

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

<sup>4</sup> **Sébastien Renaud<sup>1,2</sup>, Jacynthe Masse<sup>1,2</sup>, Jeffrey P. Norrie<sup>3</sup>, Bachar Blal<sup>3</sup> Mohamed Hijri<sup>1,2</sup>**

<sup>5</sup> *<sup>1</sup>Département de Sciences Biologiques, Institut de Recherche en Biologie Végétale, Université de Montréal, 4101*

<sup>6</sup> *Sherbrooke Est, Montreal, H1X 2B2, Quebec, Canada. <sup>2</sup>Quebec Centre for Biodiversity Science, Montreal, Quebec,*

<sup>7</sup> *Canada <sup>3</sup>Acadian Seaplant Ltd, 30 Brown Avenue, Dartmouth, Nova Scotia, Canaad, B3B 1X8*

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<sup>8</sup> Seaweeds have been used as a source of natural fertilizer and biostimulant for centuries. Here,  
<sup>9</sup> we used a commercially available *Ascophyllum nodosum* extract in order to test its effect of plant  
<sup>10</sup> productivity in peppers and tomatoes. In addition, by using a metabarcoding high throughput  
<sup>11</sup> sequencing approach, we identified Amplicon Sequence Variants in the root and soil, fungal and  
<sup>12</sup> bacterial microbiome. We find that all productivity measures of root, shoot and fruit biomass  
<sup>13</sup> differed significantly according to species, and five of those were significantly greater according  
<sup>14</sup> to the fertilization treatment. Local species richness (*a*-diversity) was the highest in the bacteria-  
<sup>15</sup> soil and fungi-soil microbiome, and the lowest in the bacteria-root microbiome. In addition, *a*-  
<sup>16</sup> diversity differed according to the fertilization treatment, but this effect was small. Species com-  
<sup>17</sup> position among sites (*b*-diversity) differed according to the fertilization treatment in all four com-  
<sup>18</sup> munities measured (fungal-root, fungal-soil, bacterial-root and bacterial-soil). Finally, we identify  
<sup>19</sup> a number of candidate taxa most strongly associated with measures of productivity. Further stud-  
<sup>20</sup> ies, for example using inoculum of microbial species linked to the presence of liquid seaweed  
<sup>21</sup> extract may help to identify a causative link between extracts, microbes and productivity.

<sup>22</sup> **Keywords:** Stella Maris®, 16S, ITS, soil microbial diversity, Illumina MiSeq, ANE, Amplicon Se-  
<sup>23</sup> quence Variants, OTU

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<sup>24</sup> INTRODUCTION

<sup>25</sup> Seaweeds (also known as marine macroalgae) have been used as a source of organic matter and  
<sup>26</sup> nutrients for centuries, especially in coastal areas (Khan et al., 2009; Craigie, 2011). Liquid seaweed  
<sup>27</sup> extracts, developed in the 1950s in order to concentrate plant growth-stimulating compounds, fa-  
<sup>28</sup> cilitate their usage (Milton, 1952). Today, most commercially available extracts are made from  
<sup>29</sup> the brown algae *Ascophyllum nodosum*, *Ecklonia maxima* or *Laminaria spp.* Unlike modern chemi-  
<sup>30</sup> cal fertilizers, seaweed extracts are biodegradable, non-toxic and come from a renewable resource  
<sup>31</sup> (Dhargalkar & Pereira, 2005). Industry-funded bodies such as the European Biostimulant Indus-  
<sup>32</sup> try Coalition and the United States Biostimulant Coalition have been working to accommodate  
<sup>33</sup> biostimulants into mainstream legal architecture. These organizations extoll benefits arising from  
<sup>34</sup> modes-of-action research, agricultural applications and positive effects on yield and quality of  
<sup>35</sup> many commercial species (i.e. fruits, vegetables, turf, ornamentals and woody species). Legal  
<sup>36</sup> recognition will further allow a fluid integration of various biostimulants, including *Ascophyllum*  
<sup>37</sup> *nodosum* Extracts (ANE) into sustainable long-term crop management programs (Craigie, 2011;  
<sup>38</sup> Jardin, 2015).

<sup>39</sup>

<sup>40</sup> Several comprehensive reviews have described the effects of seaweed extracts on agricultural  
<sup>41</sup> plant productivity (Khan et al., 2009; Craigie, 2010, 2011; Battacharyya et al., 2015). The science  
<sup>42</sup> points to wide-ranging effects from biotic to abiotic resistance, effects on growth and develop-  
<sup>43</sup> ment, and ultimately, to their impact on plant establishment, crop yield and/or quality, and shelf  
<sup>44</sup> life. At the physiological level, these extracts have been found to influence hormonal changes that  
<sup>45</sup> in turn, influence physiological processes even at very low concentrations (Wally et al., 2013).

<sup>46</sup>

<sup>47</sup> Starting in the 1990's, the development of high quality ANE has led to an increase in cause-effect  
<sup>48</sup> research, especially on plant diseases (Jayaraj & Ali, 2015). Noted increases in the activity of super-  
<sup>49</sup> oxide dismutase, glutathione peroxidase and ascorbate peroxidase helped support the argument  
<sup>50</sup> that ANE improve plant tolerance to oxidative stress (Ayad et al., 1997; Schmidt & Zhang, 1997;  
<sup>51</sup> Ayad, 1998; Allen et al., 2001). Positive effects were also found on phytoalexin production suggest-  
<sup>52</sup> ing that ANE may be involved in suppressing disease infection through increased activity of these

53 protective enzymes that target oxidizing toxins naturally emitted by disease pathogens (Lizzi et  
54 al., 1998; Jayaraj et al., 2008; Jayaraman, Norrie & Punja, 2010).

55

56 Improved plant stress resistance and tolerance to foliar and soil treatments is attributed to a cas-  
57 cade of various physiological reactions. ANE can impact plant-signalling mechanisms through a  
58 multitude of plant processes and cellular modifications including osmotic/oxidative stresses such  
59 as salinity, freezing and drought stress (Jithesh et al., 2012). ANE can also impart drought-stress  
60 tolerance to plants by reducing stomatal conductance and cellular electrolyte leakage (Shotton and  
61 Martynenko, unpublished data; Spann & Little, 2011). These results suggest that ANE can influ-  
62 ence cellular membrane maintenance leading to a higher tolerance for various osmotic stresses  
63 and can mitigate oxidative damage.

64

65 Although there is an abundance of published evidence detailing systemic plant effects from ANE,  
66 outstanding questions remain as to the effects of ANE on the soil rhizosphere. Various microbes,  
67 small arthropods, nematodes, earthworms and insects thrive in the soil rhizosphere. This vast  
68 microbial biodiversity contributes to the aggregation of soil particles, enhances nutrient cycling  
69 and delivery to plants, degrades toxic substances, allows better soil water and plays a role in plant  
70 disease management. It has been suggested that the plant immune system is composed of inher-  
71 ent surveillance systems that perceive several general microbial elicitors, which allow plants to  
72 switch from growth and development into a defense mode (Newman et al., 2013). This may allow  
73 the plant to avoid infection from potentially harmful microbes. An examination of sustainable  
74 products that can positively influence microbial interactions between plant roots and soil biota  
75 will in turn help to further understand soil borne plant-pathogens competition dynamics. The ef-  
76 fect of ANE on the bacterial profile suggests that ANE applications increased strawberry root and  
77 shoot growth, berry yield and rhizosphere microbial diversity and physiological activity (Alam et  
78 al., 2013). Similar results were found in sandy loam soils as Alam et al. (2014) showed a strong  
79 relationship between carrot growth, soil microbial populations and activity.

