

<sup>1</sup> **The effect of *Ascophyllum nodosum* extracts on tomato  
2 and pepper plant productivity and their associated  
3 fungal and bacterial communities.**

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<sup>7</sup> Root and soil microbial and fungal activity were examined in two species (pepper and tomato)  
<sup>8</sup> following treatment with *Ascophyllum nodosum* marine- plant extract. In addition, productivity  
<sup>9</sup> was measured.

<sup>10</sup> *Keywords:* Stella Marris, 16S, ITS, microbial diversity, Illumina MiSeq

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## 11 INTRODUCTION

12 Seaweeds (also known as marine algae) have been used as a source of organic matter and nutri-  
13 ents for centuries, especially in coastal areas (Khan et al., 2009; Craigie, 2011). Originally, farmers  
14 would simply collect and dry algae before directly applying them to the field to decompose nat-  
15 urally prior to seeding in the spring. Liquid seaweed extracts, developed in the 1950s in order  
16 to concentrate plant growth-stimulating compounds, facilitate their usage in modern agricultural  
17 crop production systems Milton (1952). Today, most commercially available seaweed extracts are  
18 made using a high temperature and pressure alkaline extraction technique from brown algae such  
19 as *Ascophyllum nodosum*, *Ecklonia maxima* or *Laminaria spp.*

20

21 One of the main advantages of seaweed extracts is that they are biodegradable, non-toxic and  
22 come from a renewable resources, unlike modern chemical fertilizers Dhargalkar & Pereira (2005).  
23 As such, they are commonly used as a natural fertilizer and source of phytohormones in organic  
24 and sustainable agricultural systems Craigie (2011). However, the specific molecular mechanisms  
25 underlying their mode-of-action and their effect on plant growth and productivity are complex  
26 and not well understood. In fact, scientific research into their mode-of-action on plant growth  
27 and their effect on root associated microbial and fungal communities is still in its infancy (Khan et  
28 al., 2009; Alam et al., 2013a). In turn, this likely precludes a greater acceptance of liquid seaweed  
29 extracts as a viable and renewable alternative to limit the use of modern fertilizers and pesticides  
30 in agriculture.

31

32 Studies on liquid seaweed extracts have been conducted to better understand how seaweed ex-  
33 tracts might ultimately boost plant productivity (e.g.: Khan et al., 2009; Alam et al., 2013a,b; Wally  
34 et al., 2013; Arioli, Mattner & Winberg, 2015) and a large number of phytohormones which can  
35 influence physiological processes even at very low concentrations, have been identified. Aux-  
36 ins, cytokinins, abscissic acid or gibberellins are all phytohormones present in seaweed extracts  
37 and which are hypothesized to trigger disease response pathways and increase stress tolerance  
38 in plants (Stirk & Van Staden, 2014; Arioli, Mattner & Winberg, 2015). Yet, Wally et al. (2013)  
39 concluded that phytohormone levels present within the extracts themselves were insufficient to

40 cause significant effects in plants when applied at recommended rates. Instead, bioactive compo-  
41 nents within seaweed extracts themselves such as vitamins, oligosaccharides, and micronutrients  
42 may directly modulate the plant endogenous biosynthesis pathways of phytohormones. Never-  
43 theless, the basis for these benefits is complex and still relatively poorly understood (Stirk & Van  
44 Staden, 2014; Arioli, Mattner & Winberg, 2015).

45

46 Concurrently, it is hypothesized that the application of seaweed extract may also alter micro-  
47 bial and fungal communities. This in turn, could explain the improved plant performance often  
48 observed when seaweed extracts are applied to agricultural soil (Santoyo, Orozco-Mosqueda &  
49 Govindappa, 2012; Alam et al., 2013a,b). For example, Alam et al. (2013a) demonstrated that  
50 the applicatio of *Ascophyllum* extracts increased root associated microbial activity while promot-  
51 ing plant growth and root yield in carrots. Similarly, Alam et al. (2013b) showed that seaweed  
52 extracts increased strawberry root and shoot growth, berry yield and rhizosphere microbial diver-  
53 sity. While promising, these studies come with two major caveats. First, corellation does not imply  
54 causation, and the molecular mechanisms behind the observed seaweed extract - bacterial diver-  
55 sity - plant productivity relationship was not identified precisely. Second, these studies required  
56 culturing bacteria *in vitro*, a labour intensive approach targetting a small (and biased) fraction of  
57 the total communities. Given the vast diversity of microbial populations, studies on the functional  
58 activity of complex soil microbial communities remain challenging.

59

60 The recent development of culture-independent molecular techniques should therefore permit to  
61 circumvent the inherent bias of culture based approaches by targeting the ubiquitous component  
62 of life, its DNA. In turn, this should permit to identify a larger proportion of the bacterial diversity  
63 and lead to a better understanding of the soil microbial response to seaweed extract. DNA bar-  
64 coding targeting the internal transcribed spacer (ITS) region of the nuclear ribosomal repeat and  
65 the bacterial V3-V4 region of the 16S ribosomal gene for fungi and bacteria, respectively, are now  
66 regarded as a prerequisite procedure to comprehensively understand the diversity and ecology of  
67 microbial organisms (Toju et al., 2012; Klindworth et al., 2013). Once this is performed, further  
68 studies for example using inoculum of microbial species linked to the presence of liquid seaweed  
69 extract may help to identify a causative link between extracts, microbes and productivity.

70

71 The aim of the current project was to quantify the impact of seaweed (*Ascophyllum nodosum*)  
72 extracts on plant growth. In addition, we tested how the bacterial and fungal communities re-  
73 sponded to the addition of theses extracts. We hypothesized that the inclusion of liquid seaweed  
74 extracts would improve productivity and alter significantly the bacterial and fungal communities.  
75 We used a a commercially available extract (Stella Maris®) derived from fresh *Ascophyllum no-*  
76 *dosum* algae harvested from the nutrient-laden waters of the North Atlantic off the Eastern Coast  
77 of Canada. We tested the effect of these extract on two commonly used plants (Tomato - *Solanum*  
78 *lycopersicum* and Pepper - *Capsicum annuum*) using different measures of productivity and by mea-  
79 suring soil and root bacterial anf fungal diversity using High Throughput Illumina Miseq sequenc-  
80 ing.

81

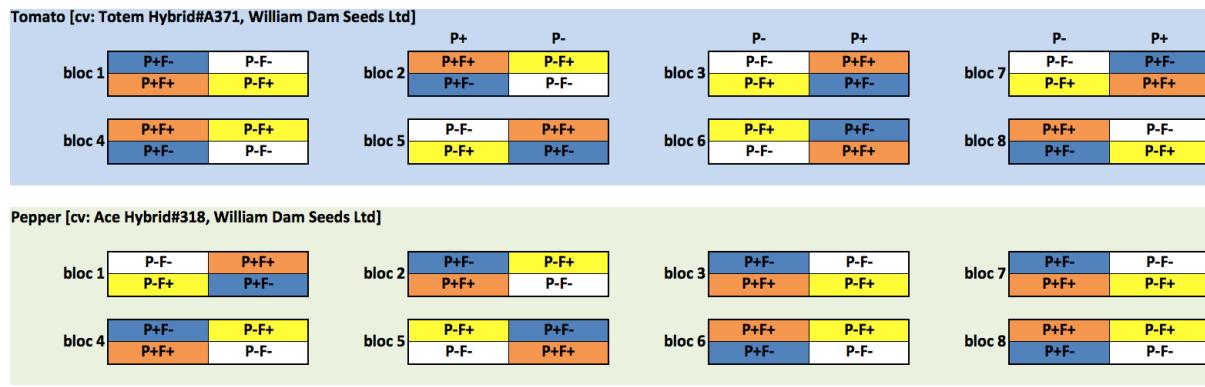
## 82 MATERIAL AND METHOD

### 83 Study design

84 Two greenhouse experiment were set up in large trays (60x30x18 cm) in November (tomato [cv:  
85 Totem Hybrid#A371, William Dam Seeds Ltd]) and December (Pepper [cv: Ace Hybrid#318,  
86 William Dam Seeds Ltd]) 2015. Soil was collected from an agricultural field under organic regime  
87 at the IRDA research station in St-Bruno (Qc, Canada) on October 7<sup>th</sup> 2015 (loamy sand soil, 15 cm  
88 top layer collected). Soil characteristics (pH, conductivity, nutrients, see Table 1) were measured  
89 by AgriDirect (Longueuil, Qc, Canada).

