Seaweed extracts strongly structured microbial communities associated with tomato and pepper roots and significantly increased crop yield

Sébastien Renaut[1,2],Jacynthe Masse[1,2], Jeffrey P. Norrie[3], Bachar Blal[3] Mohamed Hijri[1,2]

[1]Département de Sciences Biologiques, Institut de Recherche en Biologie Végétale, Université de Montréal, 4101 Sherbrooke Est, Montreal, H1X 2B2, Quebec, Canada. [2]Quebec Centre for Biodiversity Science, Montreal, Quebec, Canada [3]Acadian Seaplant Ltd, 30 Brown Avenue, Darthmouth, Nova Scotia, Canada, B3B 1X8

Seaweeds and their derivatives have been used as a source of natural fertilizer and biostimulant in agriculture and horticulture for centuries. However, their effects on soil and crop roots microbiota remain unclear. Here, we used a commercially available *Ascophyllum nodosum* extract in order to test its effect on bacterial and fungal communities of rhizospheric soils and roots of pepper and tomato plants in greenhouse trials. Two independent greenhouse trials were conducted using tomato and pepper plants grown in natural soil in a split block design with four treatments (planted, non-planted, fertilized and non-fertilized). We used amplicon sequencing targeting fungal ITS and bacterial 16S rRNA gene to determine microbial community structure changes in the rhizosphere soil and root biotopes. We find that all productivity measures of root, shoot and fruit biomass differed significantly according to crop species, and most of those were significantly greater according to the fertilization treatment. In addition, *a*-diversity differed according to fertilization, but this effect was small. Species composition among sites (*b*-diversity) differed according to fertilization in all four communities measured (fungal-root, fungal-soil, bacterial-root and bacterial-soil). Finally, we identified a number of candidate taxa most strongly correlated with crop yield increases. Further studies on isolation and characterization of these microbial taxa that are linked to the application of liquid seaweed extract may help to enhance crop yield and sustain agro-ecosystems.

Keywords: Stella Maris®, 16S, ITS, soil microbial diversity, Illumina MiSeq, ANE, Amplicon Sequence Variants, OTU

INTRODUCTION

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

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

Starting in the 1990's, high quality ANE was developped and let to an increased usage by

farmers, in addition to an increase in cause-effect research, especially on plant diseases (Jayaraj & Ali, 2015). Noted increases in the activity of superoxide dismutase, glutathione peroxidase and ascorbate peroxidase helped support the argument that ANE improve plant tolerance to oxidative stress (Ayad et al., 1997; Schmidt & Zhang, 1997; Ayad, 1998; Allen et al., 2001). Positive effects were also found on phytoalexin production suggesting that ANE may be involved in suppressing disease infection through increased activity of these protective enzymes that target oxidizing toxins naturally emitted by disease pathogens (Lizzi et al., 1998; Jayaraj et al., 2008; Jayaraman, Norrie & Punja, 2010).

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

Although there is an abundance of published evidence detailing systemic plant effects from ANE, outstanding questions remain as to the effects of ANE on the rhizosphere biology. Various microbes, small arthropods, nematodes and insects thrive in the soil rhizosphere. This microbial biodiversity then contributes to the aggregation of soil particles, enhances nutrient cycling and delivery to plants, degrades toxic substances, allows better soil water retention and plays a role in plant disease management. It has been suggested that the plant immune system is composed of inherent surveillance systems that perceive several general microbial elicitors, which allow plants to switch from growth

and development into a defense mode (Newman et al., 2013). This may allow the plant to avoid infection from potentially harmful microbes. An examination of sustainable products that can positively influence microbial interactions between plant roots and soil biota will in turn help to further understand soil borne plant-pathogens competition dynamics. The effect of ANE on the bacterial profile suggests that ANE applications increased strawberry root and shoot growth, berry yield and rhizosphere microbial diversity and physiological activity (Alam et al., 2013). Similar results were found in sandy loam soils as Alam and colleagues Alam et al. (2014) showed a strong relationship between carrot growth, soil microbial populations and activity.

The recent development of culture-independent molecular techniques and high throughput sequencing should permit to circumvent the inherent biases of culture-based approaches by targeting the ubiquitous component of life, DNA. In turn, this will help to identify a larger proportion of the microbial diversity and lead to a better understanding of the soil microbial response to seaweed extract. DNA barcoding targeting specific regions of the genome (e.g. ITS: fungi, 16s ribosomal genes: bacteria) are now regarded as a prerequisite procedure to comprehensively document the diversity and ecology of microbial organisms (Toju et al., 2012; Klindworth et al., 2013).