80

81 The recent development of culture-independent molecular techniques and high throughput se-  
82 quencing should permit to circumvent the inherent biases of culture-based approaches by target-

83 ing the ubiquitous component of life, its DNA. In turn, this will help to identify a larger proportion  
84 of the microbial diversity and lead to a better understanding of the soil microbial response  
85 to seaweed extract. DNA barcoding targeting specific regions of the genome (e.g. ITS: fungi, 16s  
86 ribosomal genes: bacteria) are now regarded as a prerequisite procedure to comprehensively doc-  
87 ument the diversity and ecology of microbial organisms (Toju et al., 2012; Klindworth et al., 2013).

88

89 Here the general objective was to quantify the impact of ANE on plant growth and test how the  
90 bacterial and fungal communities responded to the addition of theses extracts. We also aimed to  
91 identify specific taxon positively associated with increased in plant productivity following addi-  
92 tion of ANE. We hypothesized that the inclusion of liquid seaweed extracts would improve pro-  
93 ductivity and alter significantly the bacterial and fungal communities. We used a commercially  
94 available ANE, Stella Maris®, developed by Acadian Seaplants Ltd (NS, Canada). Stella Maris®  
95 is derived from the marine algae *A. nodosum*, and harvested from the nutrient-laden waters of the  
96 North Atlantic off the Eastern Coast of Canada. We tested the effect of ANE on two agricultural  
97 plants commonly grown in greenhouse conditions (tomato and pepper). Several traits related to  
98 plant productivity were measured and soil and root bacterial and fungal diversity were quantified  
99 using High Throughput Illumina Miseq sequencing.

100

101

102 MATERIAL AND METHOD

103 *Experimental design*

104 Greenhouse experiments were set up in large trays (60x30x18 cm LxWxH) using two different  
105 crops: tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.). Tomato cultivar Totem  
106 Hybrid#A371 was planted in November 16th 2015 and pepper cultivar Ace Hybrid#318 was  
107 planted in December 9th 2015. Tomato and pepper seeds were purchased from William Dam  
108 Seeds Ltd (ON, Canada). These cultivars were selected for greenhouse production. Soil was col-  
109 lected from an agricultural field under organic regime at the IRDA research station in St-Bruno  
110 (Qc, Canada, 45°32'59.6"N, 73°21'08.0"W) on October 7th 2015. The soil was a loamy sand and  
111 was collected from the 15 cm top layer. Natural soil was mixed and put into trays, filled to 15 cm  
112 in height. Soil analysis was done using a commercial service provided by AgriDirect (Longueuil,  
113 QC) and soil characteristics are shown in Table 1. Eight seeds per tray were planted and after  
114 germination, only four seedlings per tray were kept.

115

116 For each crop species, a randomized split block design (Table S1) was used with four trays set  
117 up per block and eight blocks for each experiment. Half of the trays were fertilized (fertilization  
118 treatment), as described below. Half of the trays were also planted (planting treatment) with four  
119 plants per tray, while the other trays were not planted. This allowed a direct comparison of fungal  
120 and bacteria soil communities with respect to fertilization and planting treatments.

121

122 Two different fertilization regimes were used according to the plant species. For tomatoes, plants  
123 were fertilized using multipurpose organic fertilizer (pure hen manure, 18 g per tray repeated ev-  
124 ery 4 weeks, 5-3-2) from Acti-sol (Notre-Dame-du-Bon-Conseil, QC) in addition to Stella Maris®  
125 (3.5 ml per 1L, each tray received 250 ml, repeated every 2 weeks) for the duration of the ex-  
126 periment. The other half was not fertilized. Stella Maris® is a commercial *Ascophyllum nodosum*  
127 seaweed based product and its physico- chemical composition is shown in Table S2 (DO WE  
128 HAVE THIS INFORMATION SOMEWHERE?). For the pepper experiment, the fertilization  
129 regime consisted solely of Stella Maris® (3.5 ml per 1L, each tray received 250 ml, repeated every  
130 2 weeks) for the duration of the experiment. The other half was not fertilized. Both experiments

131 were managed under organic farming practices. Thrips were controlled using *Neoseiulus cucumeris*  
132 (syn. *Amblyseius cucumeris*) (1 bag per plant), Fungus gnats were also controlled using predatory  
133 mite *Gaeolaelaps gillespiei* (1L; Natural Insect Control, ON). Plants were treated once a week with  
134 Milstop, a Potassium Bicarbonate-based foliar fungicide to control the powdery mildew on both  
135 crops.

136

137 *Plant productivity*

138 Tomato and pepper experiments were harvested on March 29th 2016. The following traits assessed  
139 plant productivity: fruit number, fruit weight, shoots fresh weight and roots fresh weight. Traits  
140 were measured on three plants chosen randomly per tray for each fertilization-control treatment,  
141 crop (tomato/pepper) and block (eight blocks) for a total of 96 samples. In addition, both shoot  
142 and root samples were dried in a 70 degrees drying oven, and dry weights were quantified after  
143 48 hours. Together, these traits are expected to represent well the plant overall productivity.

144

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

146 Soil and root samples were taken for both experiments. Soil DNA was extracted using NucleoSpin®  
147 Soil DNA extraction kit (Macherey-Nagel, BioLinx, ON) on 250 mg of soil, following the manufac-  
148 turer's protocol Roots were first washed with tap water and rinsed with sterile water. Chopped  
149 roots sub-samples (100 mg) were subjected to DNA extraction using DNeasy Plant Mini kit (Qi-  
150 agen Inc - Canada, ON), following the manufacturer's recommendations. Amplicon sequencing  
151 targeting bacterial 16S rRNA gene and fungal ITS was performed on both root and soil samples.

152

153 For fungal ITS, we used the following primers with the universal CS1 and CS2 adapters: CS1\_ITS3\_KYO2  
154 (5'-ACA CTGA CGA CAT GGT TCT ACA GAT GAA GAA CGY AGY RAA-3') and CS2\_ITS4\_KYO3  
155 (5'-TAC GGT AGC AGA GAC TTG GTC TCT BTT VCC KCT TCA CTC G-3') to produce a final  
156 amplicon size of approximately 430bp including adapters (Toju et al., 2012).

157

158 For bacterial 16S, we used the following primers with CS1 and CS2 universal adapters: 341F (5'-  
159 CCT ACG GGN GGC WGC AG-3') and 805R (5'-GAC TACC AGG GTA TCT AAT C-3') to produce  
160 a final amplicon size of approximately 460 bp and targeting specifically the bacterial V3-V4 region

<sup>161</sup> of the 16S ribosomal gene (Klindworth et al., 2013).

<sup>162</sup>

<sup>163</sup> DNA samples were then barcoded, pooled and sequenced (2X300bp, paired-end) using an Il-  
<sup>164</sup> lumina (San Diego, CA, USA) MiSeq sequencer through a commercial service provided by the  
<sup>165</sup> Genome Quebec Innovation Centre (Montreal, QC). Sequences were demultiplexed by the se-  
<sup>166</sup> quencing facility and further processed as described below.

<sup>167</sup>

### <sup>168</sup> Bioinformatics

<sup>169</sup> All bioinformatics, statistical, and graphical analyses further described were performed in R 3.5.1  
<sup>170</sup> (Team, 2018) and detailed scripts are available here ([https://github.com/seb951/Acadian\\_Seaplants](https://github.com/seb951/Acadian_Seaplants)).