90

91 For each species tested (Tomato - *Solanum lycopersicum*, Pepper - *Capsicum annuum*), a randomized  
92 split block design (Figure 1) was used with four trays set up per block (eight blocks). Half of  
93 the trays were fertilized (fertilization treatment), as described below. Half of the trays were also  
94 planted with four replicate plants each, while the other trays were left bare. This allowed a direct  
95 comparison of the fungal and bacteria soil communities with respect to the fertilization and plant-  
96 ing treatment.



97

## 98 Figure 1: experimental design

99

100 Half of the tomato plants were fertilized using multipurpose organic fertilizer (pure hen manure, 18 g per tray repeated every 4 weeks, 5-3-2) from Acti-sol (Notre-Dame-du-Bon-Conseil, 101 Qc, Canada) in addition to Stella Maris® (3.5 ml per 1L, each tray received 250 ml, repeated every 102 2 weeks) for the duration of the experiment. The other half were unfertilized. Stella Maris® is a 103 registered trademark from Acadian Seaplants Ltd. (Darmouth, NS, Canada). It is primarily com- 104 posed of *Ascophyllum nodosum* seaweed and is advertised as a natural activator of the crops' own 105 growth and defense mechanisms to improve root growth and resist temperature, drought, and 106 salinity stress in order to maximize yield and crop qualities (Acadian Seaplants Ltd. 2018). Half 107 of the pepper plants were treated using solely Stella Maris (3.5 ml per 1L, each tray received 250 108 ml, repeated every 2 weeks) for the duration of the experiment. The other half were untreated. 109

110 Thrips were managed with *Neoseiulus cucumeris* (syn. *Amblyseius cucumeris*) (100 bags), Fungus 111 gnat and thrips were also controlled using predatory mite *Gaeolaelaps gillespiei* (1L). Plants were 112 treated once a week with Oidium Milstop to control the fungus.

113

114

### 115 Plant productivity

116 At the end of the experiment, plant productivity was assessed by measuring four different traits 117 (fruit number, average fruit weight, shoots fresh weight, roots fresh weight) on three plants chosen 118 randomly per tray (for each treatment [fertilization/control], species [tomato/pepper] and block 119 [eight blocks]) for a total of 96 samples. In addition, both shoots and roots were dried in a 70 120 degrees drying oven, and dry weights were measured after 48 hours. Together, these traits are

121 expected to represent well the plant overall productivity.

122

123

124 *Sample preparation, DNA extraction and High throughput sequencing*

125 We sampled both the microbial and fungal communities from soil and root samples. Soil DNA  
126 was extracted using XXX DNA isolation kit with YYY g of soil. Roots were first washed with  
127 sterile water and DNA was extracted using XXX DNA isolation kit with YYY g of root samples.  
128 Amplicon sequencing targeting 16S rRNA gene (bacteria) and ITS (fungi) was performed on both  
129 root and soil samples.

130

131 In order to target fungi specifically, we used fungal primers ITS3\_KYO2 (5'-ACACTGACGA CATG-  
132 GTTCT ACAGATGAAGAAC GYAGYRAA-3') and ITS4\_KYO3 (5'-TACGGT AGCAGAGACTT  
133 GGTCTCTBTTV CCKCTTCACTCG-3') to produce a final amplicon size of ~430bp. This primer  
134 pair should target the Internal transcribed spacer and inhibit the amplification of plant sequences  
135 and enable the selective amplification of fungal communities from soil, mycorrhizal and other en-  
136 vironmental samples Toju et al. (2012).

137

138 Bacterial primers 341F (5'-CCTACGGG NGGCWGCAG-3') and 805R (5'-GACTACC AGGGTATC  
139 TAATC-3') producing a final amplicon size of ~464b and targeting specifically the bacterial V3-V4  
140 region of the 16S ribosomal gene were chosen. This primer pair has been used extensively in high-  
141 throughput sequencing studies in a range of environments and was shown to be the least biased  
142 among 512 primer pairs evaluated in silico for bacterial amplification Klindworth et al. (2013).

143

144 DNA samples were then barcoded, pooled and sequenced (2X300bp, paired-end) using an Illu-  
145 mina MiSeq (San Diego, CA, USA) sequencer at the Genome Quebec Innovation Centre (Montreal,  
146 Canada). Sequences were demultiplexed by the sequencing facility (Genome Quebec Innovation  
147 Centre) and further processed as described below.

148

149

150 *Bioinformatics*

151 All bioinformatics, statistical, and graphical analyses further described were performed in R 3.5.1  
152 Team & others (2018) and detailed scripts are available here ([https://github.com/seb951/Acadian\\_SeaPlants](https://github.com/seb951/Acadian_SeaPlants)).  
153

154

155 We used the R package dada2 Callahan et al. (2016) to infer *Amplicon Sequence Variants* (ASVs).  
156 Dada2 offers accurate sample inference from amplicon data with single-nucleotide resolution in  
157 an open source environments. Unlike the Operational Taxonomic Unit (OTU) approach (e.g.  
158 Schloss et al. (2009), Caporaso et al. (2010)), ASV are not treated as cluster of sequences defined  
159 with an *ad hoc* sequence similarity threshold. Instead, after sequences are quality trimmed and  
160 error-corrected, dada2 reveals the unique members of the sequenced community, thus allowing  
161 sequences and abundance counts to be compared among studies Callahan et al. (2016).

162

163 First, sequences were trimmed following strict quality thresholds (removing primers and low  
164 quality nucleotides, see parameter details in the accompanying R scripts). Following this, we  
165 applied the error model algorithm of dada2 which incorporates quality information after filtering,  
166 unlike other OTU based methods. Then dereplication, sample inference, merging of paired end  
167 reads and removal of chimera reads were performed in order to obtain a sequence (ASVs) table  
168 of abundance per sample. Taxonomy was also assigned using the Ribosomal Database Project  
169 (RDP) Naive Bayesian Classifier algorithm from Wang et al. (2007). Depending on support (min-  
170 imum bootstrap support of 80), we assigned taxonomy from Kingdom to species. We used the  
171 silva database formatted for dada2 to infer bacterial taxa Callahan (2018). We used the Com-  
172 munity (2018) fasta release (including singletons) to infer fungal taxa after formatting it to the  
173 dada2 format using a custom R script. The pipeline was run on a multithreaded (48 CPUs) com-  
174 puter infrastructure provided by Westgrid (<https://www.westgrid.ca/support/systems/cedar>)  
175 and Compute Canada ([www.computecanada.ca](http://www.computecanada.ca)). Note that the pipeline was run separately for  
176 fungal-root, fungal-soil, bacteria-soil and bacteria-root samples given the markedly different nu-  
177 cleotide compositions of the sequenced amplicons, unique taxa and specific error models of each  
178 dataset.

