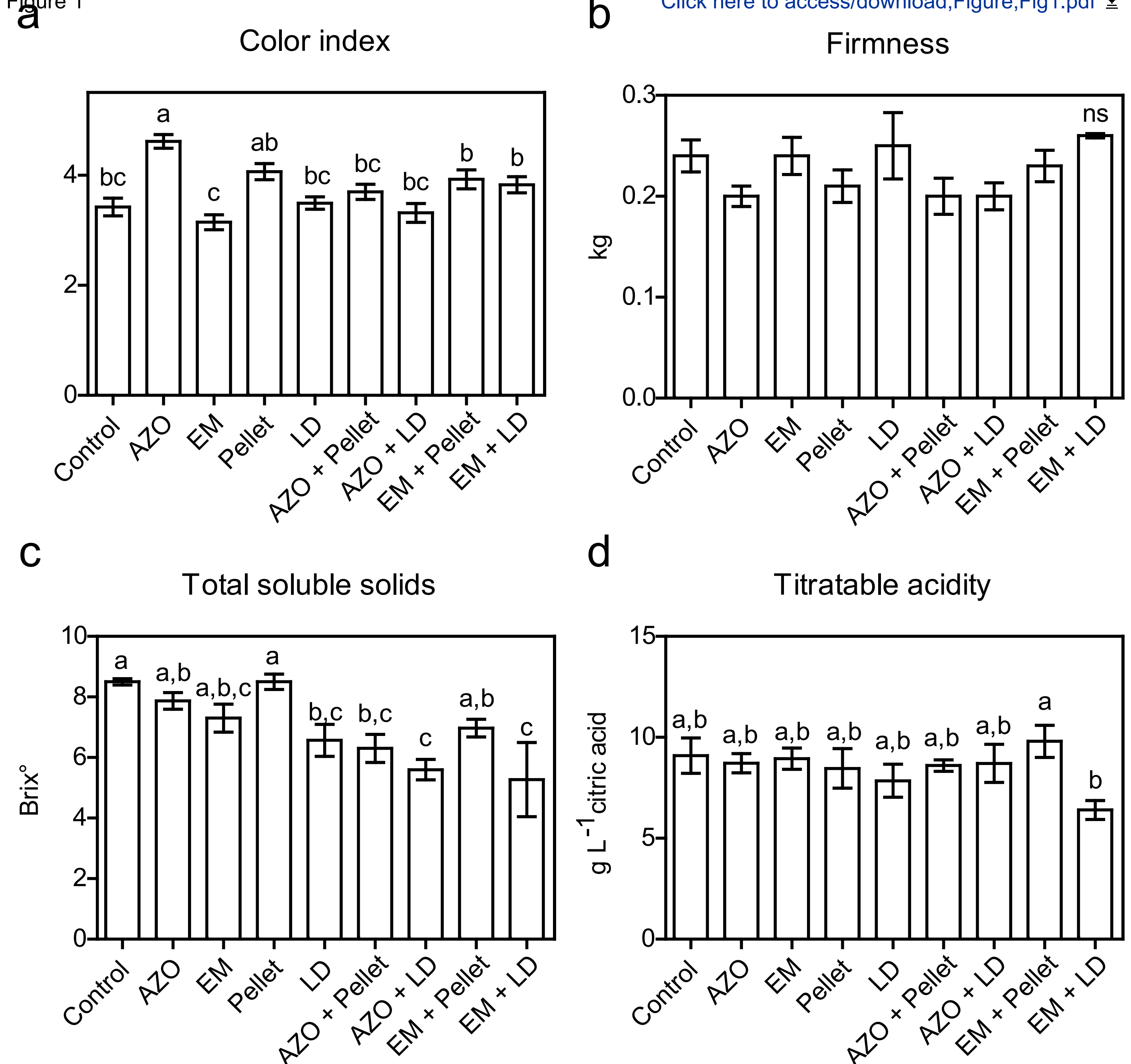

Figure 2

Click here to access/download;Figure;Fig2.pdf

a

Citric acid

mg g -1

0 10 20 30 40 50

ns

Control AZO EM Pellet LD AZO + Pellet AZO + LD EM + Pellet EM + LD

b

Malic acid

mg g -1

0 10 20 30 40

b b ab b b ab a ab b

Control AZO EM Pellet LD AZO + Pellet AZO + LD EM + Pellet EM + LD

c

Sucrose

mg g -1

0 50 100 150

b a a b b b b b b

Control AZO EM Pellet LD AZO + Pellet AZO + LD EM + Pellet EM + LD

d

Glucose

mg g -1

