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Comparative metagenomics reveals alterations in the soil bacterial community driven by N-fertilizer and Amino 16® application in lettuce

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ABSTRACT

Nutrients in the form of fertilizers and/or other additives such as amino acids, dramatically influence plant development and growth, plant nutrient composition and the level of soil pollution. Moreover, the treatment of soil microbiota is emerging as a new strategy in plant breeding to achieve desirable traits. Thus, integrated study of fertilizer application and soil microbiota might lead to a better understanding of soil-plant interactions and inform the design of novel ways to fertilize plants. Herein we report metagenomics data for soil microbiota in lettuce (*Lactuca sativa*) treated with fertilizer, amino acids or their combinations as follows: N-fertilizer + Amino16®, Amino16®, N-fertilizer and no treatment control. Data have been deposited in the NCBI Sequence Read Archive (SRA) (accession number: PRJNA388765).

Specifications	
Organism	Soil microbiota of lettuce (*Lactuca sativa*)
Sex	Not applicable
Sequencer or array type	Illumina HiSeq
Data format	Analyzed
Experimental factors	Treatment 1 = N-fertilizer + Amino16®, treatment 2 = amino16®, treatment 3 = N-fertilizer and treatment 4: crops did not receive N-fertilizer or Amino16® and coded as no treatment
Experimental features	Isolation of bacteria, metagenomics and analysis
Consent	Not applicable
Sample source location	Thessaloniki, Greece (40°32′19.2″N 22°59′56.5″E)

1. Direct link to deposited data

Metagenome sequence data from this study are available at the NCBI Sequence Read Archive (SRA) and Biosamples under accession numbers: PRJNA388765 (https://www.ncbi.nlm.nih.gov/bioproject/388765).

2. Introduction

The microbiome of the plant rhizosphere has a critical role in plant growth, nutrition, health and breeding [1]. Thus, plants offer a proportion of their photosynthetically-fixed carbon sources in order to preserve the microbiota in their rhizosphere [1]. The analysis of microbiota through omics technologies and especially through metagenomics analysis greatly supports the suggestion that the soil microbial composition is a major determinant of rhizosphere microbiome composition and plant well-being [1].

Plants communicate with their rhizosphere microbiota in a reciprocal advantageous symbiosis. Furthermore, the microbiome can change not only the nutritional status of plants but also certain physiological functions of plants like flowering [2]. Plants can alter their

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Table 1
Characteristics of soil as affected by different treatments application at Institute of Plant Breeding and Genetic Resources experimental station (Thessaloniki, Greece).

Code	Textural classification	pH	EC mS/cm	Organic C %	Organic %	$CaCO_3$%	K mg/kg	Mg mg/kg	NO_3-N mg/kg	P-Olsen mg/kg	Fe mg/kg	Mn mg/kg	Zn mg/kg	Cu mg/kg	B mg/kg
No-treatment	Sandy loam	7.9	0.465	0.91	1.81	1.4	138	635	2.25	81.1	10.8	10.3	6.22	2.00	0.48
Amino16®	Sandy loam	7.9	0.439	0.83	1.66	2.1	134	705	3.30	72.8	10.6	9.92	5.94	1.81	0.50
N-fertilizer + amino16®	Sandy loam	7.8	0.455	0.87	1.74	1.2	142	575	1.70	88.4	10.7	10.3	5.92	2.17	0.57
N-fertilizer	Sandy loam	7.9	0.559	0.71	1.43	1.8	146	685	9.65	68.8	10.0	10.8	6.40	1.91	0.45

Table 2
Original number of reads and number of reads after pre-processing analyzed in this study.