179

180 *Statistical analyses - plant productivity*

181 We tested for the effect of species (tomato vs pepper), fertilization and their interaction on six plant  
182 productivity measures (fruit number, average fruit weight, shoots fresh weight, roots fresh weight,  
183 shoots dry weight, roots dry weight). We used linear mixed effect models (LMM) in the R package  
184 `nlme` Pinheiro et al. (2017), which are more appropriate than an Analysis of Variance (ANOVA)  
185 given the current block design (blocks and replicates nested within a block were treated as random  
186 variables). All six plant productivity measures were either square root or log transformed in or-  
187 der to help satisfy the assumption of normality of the residuals in the LMM statistical framework.  
188 For the variables *fruit number* and *average fruit weight*, we also used a permutation-based 2-way  
189 ANOVA (Anderson & Legendre (1999)) given that the residuals of the LMM were not normally  
190 distributed (results were similarly significant).

191

192

### 193 *Statistical analyses - microbial and fungal diversity*

194 We analysed separately fungal-root, fungal-soil, bacterial-root and bacterial-soil ASV diversity.  
195 For each of these four datasets, we removed samples that showed poor sequencing output and  
196 contained few ASVs. In order to do this, we summed the abundance of all ASVs for each sam-  
197 ple ( $\sum_{i=1}^n ASV$ ) and eliminated samples that had fewer than the mean sum ( $\overline{\sum_{i=1}^n ASV}$ ) -  $4\sigma$  (four  
198 standard deviations). In addition, we removed ASVs from our dataset that were present in fewer  
199 than 5% of the samples (less than ten individuals in the soil samples, and less than five in the root  
200 samples). This was done to remove very rare ASVs unique to a block or replicate, but not found  
201 in the majority of a treatment and keep only *abundant* ones.

202

203 We then conducted community-based analyses looking at the effect of the fertilization treatment  
204 on the abundant ASV taxa in the tomato and pepper experiments. To reduce the complexity of  
205 the datasets, relative abundance of all taxa were calculated per family using the R package `dplyr`  
206 Wickham et al. (2015). Barplots were drawn using `ggplot2` Wickham (2016) to vizualize com-  
207 munities. ASV (*a*)-diversity was calculated for each sample using the inverse Simpson diversity  
208 index in `vegan` Oksanen et al. (2013). The effect of fertilization treatment, species (and planting  
209 for soil communities) were assessed using a linear mixed-effect (LMM) model in the R package  
210 `nlme` Pinheiro et al. (2017), given the unbalanced, replicated block design. Alpha diversity was

211 log transformed in order to help satisfy the assumption of normality of the residuals of the LMM  
212 statistical framework.

213

214 Using the community matrix data of ASVs abundance, we performed PERmutational Multivariate  
215 ANalysis Of VAriance tests (PERMANOVA; Anderson (2001)) to identify relationships between  
216 the communities according to the experimental design. ASV abundance data was Hellinger-  
217 transformed and significance was assessed using 10,000 permutations in vegan Oksanen et al.  
218 (2013). Blocks and replicates nested within blocks were factored as strata (blocks) in the model.

219

220 We also performed canonical correspondence analyses (CCAs) using Hellinger-transformed ASV  
221 abundance data in vegan Oksanen et al. (2013) to visually assess the grouping of samples, ASVs  
222 and their association with productivity variables (*species* scaling based on ASV matrix). Data were  
223 analysed separately for fungal-root, fungal-soil, bacterial-root and bacterial-soil, but also accord-  
224 ing to species (tomato/pepper), given that analyses of *a* diversity showed that tomato and pepper  
225 were markedly different. This gave a total of eight CCAs. Data were constrained based on four  
226 of the productivity measures (fruit number, average fruits weight, shoots fresh weight, roots fresh  
227 weight). We excluded the shoot & root dry weights as constraints to simplify the model and given  
228 that they were highly correlated with the fresh weighth already included as constraints ( $r^2=0.98$   
229 and 0.76 for shoot dry/fresh weights and root dry/fresh weights, respectively).

230

231 Finally, we attempted to identify candidate ASVs positively associated with productivity. As such,  
232 we identified the ten ASVs most positively associated with the measures of fruit number, shoots  
233 fresh weight and roots fresh weight from each canonical correspondence analysis for a total of 40  
234 fungal and 40 bacterial candidates ASVs. We aligned candidate sequences from these candidates  
235 ASVs using the Bioconductor R package decipher Wright (2016) and build pairwise distances ma-  
236 trices using a JC69 substitution models of DNA sequence evolution (equal base frequencies, Jukes  
237 & Cantor (1969)) in phangorn Schliep (2010). Phylogenetic trees for bacteria and fungi were plotted  
238 using ape Paradis, Claude & Strimmer (2004). This permitted to identify if similar candidate ASVs  
239 were found under different experimental conditions (soil/root, pepper/tomato), thus reinforcing  
240 their role in productivity increase, and decreasing the chance that these are false positive.



<sup>242</sup> **RESULTS**

<sup>243</sup> *Soil characteristics*

<sup>244</sup> In Table 1, we present the characteristics of the soil which was collected at the IRDA research  
<sup>245</sup> station in St-Bruno (Qc, Canada) and used in the current experimental design.

<sup>246</sup>

Table 1: Soil characteristics

Soil Characteristics	Average value
pH	6.01
Conductivity (mmhos/cm)	0.68
Nitrate (ppm N)	62.40
Ammonium (ppm)	0.09
Phosphorus (ppm)	0.41
Potassium (ppm)	29.30
Calcium (ppm)	64.40
Magnesium (ppm)	13.80
Chloride (ppm)	28.50
Sulfate (ppm)	19.30
Sodium (ppm)	17.80
Zinc (ppm)	0.12
Manganese (ppm)	0.06
Cooper (ppm)	0.81
Iron (ppm)	0.90
Aluminium (ppm)	1.66

<sup>247</sup>

<sup>248</sup> *productivity*

<sup>249</sup> We tested the effect of the fertilization treatment on six measures of overall plant growth and pro-  
<sup>250</sup> ductivity (fruit number, average fruit weight, shoots fresh weight, shoots dry weight, roots fresh