0 50 100 150

ab a a b b a a a a

Control AZO EM Pellet LD AZO + Pellet AZO + LD EM + Pellet EM + LD

e

Fructose

mg g -1

0 50 100 150 200

ab a a b b a a a a

Control AZO EM Pellet LD AZO + Pellet AZO + LD EM + Pellet EM + LD

f

Sweetness Index

0 200 400 600

b ab a d c a a ab ab

Control AZO EM Pellet LD AZO + Pellet AZO + LD EM + Pellet EM + LD

Figure 3

Click here to access/download;Figure;Fi

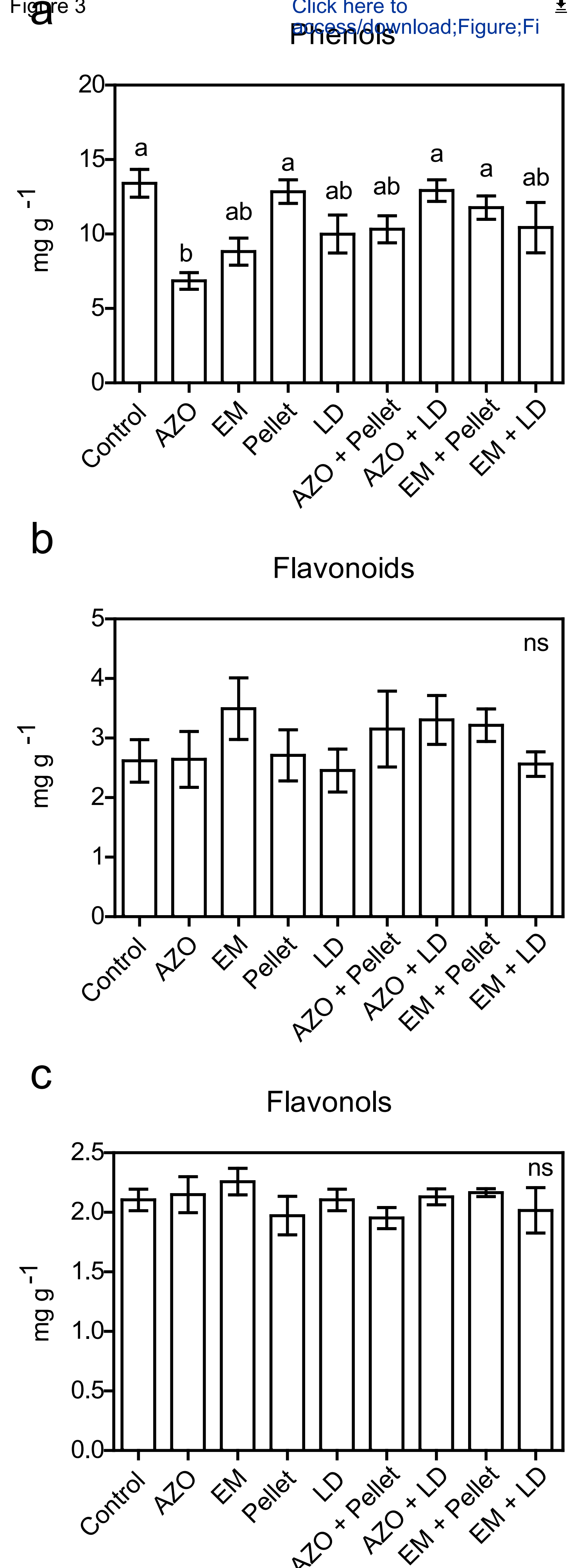

Figure 4 Click here to access/download;Figure;