Here the general objective was to quantify the impact of ANE on plant growth and test how the bacterial and fungal communities responded to the addition of these extracts. We also aimed to identify specific taxon positively correlated with increases in plant productivity following ANE amendments. We hypothesized that the addition of liquid seaweed extracts would improve productivity and alter significantly the bacterial and fungal communities. We used a commercially available ANE, Stella Maris®, developed by Acadian Seaplants Ltd (NS, Canada). Stella Maris® is derived from the marine algae *A. nodosum*, and harvested from the nutrient-laden waters of the North Atlantic off the Eastern Coast

of Canada. We tested the effect of ANE on two agricultural plants commonly grown in greenhouse conditions (tomato and pepper). Several traits related to plant productivity were measured and soil and root bacterial and fungal diversity were quantified using High Throughput Illumina (San Diego, CA, USA) Miseq sequencing.

MATERIAL AND METHOD

Experimental design

Greenhouse trials were set up in large trays (60x30x18 cm LxWxH) using two different crops: tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.). Tomato cultivar Totem Hybrid#A371 was planted in November 16th 2015 and pepper cultivar Ace Hybrid#318 was planted in December 9th 2015. Tomato and pepper seeds were purchased from William Dam Seeds Ltd (ON, Canada). These cultivars were selected for greenhouse production. Soil was collected from an agricultural field under organic regime at the IRDA research station in St-Bruno (Qc, Canada, 45º32'59.6"N, 73º21'08.0"W) on October 7th 2015. The soil was a loamy sand and was collected from the 15 cm top layer. Natural soil was mixed and put into trays, filled to 15 cm in height. Soil analysis was done using a commercial service provided by Environex (formerly AgriDirect, Longueuil, QC) and soil characteristics are shown in Table S1. Eight seeds per tray were planted and after germination, only four seedlings per tray were kept.

For each crop species, a randomized split block design (Table S2) was used with four trays set up per block and eight blocks for each trial. Half of the trays were fertilized (fertilization treatment), as described below. Half of the trays were also planted (planting treatment) with four plants per tray, while the other trays were not planted. This allowed a direct comparison of fungal and bacteria soil communities with respect to fertilization and planting treatments.

Two different fertilization regimes were used according to the plant species. For tomatoes, 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, QC) in addition to Stella Maris® (3.5 ml per 1L, each tray received 250 ml, repeated every

2 weeks) for the duration of the experiment. The other half was not fertilized, but watered with 250ml per tray instead. The physico-chemical composition of Stella Maris® is shown in Table S3. For the pepper experiment, the fertilization regime consisted solely of Stella Maris® (3.5 ml per 1L, each tray received 250 ml, repeated every 2 weeks) for the duration of the experiment. The other half was not fertilized but watered with 250 ml per tray instead. Both experiments were managed under organic farming practices. Thrips were controlled using *Neoseiulus cucumeris* (syn. *Amblyseius cucumeris*) (1 bag per plant), Fungus gnats were also controlled using predatory mite *Gaeolaelaps gillespiei* (1L; Natural Insect Control, ON). Plants were treated once a week with Milstop, a Potassium Bicarbonate-based foliar fungicide to control the powdery mildew on both crops.

Plant productivity

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

Sample preparation, DNA extraction and High throughput sequencing

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

fungal ITS was performed on both root and soil samples.

For fungal ITS, we used the following primers with the universal CS1 and CS2 adapters: CS1_ITS3_KYO2 (5'-ACA CTGA CGA CAT GGT TCT ACA GAT GAA GAA CGY AGY RAA-3') and CS2_ITS4_KYO3 (5'-TAC GGT AGC AGA GAC TTG GTC TCT BTT VCC KCT TCA CTC G-3') to produce a final amplicon size of approximately 430bp including adapters (Toju et al., 2012).

For bacterial 16S, we used the following primers with CS1 and CS2 universal adapters: 341F (5'-CCT ACG GGN GGC WGC AG-3') and 805R (5'-GAC TACC AGG GTA TCT AAT C-3') to produce a final amplicon size of approximately 460 bp and targeting specifically the bacterial V3-V4 region of the 16S ribosomal gene (Klindworth et al., 2013).