<sup>171</sup>

<sup>172</sup> We used the R package dada2 (Callahan et al., 2016) to infer *Amplicon Sequence Variants* (ASVs).  
<sup>173</sup> Dada2 offers accurate sample inference from amplicon data with single-nucleotide resolution in an  
<sup>174</sup> open source environment. Unlike the Operational Taxonomic Unit (OTU) approach (e.g. Schloss et  
<sup>175</sup> al., 2009; Caporaso et al., 2010), ASV are not treated as cluster of sequences defined with an *ad hoc*  
<sup>176</sup> sequence similarity threshold. Instead, after sequences are quality trimmed and error-corrected,  
<sup>177</sup> dada2 reveals the unique members of the sequenced community, thus allowing sequences and  
<sup>178</sup> abundance counts to be comparable among studies (Callahan et al., 2016).

<sup>179</sup>

<sup>180</sup> First, sequences were trimmed following strict quality thresholds (removing primers and low  
<sup>181</sup> quality nucleotides, see parameter details in the accompanying R scripts). Following this, we  
<sup>182</sup> applied the error model algorithm of dada2, which incorporates quality information after filter-  
<sup>183</sup> ing, unlike other OTU based methods. Then dereplication, sample inference, merging of paired  
<sup>184</sup> end reads and removal of chimera were performed in order to obtain a sequence (ASV) table of  
<sup>185</sup> abundance per sample. Taxonomy was assigned through the dada2 pipeline using the Ribosomal  
<sup>186</sup> Database Project (RDP) Naive Bayesian Classifier algorithm from Wang et al. (2007). Depending  
<sup>187</sup> on support (minimum bootstrap support of 80), we assigned taxonomy from Kingdom to species.  
<sup>188</sup> We used the silva database formatted for dada2 to infer bacterial taxa (Callahan, 2018). We used  
<sup>189</sup> the Unite (Community, 2018) fasta release (including singletons) to infer fungal taxa after format-  
<sup>190</sup> ting it to the dada2 format using a custom R script. The pipeline was run on a multithreaded

191 (48 CPUs) computer infrastructure provided by Westgrid (<https://www.westgrid.ca/support/systems/cedar>) and Compute Canada ([www.computecanada.ca](http://www.computecanada.ca)). Note that the pipeline was run  
192 separately for fungal-root, fungal-soil, bacteria-soil and bacteria-root samples given the markedly  
193 different nucleotide compositions of the sequenced amplicons, unique taxa and specific error mod-  
194 els of each dataset.

196

197 *Statistical analyses - plant productivity*

198 We tested for the effect of species (tomato vs pepper), fertilization and their interaction on six  
199 plant productivity measures (fruit number, average fruit weight, shoots fresh weight, roots fresh  
200 weight, shoots dry weight, roots dry weight). We used Linear Mixed effect Models (LMM) in the  
201 R package *nlme* (Pinheiro et al., 2017), which are more appropriate than an Analysis of Variance  
202 (ANOVA) given the current block design (blocks and replicates nested within a block were treated  
203 as random variables). All six plant productivity measures were either square root or log trans-  
204 formed in order to help satisfy the assumption of normality of the residuals in the LMM statistical  
205 framework. For the variables *fruit number* and *average fruit weight*, we also used a permutation-  
206 based 2-way ANOVA (Anderson & Legendre, 1999) given that the residuals of the LMM were not  
207 normally distributed (results were similarly significant).

208

209 *Statistical analyses - microbial and fungal diversity*

210 Fungal-root, fungal-soil, bacterial-root and bacterial-soil ASV diversity was measured separately.  
211 For each of these four datasets, we removed samples that showed poor sequencing output and  
212 contained few ASVs. In order to do this, we summed the abundance of all ASVs for each sam-  
213 ple ( $\sum_{i=1}^n \text{ASV}$ ) and eliminated samples that had fewer than the mean sum minus four standard  
214 deviations ( $\overline{\sum_{i=1}^n \text{ASV}} - 4\sigma$ ). In addition, we removed ASVs from our dataset that were present  
215 in fewer than 5% of the samples (less than ten individuals in the soil samples or less than five in  
216 the root samples). This was done to remove very rare ASVs unique to a block or replicate, but not  
217 found in the majority of samples.

218

219 We then conducted community-based analyses looking at the effect of the fertilization treatment  
220 on ASV abundance in the tomato and pepper experiments. To reduce the complexity of the

221 datasets, relative abundance of all taxa was calculated per family using the R package dplyr (Wick-  
222 ham et al., 2015). Barplots were drawn using ggplot2 (Wickham, 2016) to visualize communities.  
223 ASV alpha ( $\alpha$ )-diversity was calculated based on all ASVs (excluding rare ASVs, see paragraph  
224 above) for each sample using the inverse Simpson diversity index in vegan (Oksanen et al., 2013).  
225 The effect of the fertilization treatment, species (and planting for soil communities) were assessed  
226 using a linear mixed-effect (LMM) model in the R package nlme (Pinheiro et al., 2017), given the  
227 unbalanced, replicated block design. Alpha diversity was log transformed in order to help satisfy  
228 the assumption of normality of the residuals in the LMM statistical framework.

229

230 Using the community matrix data of ASVs abundance, we performed PERmutational Multivari-  
231 ate ANalysis Of VAriance tests (PERMANOVA; Anderson, 2001) to identify relationships between  
232 the communities according to the experimental design. ASVs abundance matrix was Hellinger-  
233 transformed and significance was assessed using 10,000 permutations in vegan (Oksanen et al.,  
234 2013). Blocks and replicates nested within blocks were factored as strata (*i.e.* blocks) in the model.

235

236 We also performed canonical correspondence analyses (CCAs) using the Hellinger-transformed  
237 ASVs abundance matrix in vegan (Oksanen et al., 2013) to visually assess the grouping of samples,  
238 ASVs and their association with productivity variables (*species* scaling based on ASV matrix). Data  
239 were analyzed separately for fungal-root, fungal-soil, bacterial-root and bacterial-soil, but also ac-  
240 cording to species (tomato/pepper), given that analyses of  $\alpha$ -diversity showed that tomato and  
241 pepper were markedly different. This gave a total of eight CCAs. Data were constrained based on  
242 four productivity measures (fruit number, average fruits weight, shoots fresh weight, roots fresh  
243 weight). We excluded the shoots & roots dry weights as constraints to simplify the model. In ad-  
244 dition, these were highly correlated with the fresh weight already included as constraints ( $r^2=0.98$   
245 and 0.76 for shoot dry/fresh weights and root dry/fresh weights, respectively).

246

247 Finally, we attempted to identify candidate ASVs positively associated with productivity. As such,  
248 we identified the ten ASVs most positively associated with the measures of fruit number, shoots  
249 fresh weight and roots fresh weight from each canonical correspondence analysis for a total of 40  
250 fungal and 40 bacterial candidate ASVs. We aligned candidate sequences from these candidates

251 ASVs using the Bioconductor R package `decipher` (Wright, 2016) and build pairwise distances ma-  
252 trices using a JC69 substitution models of DNA sequence evolution (equal base frequencies, Jukes  
253 & Cantor, 1969) in `phangorn` (Schliep, 2010). Phylogenetic trees (neighbour-joining) for bacteria  
254 and fungi were plotted using `ape` (Paradis, Claude & Strimmer, 2004). This permitted to identify  
255 if similar candidate ASVs were found under different experimental conditions (soil/root, pep-  
256 per/tomato), thus reinforcing their role in productivity increase and decreasing the false positive  
257 rate.