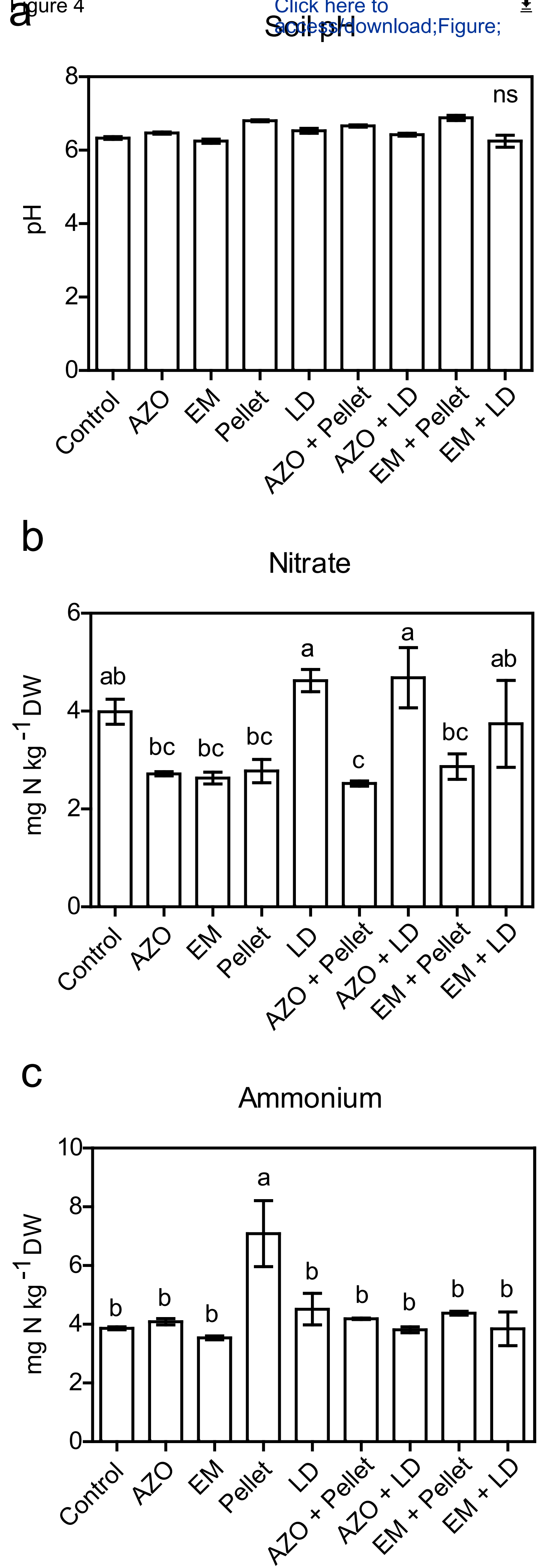

Figure 5

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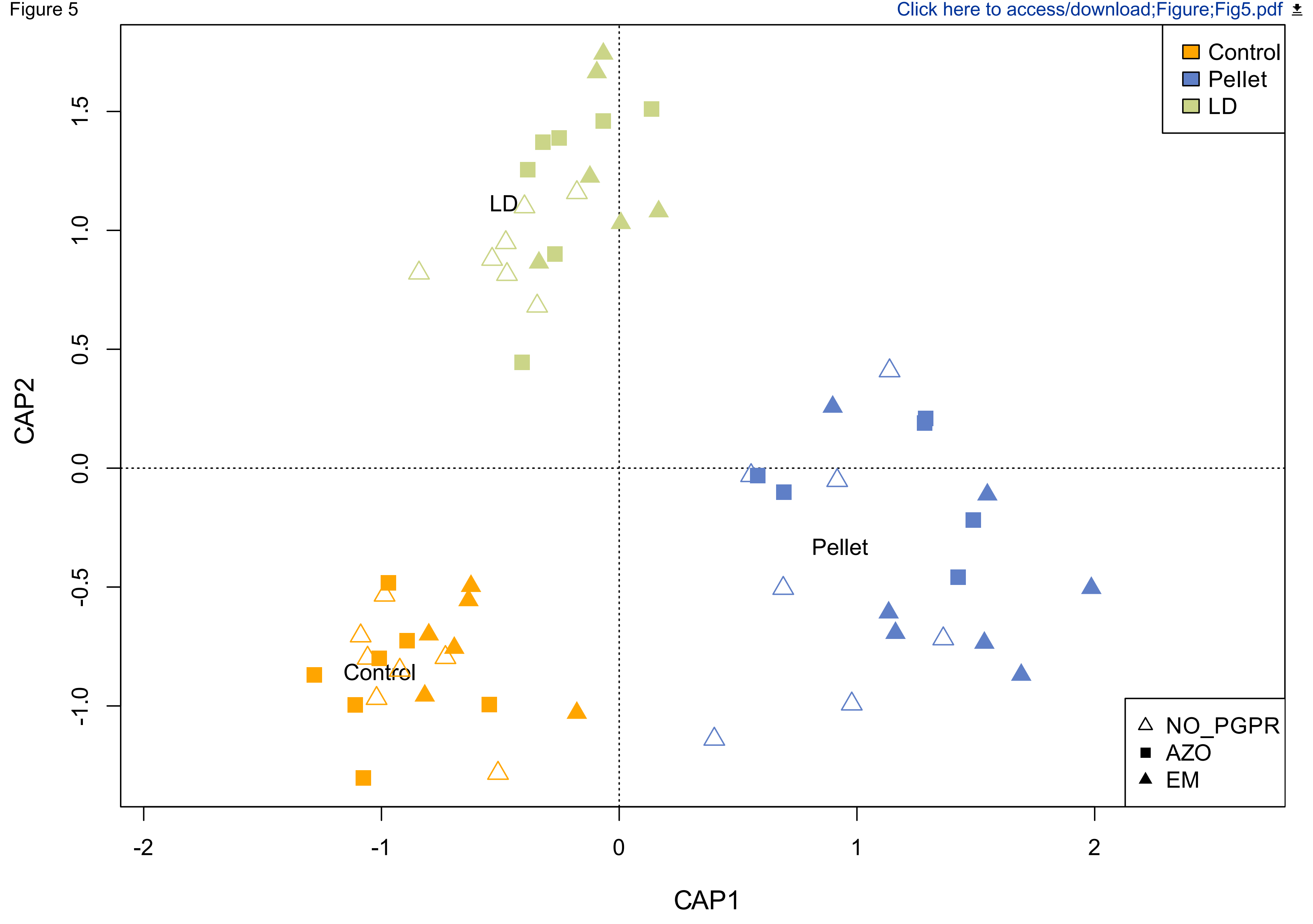