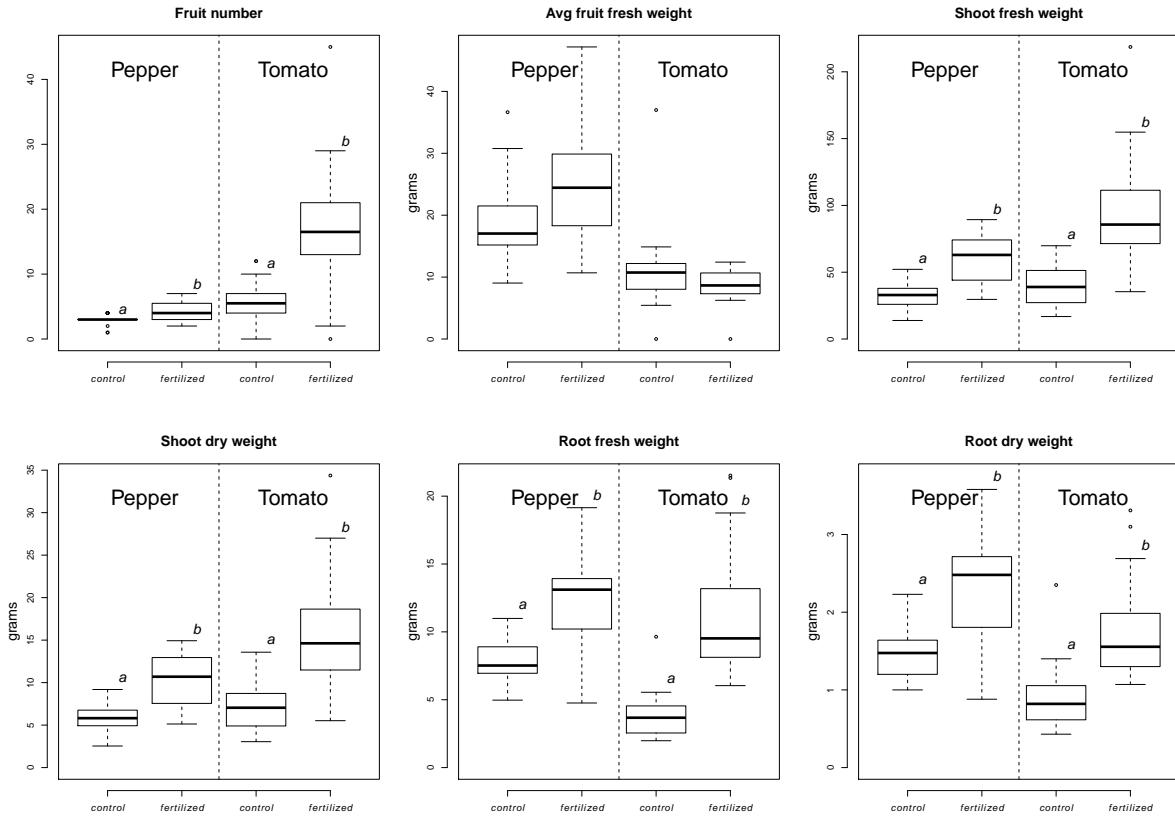
251 weight, roots dry weight) for both tomato and peppers. Visually, both above ground and below  
252 ground plant structure grew larger in fertilized plants, in addition to producing more fruits (see  
253 Figure 2 for some examples of the striking difference between fertilized and unfertilized plants).



254  
255 **Figure 2: Plant productivity. Photos were taken at the end of the experimental treatment. In**  
256 **each photo, fertilized plants are on the left. A: pepper plants, B: pepper roots, C: pepper fruits**  
257 **and D: tomato fruits.**

258  
259 Statistically, all six productivity measures significantly differed according to species, and five of  
260 those were significantly different according to the fertilization treatment. The only exception was  
261 the average fruit weight which did not differ between fertilized and control plants (LMM,  $F_{(1,69)}$   
262 = 1.27,  $p$ -value=0.26). However the model did reveal a significant interaction between treatment  
263 and plant ( $F_{(1,69)} = 9.6$ ,  $p$ -value=0.0028). In fact, when testing only the pepper plants, the effect of  
264 fertilization on average fruit weight was significantly higher in the fertilized pepper plants ( $F_{(1,23)}$   
265 = 10.84,  $p$ -value=0.0032).

266



267

268 **Figure 3: measures of plant productivity.**

269

270

271 *Sequencing*

272 A total of 2.7 million paired-end raw reads were obtained for all samples combined (976,000 for  
273 fungi-soil, 920,000 for fungi-root, 309,000 for bacteria-soil and 535,000 for bacteria-root, Table 2).

274 Note that sequencing samples were analysed separately for fungal-soil, fungal-root, bacteria-soil  
275 and bacteria-root conditions. On average, 47,664 paired-end reads were obtained per sample. Af-  
276 ter quality filters were applied, including removing chimeras, and paired-end reads were merged,  
277 an average of 19,690 sequences remained per sample. While 192 soil samples for fungi and bac-  
278 teria, and 96 root samples for fungi and bacteria were sequenced, seven fungi-soil samples, 15  
279 fungi-root samples and one bacteria-root samples were removed because they had too few reads  
280 based on our strict quality thresholds.

281

282 The dada2 pipeline inferred, on average, 170 Amplicon Sequence Variants per sample (average  
283 of 176 fungal-soil ASV, 37 fungal-root ASVs, 269 bacterial-soil ASVs and 92 bacterial-root ASVs).  
284 Many of those were unique to one of a few samples (total number of 6,112 fungal-soil, 845 fungal-  
285 root, 9,352 bacterial-soil and 2,023 bacterial-roots ASVs). After quality filtering ASVs that were  
286 found in fewer than 10% of the samples, we retained 413, 106, 811 and 325 ASVs and which  
287 comprised 94%, 95%, 89% and 98% of all reads in the fungal-soil, fungal-root, bacterial-soil and  
288 bacterial-root samples, respectively.

289

290

Table 2: Sequencing and ASV summary

	fungi_soil	fungi_root	bacteria_soil	bacteria_root
Nb_seq_sum	976,000	309,000	920,000	535,000
Nb_seq_mean	50,847	32,208	47,907	56,365
Nb_seq_mean_filtered	32,626	12,714	29,662	37,642
Nb_seq_mean_filt_merged	29,300	12,094	14,060	30,706
Nb_seq_mean_filt_merg_non_chimeras	25,476	9,849	13,521	30,408
Nb_samples	192	96	192	96
Nb_samples_trimmed	189	81	192	95
ASV_sum	6,112	845	9,352	2,023
ASV_sum_trimmed	413	106	811	325
ASV_persample	176	37	269	92