258

259 **RESULTS**

260 *Soil characteristics*

261 In Table 1, we present the characteristics of the soil collected at the IRDA research station in St-  
262 Bruno (Qc, Canada) and used in the current experimental design.

263

Table 1: Soil characteristics (in ppm unless specified otherwise)

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

264

265

266 *Plant productivity*

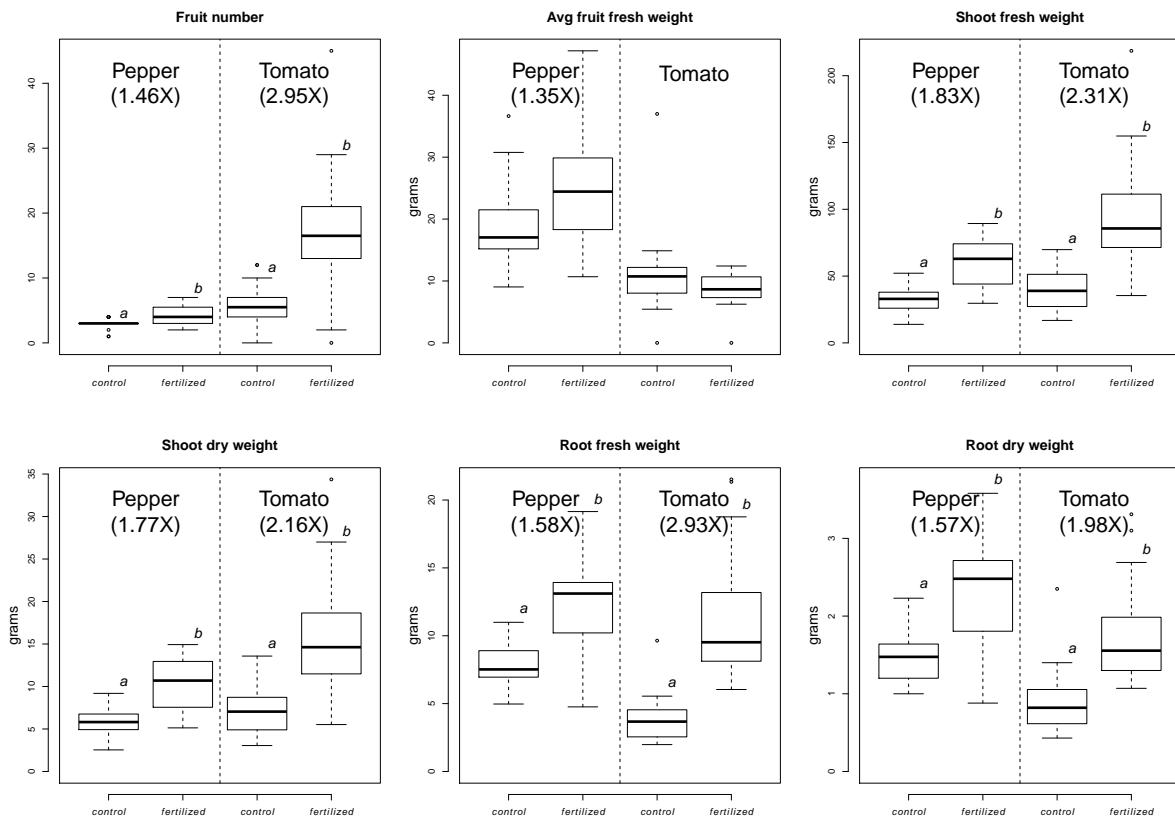
267 The effects of the fertilization treatment were tested on six measures of plant productivity (i.e. fruit  
268 number, average fruit weight, shoots fresh weight, shoots dry weight, roots fresh weight, roots  
269 dry weight) for both tomatoes and peppers. Visually, both above ground and below ground plant  
270 structure grew larger in fertilized tomato (hen manure + ANE) and pepper plants (ANE only),  
271 in addition to producing more fruits (see Figure 1 for some examples of the differences between  
272 fertilized and unfertilized plants).



273  
274 **Figure 1: Plant productivity.** Photos were taken at the end of the experimental treatment. In  
275 each photo, fertilized plants are on the left. A: pepper shoots, B: pepper roots, C: pepper fruits  
276 and D: tomato fruits.

277

278 Statistically, all six productivity measures differed significantly according to species, and five of  
279 those were significantly different according to the fertilization treatment (Figure 2). The fertil-  
280 ization effect was stronger in the tomato plants (see fold changes in Figure 2), likely due to the  
281 fact that these plants were fertilized with both hen manure and ANE. The only exception was  
282 the average fruit weight that did not differ between fertilized and control plants (LMM,  $F_{(1,69)} =$   
283 1.27,  $p\text{-value}=0.26$ ). However, the model did reveal a significant interaction between treatment  
284 and plant ( $F_{(1,69)} = 9.6$ ,  $p\text{-value}=0.0028$ ). In fact, when testing only the pepper plants, the effect of  
285 fertilization on average fruit weight was significantly higher in the fertilized pepper plants ( $F_{(1,23)}$   
286 = 10.84,  $p\text{-value}=0.0032$ ).



289 **Figure 2: Measures of plant productivity.** *a* and *b* subscripts above boxplots denote significant  
290 differences according to the fertilization treatment. Fold changes between the mean of  
291 the control and fertilized plants were also noted for significant changes (for pepper and tomato  
292 separately).

### 295 Sequencing

296 A total of 2.7 million paired-end raw reads were obtained for all samples combined (976,000 for  
297 fungi-soil, 920,000 for fungi-root, 309,000 for bacteria-soil and 535,000 for bacteria-root, Table 2).  
298 Note that sequencing samples were analyzed separately for fungal-soil, fungal-root, bacteria-soil  
299 and bacteria-root conditions. On average, 47,664 paired-end reads were obtained per sample. Af-  
300 ter quality filters were applied, including removing chimeras, and paired-end reads were merged,  
301 an average of 19,690 sequences remained per sample. From 192 soil samples for fungi and bacte-  
302 ria, and 96 root samples for fungi and bacteria sequenced, seven fungi-soil samples, 15 fungi-root

303 samples and one bacteria-root samples were removed because they had too few reads based on our  
304 strict quality thresholds.

305

306 The dada2 pipeline inferred, on average, 170 Amplicon Sequence Variants per sample (average  
307 of 176 fungal-soil ASV, 37 fungal-root ASVs, 269 bacterial-soil ASVs and 92 bacterial-root ASVs).  
308 Many of those were unique to one or a few samples (total number of 6,112 fungal-soil, 845 fungal-  
309 root, 9,352 bacterial-soil and 2,023 bacterial-roots ASVs). After quality filtering out ASVs found  
310 in fewer than 10% of the samples, we retained 413, 106, 811 and 325 ASVs. These retained ASVs  
311 comprised 94%, 95%, 89% and 98% of all reads in the fungal-soil, fungal-root, bacterial-soil and  
312 bacterial-root samples, respectively.