Figure 6

Click here to access/download;Figure;Fig6.pdf

Control
Pellet
LD

LD
EM
Pellet
NO_PGPR
AZO
Control

NO_PGPR
AZO
EM

CAP2
1.0
0.5
0.0
-0.5
-1.0
-1.5

-2
-1
0
1
2
3
CAP1

Figure 7

Click here to access/download;Figure;Fig7.pdf

a

AZO | EM | NO_PGPR

Abundance: 0.00, 0.25, 0.50, 0.75, 1.00

AZO, AZO+LD, AZO+Pellet, EM, EM+LD, EM+Pellet, Control, LD, Pellet

Samples_ID

Phylum: Acidobacteriota, Actinobacteriota, Bacteroidota, Chloroflexi, Firmicutes, Gemmatimonadota, Proteobacteria, Rare_phyla, Verrucomicrobiota

b

AZO | EM | NO_PGPR

Abundance: 0.00, 0.25, 0.50, 0.75, 1.00

AZO, AZO+LD, AZO+Pellet, EM, EM+LD, EM+Pellet, Control, LD, Pellet

Samples_ID

Genus: *Acidibacter*, *Bradyrhizobium*, *Candidatus_Udaeobacter*, *Ellin6067*, *Gaiella*, Others, Rare_genera, *RB41*, *Vicinamibacter*

c

AZO | EM | NO_PGPR

Abundance: 0.00, 0.25, 0.50, 0.75, 1.00

AZO, AZO+LD, AZO+Pellet, EM, EM+LD, EM+Pellet, Control, LD, Pellet

Samples_ID

Phylum: Ascomycota, Basidiomycota, Chytridiomycota, Mortierellomycota, Others, Rare_phyla

d

AZO | EM | NO_PGPR

Abundance: 0.00, 0.25, 0.50, 0.75, 1.00

AZO, AZO+LD, AZO+Pellet, EM, EM+LD, EM+Pellet, Control, LD, Pellet

Samples_ID

Genus: *Exophiala*, *Fusarium*, *Mortierella*, Others, Rare_genera, *Tetracladium*

Figure 8

Click here to access/download;Figure;Fig8.pdf

a

Bacteroidota
300
200
100
c
a
b
Control
Pellet
LD

Proteobacteria
500
400
300
a
b
c
Control
Pellet
LD

Firmicutes
75
50
25
0
b
b
a
Control
Pellet
LD

b

Acidibacter
75
50
25
0
b
a
b
Control
Pellet
LD

RB41
100
50
0
b
b
a
Control
Pellet
LD

c

Basidiomycota
750
500
250
a
b
a
Control
Pellet
LD

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Supplementary Material
Supplementary_materials.docx