291

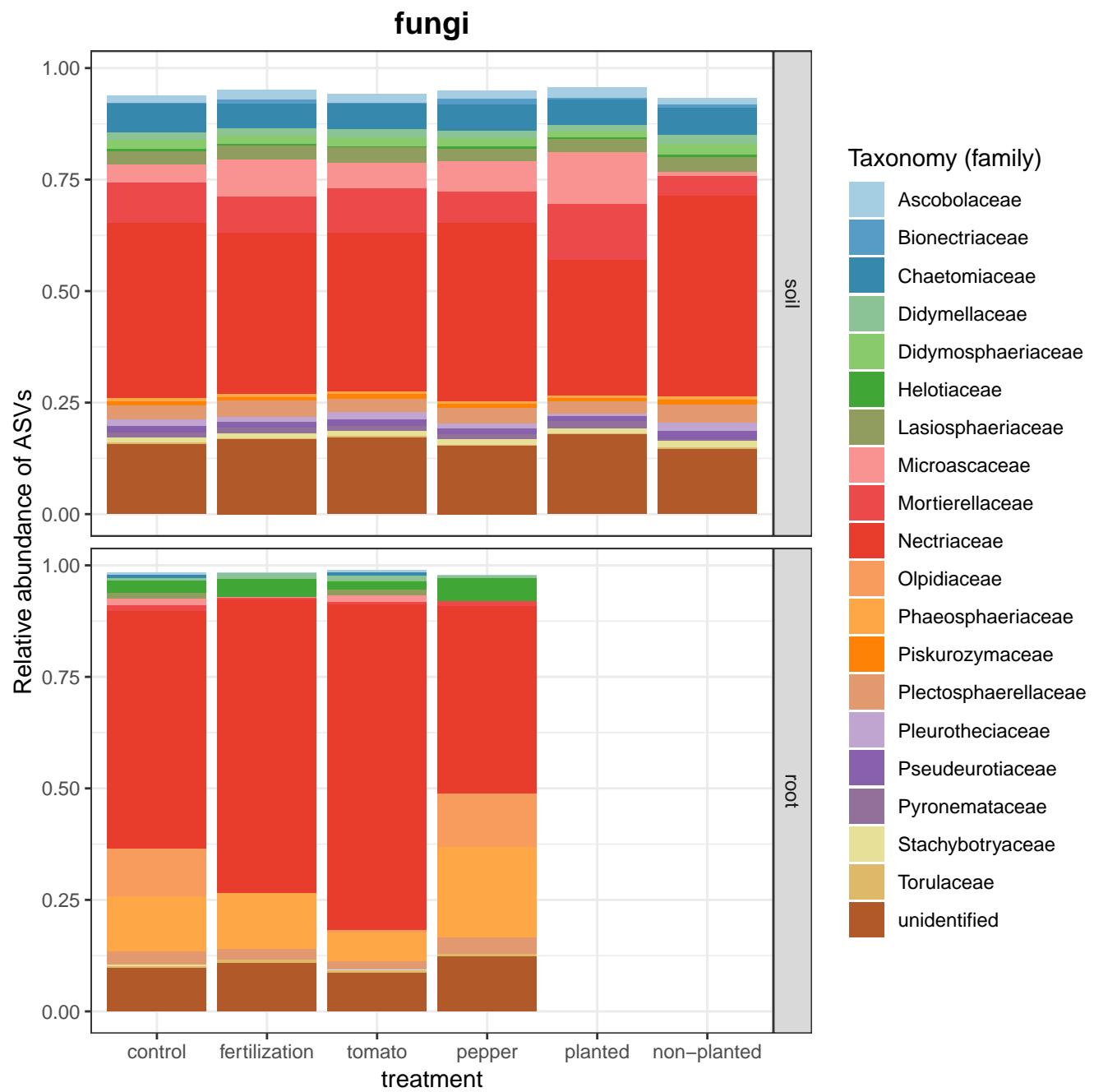
292

### 293 *Root, soil, microbial and bacterial diversity*

294 We then analysed the whole community structure and report the relative abundance of taxa (fam-  
295 ily) for the fungal-soil, fungal-root, bacteria-soil and bacteria-root conditions (Figure 4). Fungal  
296 communities were dominated by Nectriaceae, both in the root and soil samples. The bacterial

297 family Bacilaceae dominated to a lesser extent the soil communities. Bacterial root communities  
 298 were largely dominated by the Cyanobacteria phylum (identified as *chloroplast* according to the  
 299 Ribosomal Database Project Naive Bayesian Classifier and the silva database). In fact, these ASVs  
 300 are likely sequenced chloroplasts from the plants themselves, despite the fact that the primer pair  
 301 used should have primarily targeted the bacterial V3-V4 region of the 16S ribosomal gene.

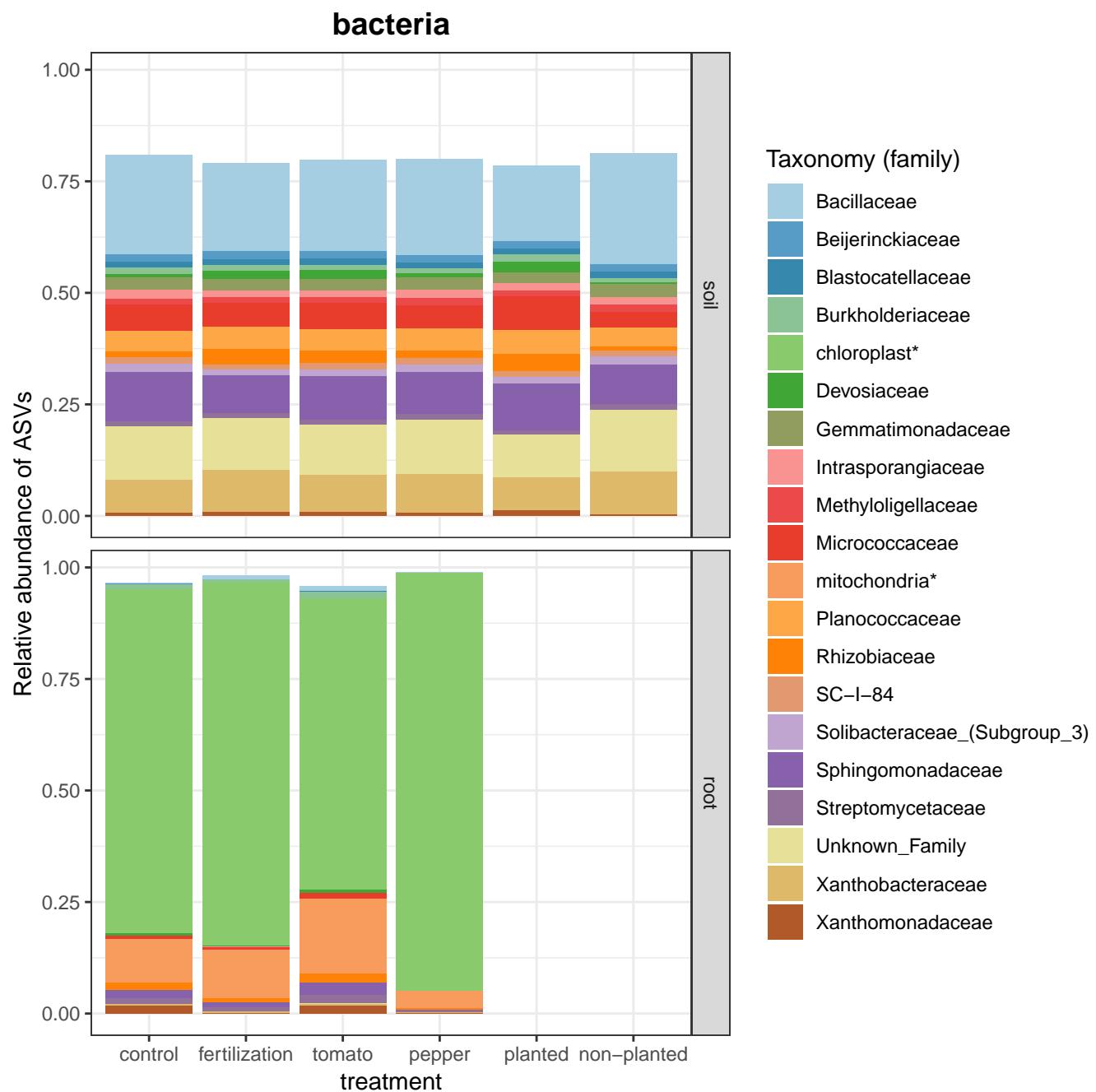
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303