DNA samples were then barcoded, pooled and sequenced (2X300bp, paired-end) using an Illumina MiSeq sequencer through a commercial service provided by the Genome Quebec Innovation Centre (Montreal, QC). Sequences were demultiplexed by the sequencing facility and further processed as described below.

Bioinformatics

All bioinformatics, statistical, and graphical analyses further described were performed in R 3.5.1 (R Core Team, 2018) and detailed scripts are available here (https://github.com/seb951/Acadian_Seaplants).

We used the `R` package `DADA2` (Callahan et al., 2016) to infer *Amplicon Sequence Variants* (ASV). `DADA2` offers accurate sample inference from amplicon data with single-nucleotide resolution in an open source environment. Unlike the Operational Taxonomic Unit (OTU) approach (e.g. Schloss et al., 2009; Caporaso et al., 2010), ASV are not treated as cluster of

sequences defined with an *ad hoc* sequence similarity threshold. Instead, after sequences are quality trimmed and error-corrected, DADA2 reveals the unique members of the sequenced community, thus allowing sequences and abundance counts to be comparable among studies (Callahan et al., 2016).

First, sequences were trimmed following strict quality thresholds (removing primers and low quality nucleotides, see parameter details in the accompanying R scripts). Following this, we applied the error model algorithm of DADA2, which incorporates quality information after filtering, unlike other OTU based methods. Then dereplication, sample inference, merging of paired end reads and removal of chimera were performed in order to obtain a sequence (ASV) table of abundance per sample. Taxonomy was assigned through the DADA2 pipeline using the Ribosomal Database Project (RDP) Naive Bayesian Classifier algorithm from Wang *et al.* (2007). Depending on support (minimum bootstrap support of 80), we assigned taxonomy from Kingdom to species. We used the silva database formatted for DADA2 to infer bacterial taxa (Callahan, 2018). We used the Unite (Community, 2018) fasta release (including singletons) to infer fungal taxa after formatting it to the DADA2 format using a custom R script. The pipeline was run on a multithreaded (48 CPUs) computer infrastructure provided by Westgrid (https://www.westgrid.ca/support/systems/cedar) and Compute Canada (www.computecanada.ca). Note that the pipeline was run separately for fungal-root, fungal-soil, bacteria-soil and bacteria-root samples given the markedly different nucleotide compositions of the sequenced amplicons, unique taxa and specific error models of each dataset.

Statistical analyses - plant productivity

We tested for the effect of species (tomato vs pepper), fertilization and their interaction on six plant productivity measures (fruit number, average fruit weight, shoots fresh weight, roots fresh weight, shoots dry weight, roots dry weight). We used Linear Mixed effect

Models (LMM) in the R package NLME (Pinheiro et al., 2017), which are more appropriate than an Analysis of Variance (ANOVA) given the current block design (blocks and replicates were treated as random variables). All six plant productivity measures were either square root or log transformed in order to help satisfy the assumption of normality of the residuals in the LMM statistical framework. For the variables *fruit number* and *average fruit weight*, we also verified statistical significance using a permutation-based 2-way ANOVA (Anderson & Legendre, 1999) given that the residuals of the LMM were not normally distributed. Results were similar according to the 2-way ANOVA.

Statistical analyses - microbial and fungal diversity

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

We then conducted community-based analyses looking at the effect of the fertilization treatment on ASV abundance in the tomato and pepper experiments. To reduce the complexity of the datasets, relative abundance of all taxa was calculated per family using the R package DPLYR (Wickham et al., 2015). Barplots were drawn using GGPLOT2 (Wickham, 2016) to visualize communities. ASV alpha (a)-diversity was calculated based on all ASV (excluding rare ASV, see paragraph above) for each sample using the inverse Simpson diversity index in VEGAN (Oksanen et al., 2013). The effect of the fertilization treatment,

species (and planting for soil communities) were assessed using a Linear Mixed effect Model (LMM) model in the R package `NLME` (Pinheiro et al., 2017), given the unbalanced, replicated block design. Alpha diversity was *log* transformed in order to help satisfy the assumption of normality of the residuals in the LMM statistical framework.