313

314

Table 2: Summary of sequencing and bioinformatics identi-  
fication of ASVs

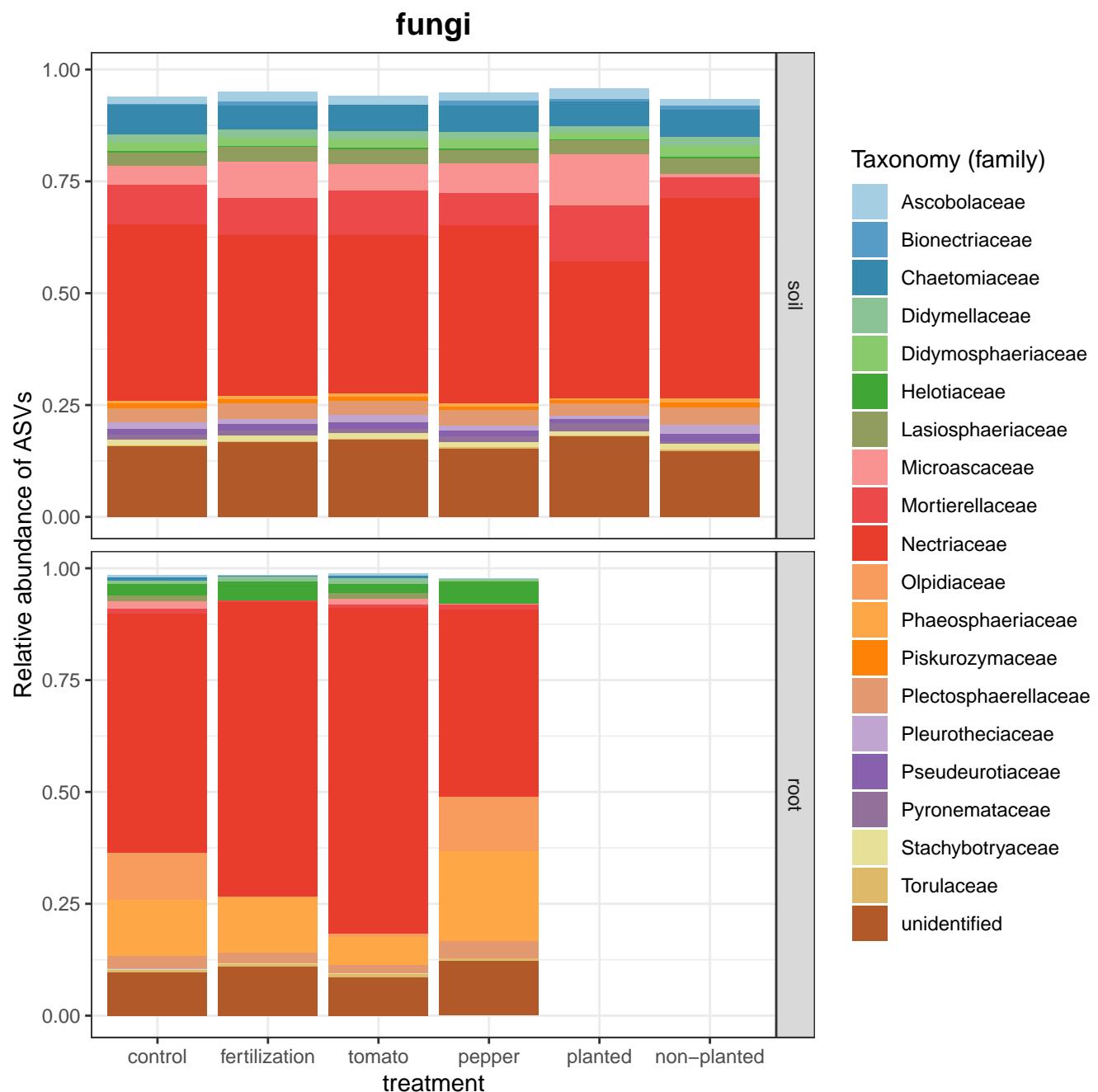
	fungi-soil	fungi-root	bacteria-soil	bacteria-root
No sequences (sum)	976,000	309,000	920,000	535,000
No sequences (mean)	50,847	32,208	47,907	56,365
No seq. filtered (mean)	32,626	12,714	29,662	37,642
No seq. filt. merged (mean)	29,300	12,094	14,060	30,706
No seq. filt. merg. no chimeras (mean)	25,476	9,849	13,521	30,408
No samples	192	96	192	96
No samples trimmed	189	81	192	95
No ASVs (sum)	6,112	845	9,352	2,023
No ASVs trimmed (sum)	413	106	811	325
ASV per sample (mean)	176	37	269	92

315

316 *Root, soil, microbial and bacterial diversity*

317 The entire community structure measured in the soil was then analyzed and the relative abundance  
 318 of taxa (family) for the fungal-soil, fungal-root, bacteria-soil and bacteria-root conditions  
 319 was reported (Figure 3a & b). Fungal communities were dominated by Nectriaceae, both in the  
 320 root and soil samples. The bacterial family Bacillaceae dominated to a lesser extent the soil commu-  
 321 nities. Bacterial root communities were largely dominated by the Cyanobacteria phylum (identi-  
 322 fied as *chloroplast* in the silva database according to the RDP Bayesian Classifier).