304 **Figure 4a: Barplots fo the relative abundance of fungal ASVs for fungi**

305



306

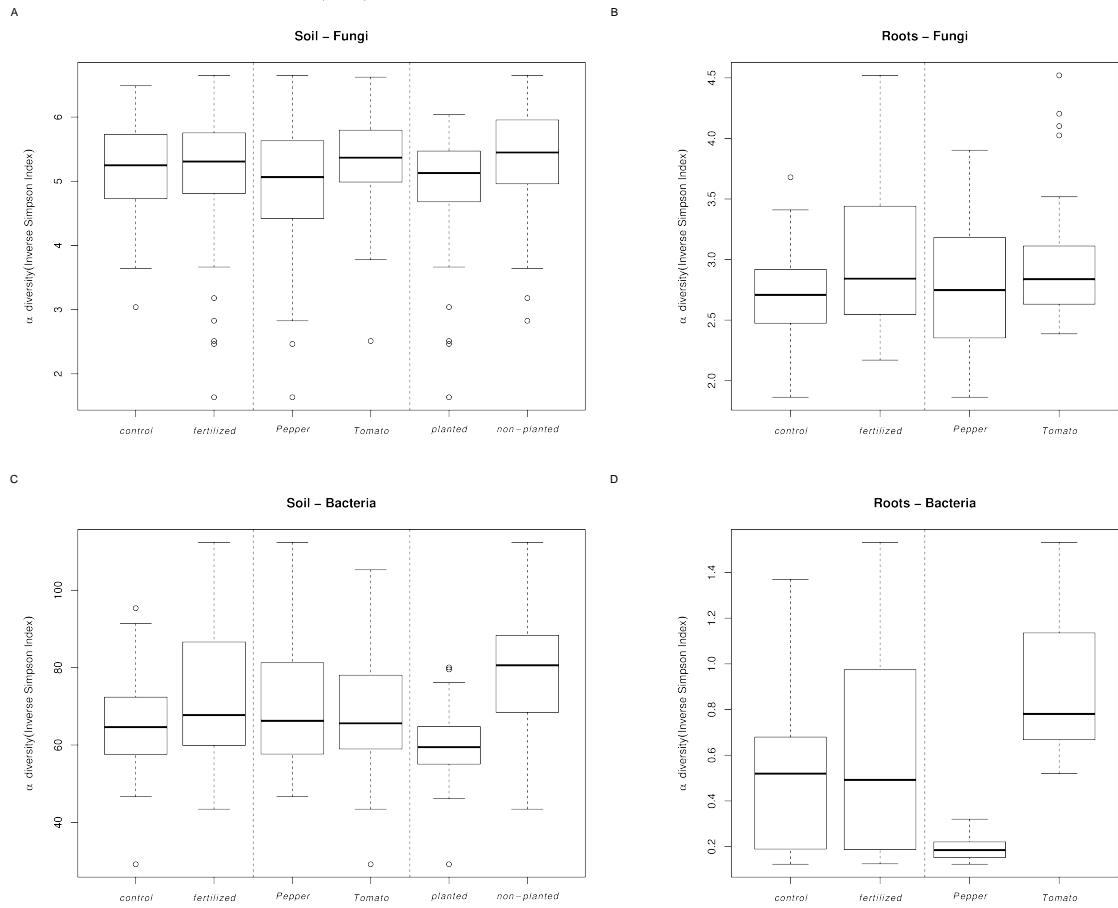
307 **Figure 4b: Barplots fo the relative abundance of bacterial ASVs for bacteria**

308

309 *Local (a-diversity)*

310 The diversity of each site (*a*-diversity) was calculated separately for each sample and under each  
 311 experimental conditions (fungi-soil, fungi-root, bacteria-soil and bacteria-root, Figure 5). Linear  
 312 mixed effects models were used to assess significance. In soil samples, fungal diversity did not

313 differ with respect to the fertilization ( $F_{(1,161)}=0.17$ ,  $p\text{-value}=0.6853$ ), but did so with respect to  
 314 planting ( $F_{(1,161)}=9.00$ ,  $p\text{-value}<0.0032$ ) treatment and species ( $F_{(1,161)}=13.03$ ,  $p\text{-value}=0.0003$ ). In  
 315 root samples, fungal diversity differed with respect to the fertilization treatment ( $F_{(1,56)}=10.1$ ,  $p\text{-}  
 316 value}=0.003$ ), and the species tested ( $F_{(1,56)}=4.5$ ,  $p\text{-value}=0.04$ ). In soil samples, bacterial diver-  
 317 sity differed with respect to the fertilization treatment ( $F_{(1,165)}=17.13$ ,  $p\text{-value}<0.0001$ ), planting  
 318 ( $F_{(1,165)}=139.0$ ,  $p\text{-value}<0.0001$ ) but not species ( $F_{(1,165)}=1.89$ ,  $p\text{-value}=0.17$ ). In root samples, bac-  
 319 terial diversity differed with respect to the fertilization treatment ( $F_{(1,67)}=17.27$ ,  $p\text{-value}=0.0001$ ),  
 320 and the species tested ( $F_{(1,67)}=359.69$ ,  $p\text{-value}<0.0001$ ).



321  
 322 **Figure 5: Boxplot of alpha diversity according to the treatment, species and planting effect for**  
 323 **fungal-root, fungal-soil, bacteria-soil and bacteria-root.**

324

325

326 *Differences in species composition among sites*

327 Using a PERMANOVA statistical framework, we identified that for all conditions, communities  
 328 differed with respect to the fertilization treatment (Table 3). Soil fungal and bacterial communities  
 329 differed the most according to whether the tray was planted (greatest % of variance explained,  
 330 Table 3), while root communities differed the most between tomato and pepper plants.

Table 3: summary of PERMANOVAs\*

	fungi-soil	fungi-root	bacteria-soil	bacteria-root
fertilization	0.02 (2e-04)	0.08 (1e-04)	0.04 (1e-04)	0.07 (1e-04)
planted	0.21 (1e-04)	NA	0.13 (1e-04)	NA
species	0.02 (1e-04)	0.26 (1e-04)	0.02 (3e-04)	0.52 (1e-04)
fertilization:planted	0.01 (0.003)	NA	0.02 (1e-04)	NA
fertilization:species	0.01 (0.006)	0.04 (0.002)	0.03 (1e-04)	0.05 (2e-04)
planted:species	0.01 (0.09)	NA	0.01 (0.004)	NA
fertilization:planted:species	0.01 (0.16)	NA	0.01 (0.04)	NA

331 \* $r^2$  [percentage of variance explained by the term in the model] and associated  $p$ -values in  
 332 parentheses.