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

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

Finally, we attempted to identify candidate ASV positively associated with productivity. As such, we identified the ten ASV most positively associated with the measures of fruit number, shoots fresh weight and roots fresh weight from each canonical correspondence analysis for a total of 40 fungal and 40 bacterial candidate ASV. We aligned candidate se-

quences from these candidates ASV using the Bioconductor R package DECIPHER (Wright, 2016) and build pairwise distances matrices using a JC69 substitution models of DNA sequence evolution (equal base frequencies, Jukes & Cantor, 1969) in PHANGORN (Schliep, 2010). Phylogenetic trees (neighbour-joining) for bacteria and fungi were plotted using APE (Paradis, Claude & Strimmer, 2004). This permitted to identify if similar candidate ASV were found under different experimental conditions (soil/root, pepper/tomato), thus reinforcing their role in productivity increase and decreasing the false positive rate.

RESULTS

The effects of the fertilization treatment were determined by measuring six agronomic parameters (fruit number, average fruit weight, shoots fresh weight, shoots dry weight, roots fresh weight, roots dry weight) for both tomatoes and peppers. We observed a significant increase of all these agronomic parameters for fertilized plants except for the average fruit fresh weight for tomato that did not differ between fertilized and control plants (LMM, $F_{(1,69)} = 1.27$, p-value=0.26, Figure 1 and Figure S1). The fertilization effect was stronger in the tomato plants (fold changes between fertilized and control plants shown in Figure 1), likely due to the fact that these plants were fertilized with both hen manure and ANE. In addition, the model revealed a significant interaction between treatment and plant ($F_{(1,69)} = 9.6$, p-value=0.0028). In fact, when testing only the pepper plants, the effect of fertilization on average fruit weight was significantly higher in the fertilized pepper plants ($F_{(1,23)} = 10.84$, p-value=0.0032).

Amplicon Sequencing

A total of 2.7 million paired-end raw reads were obtained for all samples combined (976,000 for fungi-soil, 920,000 for fungi-root, 309,000 for bacteria-soil and 535,000 for bacteria-root, Table S4). We analyzed separately the sequence datasets for fungal-soil, fungal-root, bacteria-soil and bacteria-root conditions. On average, 47,664 paired-end reads were obtained per sample. After quality filters were applied, including removing chimeras, and paired-end reads were merged, an average of 19,690 sequences remained per sample. From 192 soil samples for fungi and bacteria, and 96 root samples for fungi and bacteria sequenced, seven fungi-soil samples, 15 fungi-root samples and one bacteria-root samples were removed because they had to few reads based on our strict quality thresholds.

The DADA2 pipeline inferred, on average, 170 Amplicon Sequence Variants (ASV) per sample (average of 176 fungal-soil ASV, 37 fungal-root ASV, 269 bacterial-soil ASV and 92 bacterial-root ASV). Many of these were unique to one or a few samples (total number of 6,112 fungal-soil, 845 fungal-root, 9,352 bacterial-soil and 2,023 bacterial-roots ASV). After quality filtering, we retained 413, 106, 811 and 325 ASV respectively for fungal-soil, fungal-root, bacterial-soil and bacterial-roots. These retained ASV comprised 94%, 95%, 89% and 98% of all reads in the fungal-soil, fungal-root, bacterial-soil and bacterial-root samples, respectively.

Fungal and bacterial diversity in root and soil biotopes

The microbial community structures of soil and root samples were analyzed and the relative abundance of their taxa was determined at the family level (Figures 2 & 3). Fungal communities were dominated by Nectriaceae, both in the root and soil samples, while the bacterial family Bacilaceae dominated to a lesser extent the soil samples. Bacterial root communities were largely dominated by Cyanobacteria (identified as *chloroplast* in the silva database according to the RDP Bayesian Classifier).

Local (a-diversity)

The *a*-diversity of each biotope (soil or root) was calculated separately for each sample and under each experimental condition (fungi-soil, fungi-root, bacteria-soil and bacteria-root, Figure 4). Linear mixed effects models showed that the *a*-diversity was significantly higher in the soil biotope that in the roots for both fungi and bacteria.

In soil samples, fungal *a*-diversity was significantly different in planted compared to non-planted treatments ($F_{(1,161)}$=9.0, p-value=0.0032) and tomato versus pepper ($F_{(1,161)}$=13.03, p-value=0.0003), while no significant change was observed in fertilized versus non-fertilized treatments ($F_{(1,161)}$=0.17, p-value=0.6853). In root samples, fungal *a*-diversity was signifi-

cantly different in fertilized versus non-fertilized treatments ($F_{(1,56)}$=10.1, p-value=0.003) and tomato versus pepper ($F_{(1,56)}$=4.5, p-value=0.04).