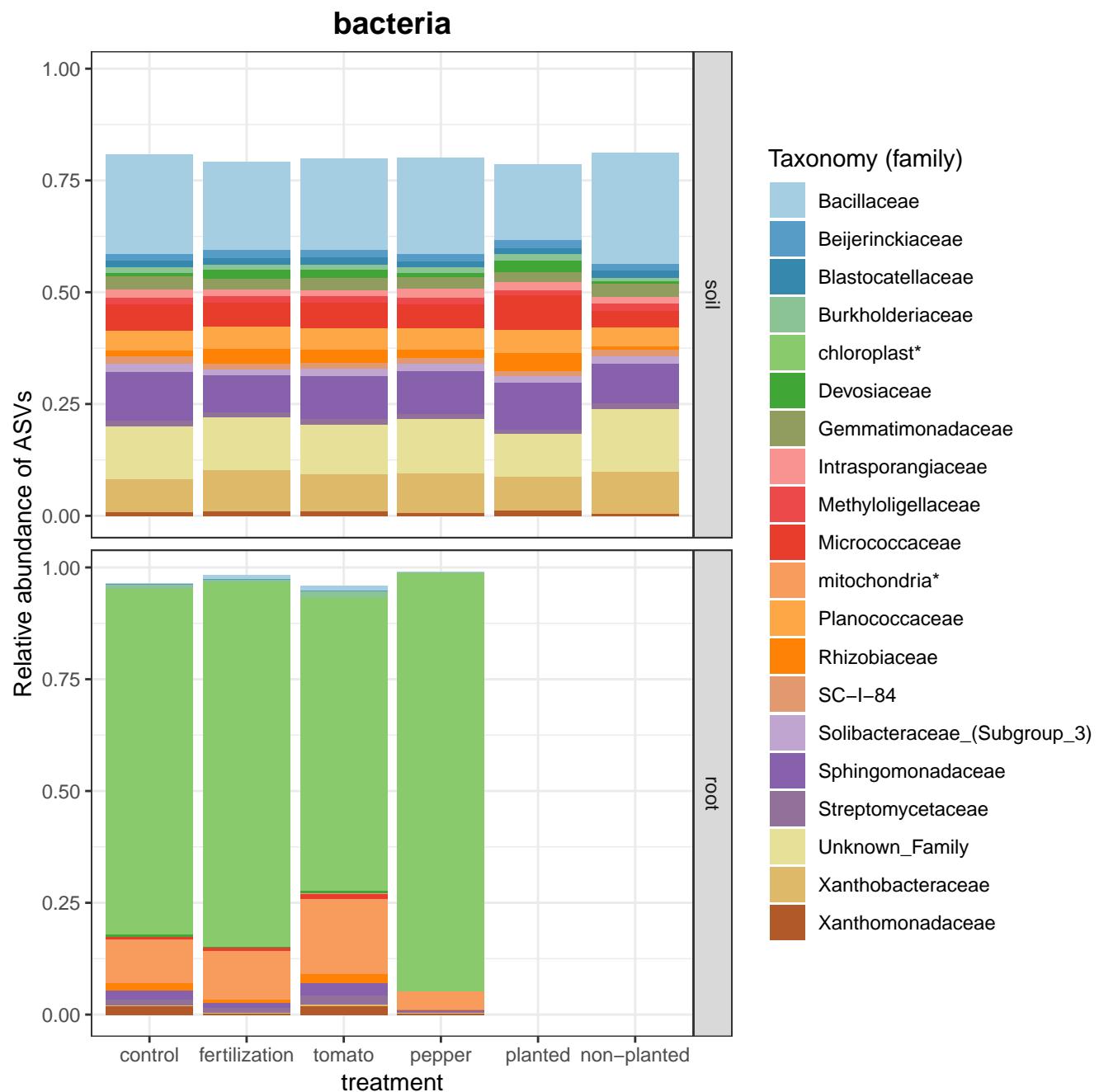
323



324

325 **Figure 3a: Barplots of the relative abundance of fungal ASVs for fungi**

326



327

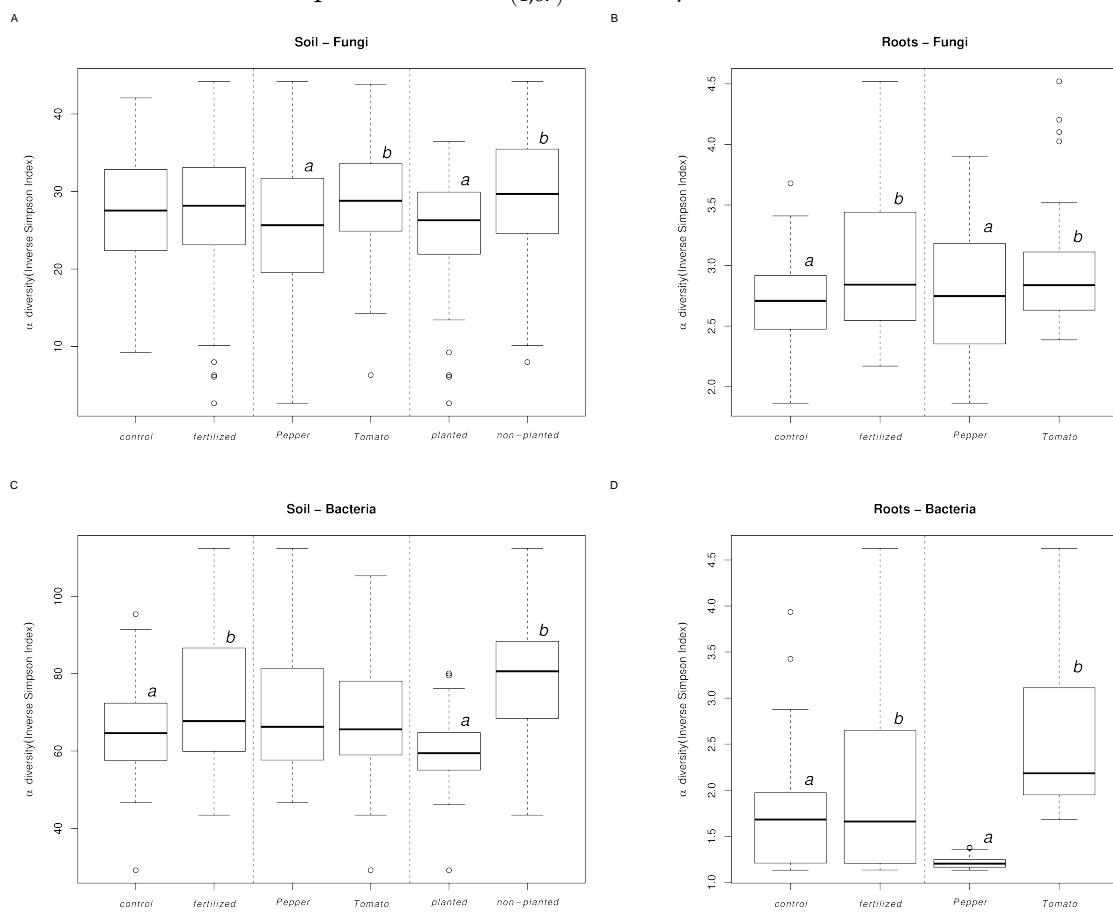
328 **Figure 3b: Barplots of the relative abundance of bacterial ASVs for bacteria**

329

330 *Local ( $\alpha$ -diversity)*

331 The diversity of each site ( $\alpha$ -diversity) was calculated separately for each sample and under each  
332 experimental condition (fungi-soil, fungi-root, bacteria-soil and bacteria-root, Figure 4). Total  $\alpha$ -

diversity was the highest in the bacteria-soil and fungi-soil samples, and the lowest in the bacteria-root. Linear mixed effects models were used to assess significance. In soil samples, fungal diversity did not differ with respect to the fertilization ( $F_{(1,161)}=0.17$ ,  $p\text{-value}=0.6853$ ), but did so with respect to planting ( $F_{(1,161)}=9.00$ ,  $p\text{-value}<0.0032$ ) and species ( $F_{(1,161)}=13.03$ ,  $p\text{-value}=0.0003$ ) treatments. In root samples, fungal diversity differed with respect to the fertilization treatment ( $F_{(1,56)}=10.1$ ,  $p\text{-value}=0.003$ ), and the species tested ( $F_{(1,56)}=4.5$ ,  $p\text{-value}=0.04$ ). In soil samples, bacterial diversity differed with respect to the fertilization ( $F_{(1,165)}=17.13$ ,  $p\text{-value}<0.0001$ ), planting ( $F_{(1,165)}=139.0$ ,  $p\text{-value}<0.0001$ ) but not species ( $F_{(1,165)}=1.89$ ,  $p\text{-value}=0.17$ ) treatments. In root samples, bacterial diversity differed with respect to the fertilization treatment ( $F_{(1,67)}=17.27$ ,  $p\text{-value}=0.0001$ ), and the species tested ( $F_{(1,67)}=359.69$ ,  $p\text{-value}<0.0001$ ).



343

Figure 4: Boxplot of  $\alpha$ -diversity according to the treatment, species and planting effect for fungal-root, fungal-soil, bacteria-soil and bacteria-root. *a* and *b* subscripts above boxplots denote significant differences.

347

348

349 *Differences in species composition among sites*

350 Using a PERMANOVA statistical framework, we identified that for all conditions, communities  
351 differed with respect to the fertilization treatment (Table 3). Soil fungal and bacterial commu-  
352 nities differed the most according to whether the tray was planted (greatest % of variance ex-  
353 plained by factor, Table 3), while root communities differed the most with respect to the species  
354 (tomato/pepper) factor.