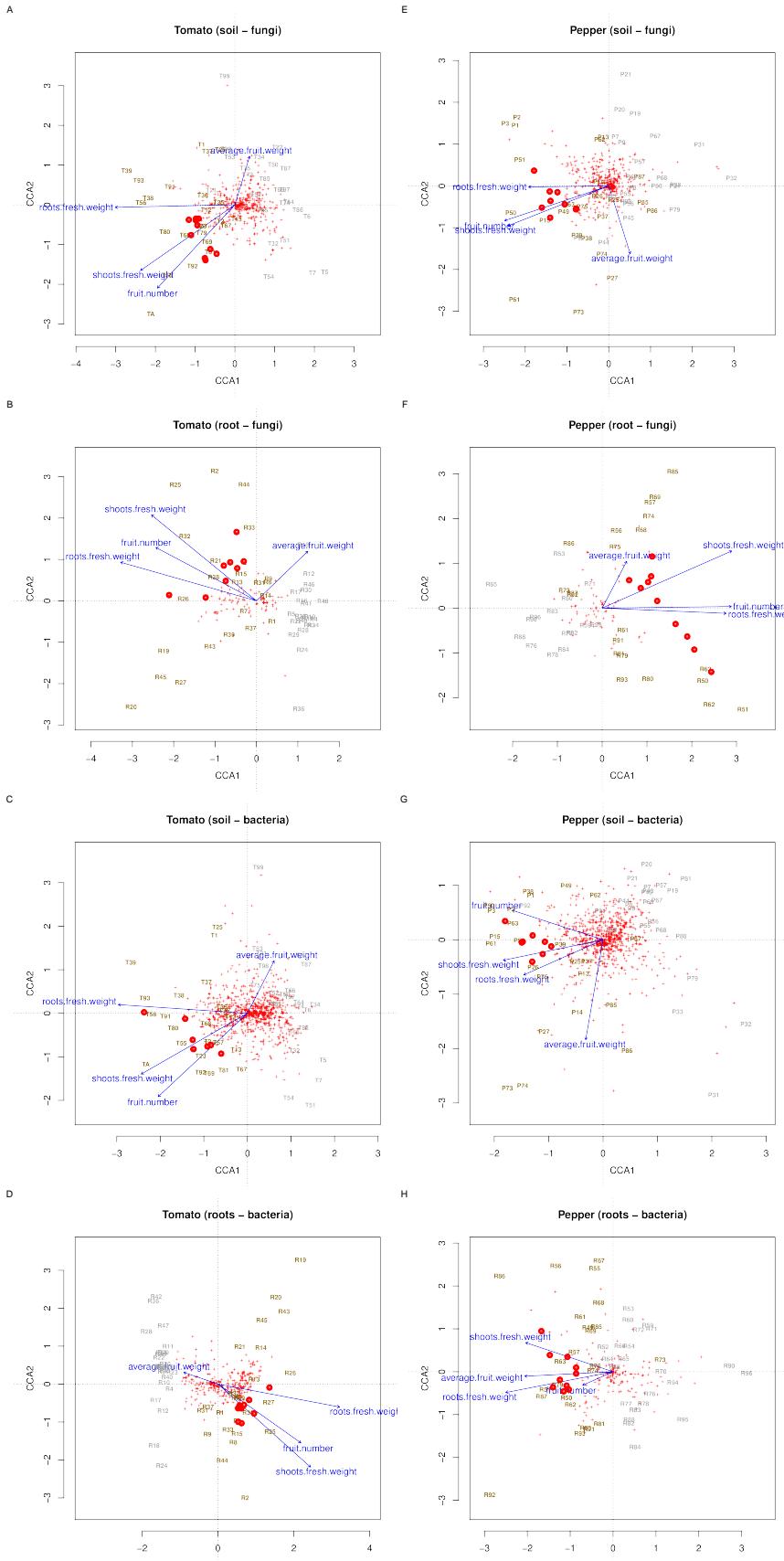
333

334 *Canonical correspondence analyses and candidate ASVs*

335 Canonical correspondence analyses indicated how fertilized samples clustered together according  
 336 to their fungal or bacterial communities (Figure 6). It also shows a similar association of three of  
 337 the constrain variables (productivity measures of root fresh weight, shoots fresh weight and fruit  
 338 number), while average fruit weight behave differentially (in fact nearly orthogonally to the other  
 339 three constrains in most ordinations).

340

341



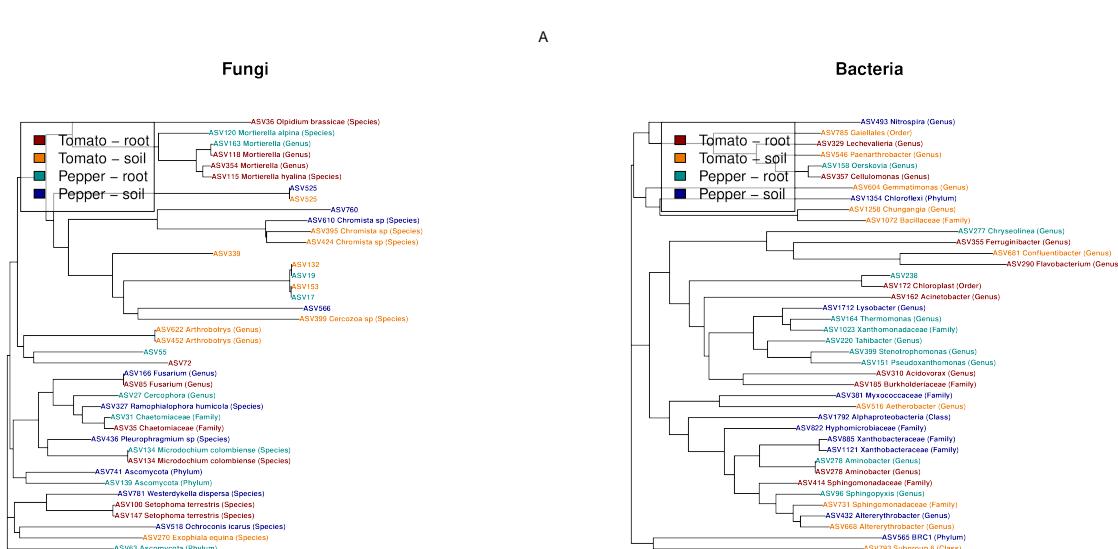
343 **Figure 6: Canonical correspondence analyses for tomato (A-D) and peppers (E-H) for soil-fungi,**  
344 **root-fungi, soil-bacteria and root-bacteria.** Samples were labelled and colored in gray (unfer-  
345 **tilized) or dark yellow (fertilized).** Red crosses represent individual ASVs, while red points  
346 **represent the ten ASVs most closely associated wih the three productivity measures of root**  
347 **fresh weight, shoots fresh weight and fruit number.** Blue arrows are the four productivity mea-  
348 **sures used as constrains in the ordinations.**

349

350 Next, we identified, for each ordination, the ten ASVs most closely related to the three con-  
351 strains which behaved in a similar fashion (productivity measures of root fresh weight, shoots  
352 fresh weight and fruit number). These ASVs were considered as putative candidates sequences  
353 most positively impacted (increase presence of the ASV) by fertilization. We further analysed the  
354 corresponding sequences for these eighty candidates (ten candidates \* eight ordinations) ASVs in  
355 two separate alignments (one for fungi and one for bacterial ASVs) and their accompanying phylo-  
356 genetic trees. In fungi, we identified one cluster of ASVs taxonomically assigned to *Mortierella* (soil  
357 saprotrophs in the phylum Zygomycota) positively associated to productivity in both tomato and  
358 pepper roots. In addition, we identified a cluster of four different ASVs in tomato soil (ASV132,  
359 ASV153) and pepper-root (ASV19 & AV17) closely related phylogenetically. Given that no tax-  
360 onomy was assigned to these sequences through the dada2 RDP bootstrap approach, we used a  
361 BLASTn Altschul et al. (1997) approach (against NCBI nr) to identify the most closely related se-  
362 quences. We identified this cluster of ASVs as *Rhogostoma schuessleri* (BLASTn, *e-value*=4e-76), a  
363 protist in the phylum Cercozoa, which are known to be present in the soil and phyllosphere Du-  
364 mack et al. (2017).

365

<sup>366</sup> In bacteria-roots, we identified a number of different ASVs most positively impacted (increase  
<sup>367</sup> presence of the ASV) by fertilization. For example, we identified...



<sup>370</sup> **Figure 7: Neighbor-Joining trees of candidates ASVs (fungi & roots) associated with produc-**  
<sup>371</sup> **tivity measures**

<sup>372</sup>

373 **DISCUSSION**

- 374 • Overall increases in productivity in both species (but mention that tomato were fertilized with  
375 hen manure as well).
- 376 • A few words about the goal of the sequencing: very broad approach looking at lots of factors  
377 (fertilization, species, planting root, soil, fungal, bacterial).
- 378 • A few words about the *dada2* approach and its advantages (it's reproducible, open & R based. It  
379 identifies unique (non-clustered) ASVs which can be compared among species) & disadvantages  
380 (essentially, the main disadvantage is that it identifies non-clustered ASVs, which means that most  
381 ASVs are unique, and not found anywhere else, which makes it hard to compare: see *Sequencing*  
382 paragraph in results). Also mention that most ASVs are unique AND very rare, such that when  
383 we remove the rare ones (found in <10% of the samples), we discard ~90% of ASV, but keep >95%  
384 of reads. So this is still a very valuable approach. • Also, talk about advantages / disadvantages  
385 of the RDP bayesian classifier approach to taxonomy rather than a BLAST approach (a BLAST  
386 approach will always give you a result even if this result is false, an RDP will only give you a tax-  
387 onomy (from Kingdom to species) if there is enough power/confidence in it). Also any taxonomy  
388 classification largely depends on the database you have.
- 389 • Talk about effect of treatment on root + soil on overall (alpha) diversity. • Talk about effect of  
390 treatment on root + soil on differences in species composition among sites (beta diversity) • Talk  
391 about the fact in the roots, we most likely sequenced the plant itself, rather than the bacteria.
- 392 • Discuss some of the candidate ASVs identified based on the ordinations and Figure 7.
- 393 • Follow-up work: ?