In soil samples, bacterial *a*-diversity was significantly different in fertilized versus non-fertilized treatments ($F_{(1,165)}$=17.13, p-value<0.0001), in planted compared to non-planted treatments ($F_{(1,165)}$=139.0, p-value<0.0001), while no significant change was observed in tomato versus pepper ($F_{(1,165)}$=1.89, p-value=0.17). In root samples, bacterial*a*-diversity was significantly different in fertilized versus non-fertilized treatments ($F_{(1,67)}$=17.27, p-value=0.0001), and tomato versus pepper ($F_{(1,67)}$=359.69, p-value<0.0001).

Differences in species composition among sites

Using a PERMANOVA, the fertilization treatment had a highly significant effect on both fungal and bacterial community structures (Table 1). Planting also had a significant effect (greatest % of variance was explained by the planted factor) on fungal and bacterial community structures. Plant identity (tomato/pepper) significantly influenced the fungal and bacterial community structures in roots.

Canonical correspondence analyses (CCAs, Figures 5 for fungi and Figure 6 for bacteria) illustrated that roots fresh weight, shoots fresh weight and fruit number responded similarly, while average fruit weight behaved differentially as noted previously in (in fact nearly orthogonally to the other three parameters in most ordinations). In addition, it showed that fertilized samples clustered together and were positively correlated with increases in these four productivity measures.

Next, we identified, for each ordination, the ten ASV most closely related to the three constraints of the model (roots fresh weight, shoots fresh weight and fruit number). These ASV were considered as putative candidate taxa most positively impacted by increases in productivity due to the fertilization treatment. We further analyzed the corresponding sequences for these eighty candidate ASV (ten candidates * eight ordinations) in two

separate alignments (one for fungi and one for bacterial ASV) and their accompanying phylogenetic trees.

In fungi, we identified one cluster of ASV taxonomically assigned to *Mortierella* (soil saprotrophs in the phylum Mucoromycota) positively associated to productivity in both tomato and pepper roots (Figure S2). In addition, we identified a cluster of four different fungal ASV in tomato soil (ASV132, ASV153) and pepper-root (ASV19 & ASV17) closely related phylogenetically. Given that no taxonomy was assigned to these sequences through the `DADA2` RDP bootstrap approach, we used a BLASTn (Altschul et al., 1997) approach to identify the most closely related sequences against NCBI nr. However, the most closely related reference sequences were from uncultured fungus clones (BLASTn, 88% identity, e-value=1e-55). The remaining ASV were identified as several different species such as *Fusarium sp.*, *Microdochium colombiense* or *Setophoma terrestris*, known as endophytes or pathogens.

In bacteria-roots, we identified a large diversity of ASV positively correlated (increased abundance of these ASV) with the four measures of productivity. Phylogenetic analyses did not reveal clusters of ASV associated with increases in productivity in the four different experimental conditions (Figure S3).

DISCUSSION

In the current study, we investigated the effects of *Ascophyllum nodosum* extracts (ANE) on root, shoot and fruit biomass in addition to identifying bacterial and fungal communities in tomato and pepper. Overall parameters related to plant growth (root, shoot and fruit weights) significantly increased in both plant species in response to ANE application. These results corroborate previous studies documenting the impact of ANE on productivity in strawberries (Alam et al., 2013) and carrots (Alam et al., 2014).

In the tomato experimental set up, the effect of fertilization was especially high, likely due to the fact that plants were also fertilized with hen manure in addition to ANE (see Figure 1). This was not the case for the pepper plants and the increase in productivity was solely due to the addition of ANE. The commercial extract used in this investigation contained about 0.1% nitrogen, 0.2% phosphorus, 5% potassium, along with several micronutrients (Table S3) and it is sold as a complement to fertilizers because it contains all microelements required for plant growth. In the current experimental setup, ANE was diluted to 3.5 ml/L prior to application (250 ml per tray every two weeks). In fact, in the tomato plants the amounts of N and P supplied via the application of ANE were 200-1000 times less than from the hen manure itself. As such, these nutrients were given at very low concentrations relative to the crop requirements and are not expected to significantly impact growth relative to a regular agricultural fertility program (**???**). Instead, organic molecules such as betaines, polyamines, cytokinins, auxins, oligosaccharides, amino acids and vitamins present in ANE have been found to have overall beneficial productivity effects on plant growth (Khan et al., 2009; Craigie, 2010, 2011; Battacharyya et al., 2015).