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

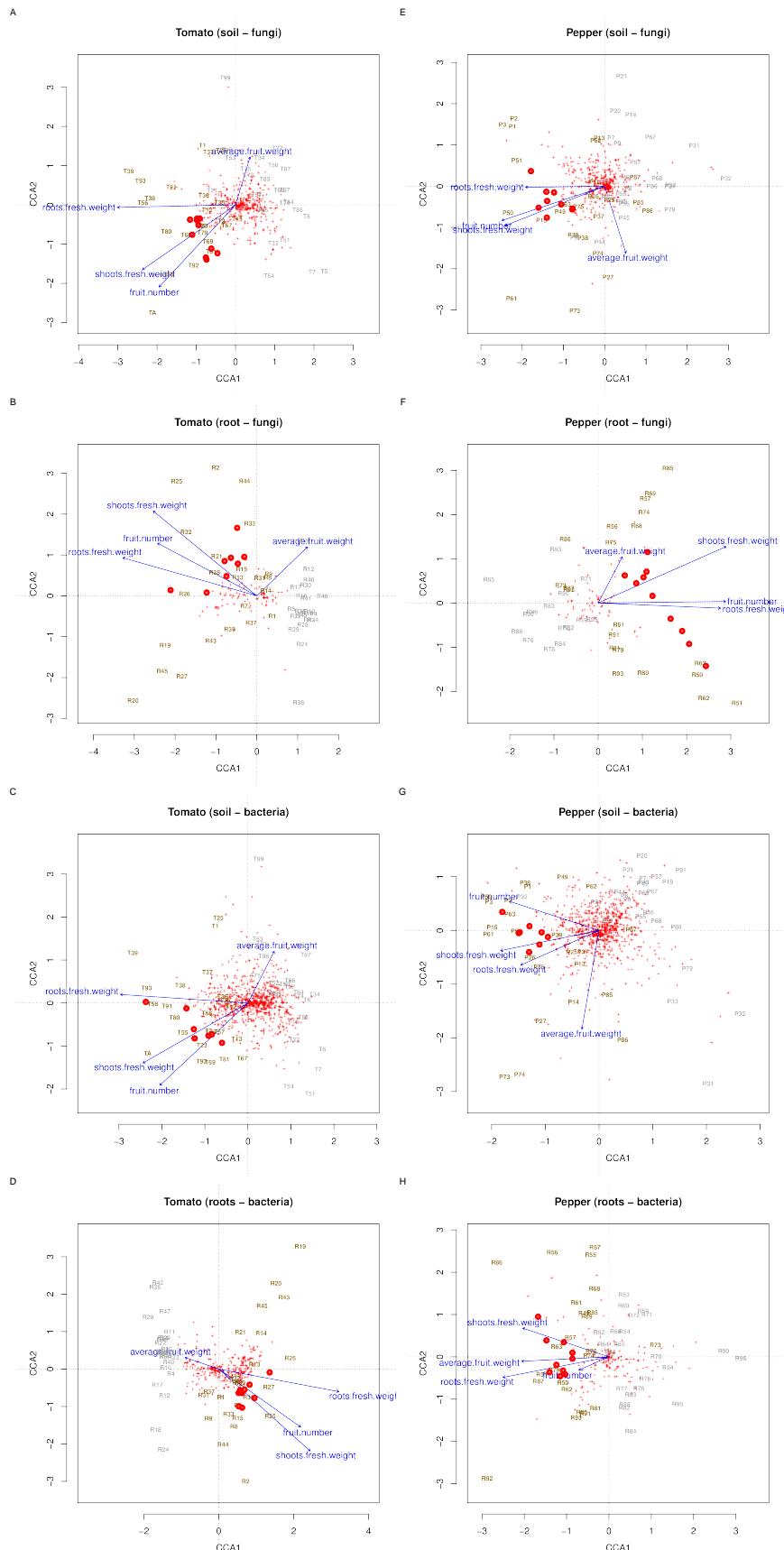
355 \* $r^2$  (percentage of variance explained by the term in the model) and associated p-values"

356

357 *Canonical correspondence analyses and candidate ASVs*

358 Canonical correspondence analyses (CCAs) indicated how fertilized samples clustered together  
359 according to their fungal or bacterial communities (Figure 5a-h). In addition, it illustrates how  
360 three of the constrain variables (productivity measures of root fresh weight, shoots fresh weight  
361 and fruit number) responded similarly, while average fruit weight behave differentially as noted  
362 previously in Figure 2 (in fact nearly orthogonally to the other three constraints in most ordina-  
363 tions).

364

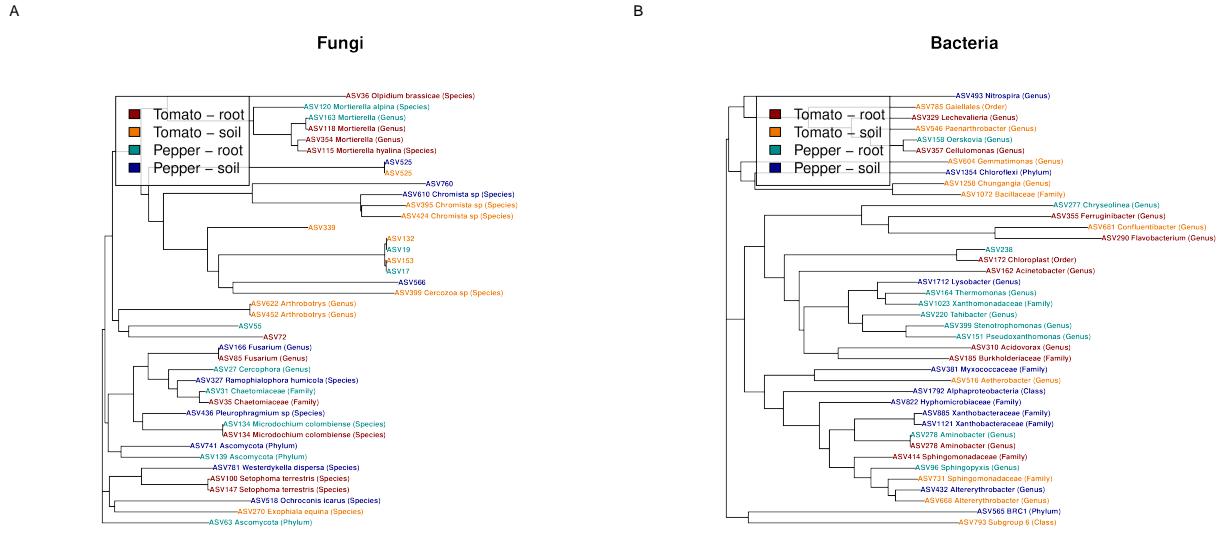


366 **Figure 5: Canonical correspondence analyses for tomato (A-D) and peppers (E-H) for soil-fungi,**  
367 **root-fungi, soil-bacteria and root-bacteria.** Samples were labeled and colored in gray (unfertil-  
368 **ized) or dark yellow (fertilized). Red crosses represent individual ASVs, while red points rep-**  
369 **resent the ten ASVs most closely associated with the three productivity measures of root fresh**  
370 **weight, shoots fresh weight and fruit number. Blue arrows are the four productivity measures**  
371 **used as constraints in the ordinations.**

372

373 Next, we identified, for each ordination, the ten ASVs most closely related to the three constraints  
374 of root fresh weight, shoots fresh weight and fruit number. These ASVs were considered as puta-  
375 tive candidate sequences most positively impacted (increase presence of the ASV) by fertilization.  
376 We further analyzed the corresponding sequences for these eighty candidate ASVs (ten candi-  
377 dates \* eight ordinations) in two separate alignments (one for fungi and one for bacterial ASVs)  
378 and their accompanying phylogenetic trees. In fungi, we identified one cluster of ASVs taxonom-  
379 ically assigned to *Mortierella* (soil saprotrophs in the phylum Zygomycota) positively associated  
380 to productivity in both tomato and pepper roots (Figure 6a). In addition, we identified a cluster  
381 of four different fungal ASVs in tomato soil (ASV132, ASV153) and pepper-root (ASV19 & AV17)  
382 closely related phylogenetically. Given that no taxonomy was assigned to these sequences through  
383 the dada2 RDP bootstrap approach, we used a BLASTn (Altschul et al., 1997) approach (against  
384 NCBI nr) to identify the most closely related sequences. We identified this cluster of ASVs as  
385 *Rhogostoma schuessleri* (BLASTn, *e*-value=4e-76), a protist in the phylum Cercozoa, which is known  
386 to be present in the rhizo and phyllo-sphere (Dumack et al., 2017). A number of putative plant  
387 pathogenic fungi were also identified such as *Fusarium sp.*, *Microdochium colombiense* or *Setophoma*  
388 *terrestris*.  
389 In bacteria-roots, we identified a large number of different ASVs most positively impacted (in-  
390 crease presence of the ASV) by fertilization (Figure 6b).