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396 sion about analyses + seaweed extracts.

397 REFERENCES

- 398 Alam MZ., Braun G., Norrie J., Hodges DM. 2013a. Ascophyllum extract application can promote  
399 plant growth and root yield in carrot associated with increased root-zone soil microbial activity.  
400 *Canadian Journal of Plant Science* 94:337–348.
- 401 Alam MZ., Braun G., Norrie J., Hodges DM. 2013b. Effect of ascophyllum extract application  
402 on plant growth, fruit yield and soil microbial communities of strawberry. *Canadian Journal of Plant*  
403 *Science* 93:23–36.
- 404 Altschul SF., Madden TL., Schäffer AA., Zhang J., Zhang Z., Miller W., Lipman DJ. 1997.  
405 Gapped blast and psi-blast: A new generation of protein database search programs. *Nucleic acids*  
406 *research* 25:3389–3402.
- 407 Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Aus-  
408 tral ecology* 26:32–46.
- 409 Anderson MJ., Legendre P. 1999. An empirical comparison of permutation methods for tests  
410 of partial regression coefficients in a linear model. *Journal of statistical computation and simulation*  
411 62:271–303.
- 412 Arioli T., Mattner SW., Winberg PC. 2015. Applications of seaweed extracts in australian agri-  
413 culture: Past, present and future. *Journal of applied phycology* 27:2007–2015.
- 414 Callahan B. 2018. Silva for dada2: Silva taxonomic training data formatted for dada2 (silva  
415 version 132). *Zenodo*. DOI: [10.5281/zenodo.1172783](https://doi.org/10.5281/zenodo.1172783).
- 416 Callahan BJ., McMurdie PJ., Rosen MJ., Han AW., Johnson AJA., Holmes SP. 2016. DADA2:  
417 High-resolution sample inference from illumina amplicon data. *Nature methods* 13:581.
- 418 Caporaso JG., Kuczynski J., Stombaugh J., Bittinger K., Bushman FD., Costello EK., Fierer N.,  
419 Pena AG., Goodrich JK., Gordon JI., others. 2010. QIIME allows analysis of high-throughput  
420 community sequencing data. *Nature methods* 7:335.
- 421 Community U. 2018.UNITE general fasta release. version 01.12.2017. Available at <https://files.pluto.ut.ee/doi/C8/E4/C8E4A8E6A7C4C00EACE3499C51E550744A259A98F8FE25993B1C7B9E7D2170B2.zip>
- 422 Craigie JS. 2011. Seaweed extract stimuli in plant science and agriculture. *Journal of Applied*

- 425 *Phycology* 23:371–393.
- 426 Dhargalkar V., Pereira N. 2005. Seaweed: Promising plant of the millennium.
- 427 Dumack K., Flues S., Hermanns K., Bonkowski M. 2017. Rhogostomidae (cercozoa) from soils,
- 428 roots and plant leaves (*arabidopsis thaliana*): Description of rhogostoma epiphylla sp. nov. and r.
- 429 cylindrica sp. nov. *European journal of protistology* 60:76–86.
- 430 Jukes T., Cantor C. 1969. Evolution of protein molecules, pp. 21–132 in mammalian protein
- 431 metabolism, edited by munro hn.
- 432 Khan W., Rayirath UP., Subramanian S., Jithesh MN., Rayorath P., Hedges DM., Critchley AT.,
- 433 Craigie JS., Norrie J., Prithiviraj B. 2009. Seaweed extracts as biostimulants of plant growth and
- 434 development. *Journal of Plant Growth Regulation* 28:386–399.
- 435 Klindworth A., Pruesse E., Schweer T., Peplies J., Quast C., Horn M., Glöckner FO. 2013. Evalu-
- 436 ation of general 16S ribosomal rna gene pcr primers for classical and next-generation sequencing-
- 437 based diversity studies. *Nucleic acids research* 41:e1–e1.
- 438 Milton R. 1952. Improvements in or relating to horticultural and agricultural fertilizers. *British*
- 439 *Patent* 664989.
- 440 Oksanen J., Blanchet FG., Kindt R., Legendre P., Minchin PR., O'hara R., Simpson GL., Solymos
- 441 P., Stevens MHH., Wagner H., others. 2013. Package “vegan”. *Community ecology package, version*
- 442 2.
- 443 Paradis E., Claude J., Strimmer K. 2004. APE: Analyses of phylogenetics and evolution in r
- 444 language. *Bioinformatics* 20:289–290.
- 445 Pinheiro J., Bates D., DebRoy S., Sarkar D., Team RC. 2017. Nlme: Linear and nonlinear mixed-
- 446 effects models. r package version 3.1-128. 2016. *R software*.
- 447 Santoyo G., Orozco-Mosqueda M del C., Govindappa M. 2012. Mechanisms of biocontrol and
- 448 plant growth-promoting activity in soil bacterial species of bacillus and pseudomonas: A review.
- 449 *Biocontrol Science and Technology* 22:855–872.
- 450 Schliep KP. 2010. Phangorn: Phylogenetic analysis in r. *Bioinformatics* 27:592–593.
- 451 Schloss PD., Westcott SL., Ryabin T., Hall JR., Hartmann M., Hollister EB., Lesniewski RA.,
- 452 Oakley BB., Parks DH., Robinson CJ., others. 2009. Introducing mothur: Open-source, platform-
- 453 independent, community-supported software for describing and comparing microbial communi-

- 454 ties. *Applied and environmental microbiology* 75:7537–7541.
- 455 Stirk WA., Van Staden J. 2014. Plant growth regulators in seaweeds: Occurrence, regulation  
456 and functions. *Advances in Botanical Research* 71:125–159.
- 457 Team RC., others. 2018. R: A language and environment for statistical computing.
- 458 Toju H., Tanabe AS., Yamamoto S., Sato H. 2012. High-coverage its primers for the dna-based  
459 identification of ascomycetes and basidiomycetes in environmental samples. *PLoS one* 7:e40863.
- 460 Wally OS., Critchley AT., Hiltz D., Craigie JS., Han X., Zaharia LI., Abrams SR., Prithiviraj  
461 B. 2013. Regulation of phytohormone biosynthesis and accumulation in arabidopsis following  
462 treatment with commercial extract from the marine macroalga *ascophyllum nodosum*. *Journal of*  
463 *plant growth regulation* 32:324–339.
- 464 Wang Q., Garrity GM., Tiedje JM., Cole JR. 2007. Naive bayesian classifier for rapid assign-  
465 ment of rRNA sequences into the new bacterial taxonomy. *Applied and environmental microbiology*  
466 73:5261–5267.
- 467 Wickham H. 2016. *Ggplot2: Elegant graphics for data analysis*. Springer.
- 468 Wickham H., Francois R., Henry L., Müller K. 2015. Dplyr: A grammar of data manipulation.  
469 *R package version 0.4.3*.
- 470 Wright ES. 2016. Using decipher v2. 0 to analyze big biological sequence data in r. *R Journal* 8.