One of primary goal of the study was to document how bacterial and fungal communities responded to the addition of ANE. We used a metabarcoding high throughput sequencing

approach targeting DNA regions specific to fungi (ITS) and bacteria (16S). Then, we identified bacterial and fungal taxa present in the samples using a relatively novel bioinformatics approach developed by Callahan et al. (2016). The approach, based on the widely used programming language R (R Core Team, 2018), identifies unique, non-clustered sequences (ASV) that are then comparable among studies. In addition, the current analytical pipeline uses a bayesian classifier for taxonomy rather than the widely used BLAST approach, thus providing more conservative, but more accurate taxonomic identifications (Wang et al., 2007).

In the current experimental set up for both plants, most ASV identified were rare and unique to one or a few sample. In fact, approximately 90% of all ASV were discarded given that they were found in singletons or present in very few samples and were thus not representative of a particular experimental treatment. These 'rare' ASV comprised a small minority of all sequencing reads (approximately 5% of all sequences), a pattern reminiscent of the early species abundance models showing that in most ecological communities, few species are exceptionally abundant whereas most are rare (Fisher, Corbet & Williams, 1943).

The fertilization treatment had a significant effect on both fungal and bacterial *a*-diversity (total number of ASV) in the root biotope. In the soil biotope, it only had a significant effect for bacteria (Figure 4). Nectriaceae, a family of fungi in the order Hypocreales and often encountered as saprotrophes on decaying organic matter comprised most of the diversity both in the soil and plant roots (between 25-70% of the total number of sequencing reads, Figure 2). With respect to bacterial communities of the soil, theses were much more diverse and comprised many different families (Figure 3). Surprisingly, most sequencing reads in the bacterial communities of roots likely originate from the plants themselves (identified as chloroplastic or mitochondrial in origin in Figure 3), despite the fact that

the DNA primers pair used should have primarily targeted the bacterial V3-V4 region of the 16S ribosomal gene.

Fertilization treatment significantly influenced fungal and bacterial community composition (*b*-diversity) among root and soil biotopes. This fertilization effect was small (2-7% of variance explained in the models, Table 1) but significant, implying that the adddition of ANE (pepper) or ANE and hen manure (tomato) has a small impact on microbial communities. In fact, most of the variance in soil communities was explained by the planting effect, showing how plants can alter their microbiome. In the root biotope, the microbial communities were strongly influenced by plant identity, which is in line with numerous studies which reported that plants select their microbial communities (Chaparro, Badri & Vivanco, 2014; Reinhold-Hurek et al., 2015).

We also aimed to identify candidate taxa positively correlated with increased plant productivity in response to ANE application. In fungi, one cluster of ASV taxonomically assigned to *Mortierella* (soil saprotrophs in the phylum Zygomycota) was positively correlated to productivity in both tomato and pepper roots. In their study, Chung et al. (2007) showed how higher plant species richness and increase in productivity led to greater microbial biomass and greater number of saprophytic and arbuscular mycorrhizal fungi. Perhaps, this can be explained by the fact that microbial communities experienced greater substrate availability, potentially increasing their activity, and the activity of saprophytic fungi feeding on organic matter.

In addition, we identified several fungal ASV in tomato soil and pepper-root linked to increases in productivity. A number of putative plant pathogenic fungi were also identified such as *Fusarium sp.*, *Microdochium colombiense* or *Setophoma terrestris* (Figure S2). In bacteria roots samples, a diverse number of ASV were positively impacted by fertilization

(Figure S3). The specific role of those taxa on crop productivity will need further investigations.

It is now well established that seaweed extracts have a positive effect on agricultural plant productivity. Concurrently, DNA barcoding permits a more comprehensive understanding of the diversity and ecology of microbial organisms and how they interact. In fact, plants and microbes should likely be redefined as *holobionts*, an assemblage of different species that form an ecological unit (Margulis & Fester, 1991). In this study, we showed that the addition of ANE increased plant productivity. It also increased, by a small, but significant margin, the fungal and bacterial (only in the rhizosphere) biodiversity and changed the microbial community structure in the roots and in the rhizosphere of the plants. Finally, we identified bacterial and fungal taxa, especially saprotroph, that were positivity associated with plant productivity. Further studies, for example using inoculum of microbial species linked to increases in productivity and the presence of liquid seaweed extract, may help to identify a causative link between extracts, microbes and productivity.

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