391



392

393 Figure 6: Neighbor-Joining trees of candidates ASVs (A:fungi, B:roots) positively associated  
394 with productivity measures. The most accurate taxonomy assigned according to the RDP bayesian  
395 classifier (from Phylum to species) was added as tip labels.

396

397 **DISCUSSION**

398 In the current study, we investigated the effects of *Ascophyllum nodosum* extracts (ANE) on root,  
399 shoot and fruit biomass in addition to identifying bacterial and fungal communities in tomato  
400 and pepper. Overall measures of root, shoot and fruit productivity increased in both plant species  
401 following the addition of ANE. As such, our results corroborate previous studies documenting the  
402 impact of ANE on productivity in strawberries (Alam et al., 2013) and carrots (Alam et al., 2014).

403

404 In the tomato experimental set up, the effect of fertilization was especially high, likely due to the  
405 fact that plants were also fertilized with hen manure (5-3-2, 18g per tray / 2 weeks) in addition to  
406 ANE (see Figure 2). This was not the case for the pepper plants and the increase in productivity  
407 was solely due to the addition of ANE. The commercial extract (Stella Maris®, Acadian Seaplants  
408 Ltd) used contained about 1% nitrogen, 0.5% phosphorus, 15% potassium, 0.4% calcium, 0.4%  
409 magnesium, 155 ppm iron, 121 ppm manganese, 5 ppm copper, 91 ppm zinc, and 124 ppm boron.  
410 In the current experimental setup, ANE was diluted to 3.5 g / L prior to application (250ml per  
411 tray / 2 weeks). In fact, in the tomato plants the amounts of Nitrogen and Phosphorus supplied  
412 via the application of ANE were ~100 less than from the hen manure itself. As such, these nutrients  
413 were given at very low concentrations relative to the crop requirements and are not expected to  
414 significantly impact growth relative to a regular agricultural fertility program (Alam et al., 2013).  
415 Instead, bioactive compounds such as betaines, polyamines, cytokinins, auxins, oligosaccharides,  
416 amino acids and vitamins have been found to have overall beneficial productivity effects on plant  
417 growth (Khan et al., 2009; Craigie, 2010, 2011; Battacharyya et al., 2015)

418

419 Here, one of primary goal of the study was to document how bacterial and fungal communities  
420 responded to the addition of ANE. We used a metabarcoding high throughput sequencing ap-  
421 proach targeting DNA regions specific to all fungi (ITS) and bacteria (16S). Then, we identified  
422 bacterial and fungal taxa present in the samples using a relatively novel bioinformatics approach  
423 developed by Callahan et al. (2016). The approach, based on the widely used programming lan-  
424 guage R (Team, 2018), identifies unique, non-clustered sequences (ASVs) that are then comparable  
425 among studies. In addition, the current analytical pipeline uses a bayesian classifier for taxonomy

426 rather than the widely used BLAST approach, thus providing more conservative, but more accu-  
427 rate taxonomic identifications (Wang et al., 2007).

428

429 In the current experimental set up, most ASVs identified were rare and unique to one or a few  
430 sample. In fact, ~90% of all ASVs were discarded given that they were found in very few sam-  
431 ples and were thus not representative of a particular experimental treatment. Nevertheless, these  
432 'rare' ASVs comprised a small minority of all sequencing reads (~5% of all sequences), a pattern  
433 reminiscent of the early species abundance models showing that in most ecological communities,  
434 few species are exceptionally abundant whereas most are rare (Fisher, Corbet & Williams, 1943).

435

436 The total number of ASVs per site (*a*-diversity) for bacteria and fungi significantly differed with  
437 respect to the fertilization treatment in root and soil (only for bacteria) samples, but these effects  
438 were small (Figure 5). Nectriaceae, a family of fungi in the order Hypocreales and often encoun-  
439 tered as saprophytes on decaying organic matter comprised most of the diversity both in the soil  
440 and plant roots (between 25-70% of the total number of sequencing reads, Figure 4a). With respect  
441 to soil bacteria, communities were much more diverse and comprised many different families  
442 (Figure 4b). Surprisingly, most sequencing reads in the root-bacteria communities likely originate  
443 from the plants themselves (identified as chloroplastic or mitochondrial in origin in Figure 4b),  
444 despite the fact that the DNA primers pair used should have primarily targeted the bacterial V3-  
445 V4 region of the 16S ribosomal gene.

446

447 Species composition among sites (*b*-diversity) differed according to the fertilization treatment in  
448 all four communities (fungal-root, fungal-soil, bacterial-root and bacterial-soil). This fertilization  
449 effect was small (2-7% of variance explained in the models, Table 3), but significant implying that  
450 the addition of ANE (pepper) or ANE and hen manure (tomato) has a small impact on microbial  
451 communities. In fact, most of the variance in soil communities was explained by the planting ef-  
452 fect, showing how plants can alter their microbiome. In root communities, the communities were  
453 strongly dependent of the actually species that was planted (tomato/pepper).

454

455 **(This paragraph still needs work)** We also aimed to identify specific candidate taxa positively as-

456 sociated with increased plant productivity following the addition of ANE. Here, we discuss some  
457 of the candidates. In fungi, we identified one cluster of ASVs taxonomically assigned to *Mortierella*  
458 (soil saprotrophs in the phylum Zygomycota) positively associated to productivity in both tomato  
459 and pepper roots. In addition, we identified several fungal ASVs in tomato soil and pepper-root  
460 linked to productivity. These were assigned to *Rhogostoma schuessleri* (*BLASTn*, *e-value*=4e-76), a  
461 protist in the phylum Cercozoa, which is known to be present in the rhizo and phyllo-sphere (Du-  
462 mack et al., 2017). Surprisingly, a number of putative plant pathogenic fungi were also identified  
463 such as *Fusarium sp.*, *Microdochium colombiense* or *Setophoma terrestris*. In bacteria roots samples, a  
464 diverse number of ASVs were positively impacted by fertilization (Figure 6).

465

466 **(conclusion still needs work too)** It is now well established that seaweed extracts have a posi-  
467 tive effect on agricultural plant productivity. Concurrently, DNA barcoding now permit a more  
468 comprehensive understanding of the diversity and ecology of microbial organisms and how they  
469 interact. In fact, plants and microbes should likely be redefined as *holobionts*, an assemblage of dif-  
470 ferent species that form an ecological unit (Margulis & Fester, 1991). Further studies, for example  
471 using inoculum of microbial species linked to the presence of liquid seaweed extract, may help to  
472 identify a causative link between extracts, microbes and productivity.

473

474

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