Sample	Type	Original # of reads (forward/reverse)	# of reads after preprocessing (forward/reverse)	Percentage retained
Soil No Treatment	V2	495,267/495,267	474,395/474,395	95.78
Soil No Treatment	V4	483,697/483,697	479,585/479,585	99.14
Soil Treatment #1	V2	401,662/401,662	390,052/ 390,052	97.10
Soil Treatment #1	V4	612,515/612,515	607,017/ 607,017	99.10
Soil Treatment #2	V2	477,101/477,101	456,727/456,727	95.72
Soil Treatment #2	V4	453,369/453,369	449,500/449,500	99.14
Soil Treatment #3	V2	453,427/453,427	440,780/440,780	97.21
Soil Treatment #3	V4	426,695/426,695	422,748/422,748	99.07

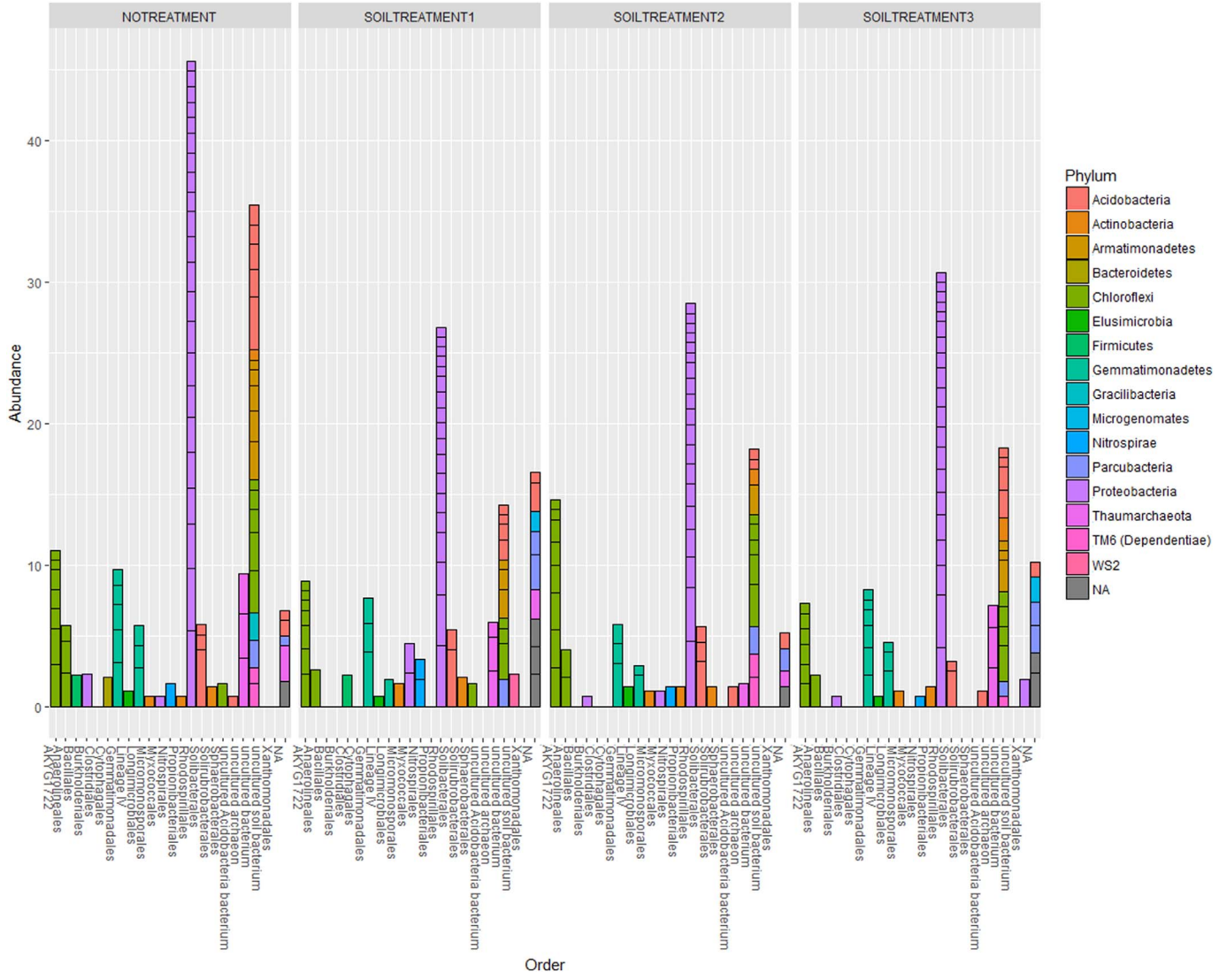

Fig. 1. Graphical representation of different bacterial phyla abundance per treatment.

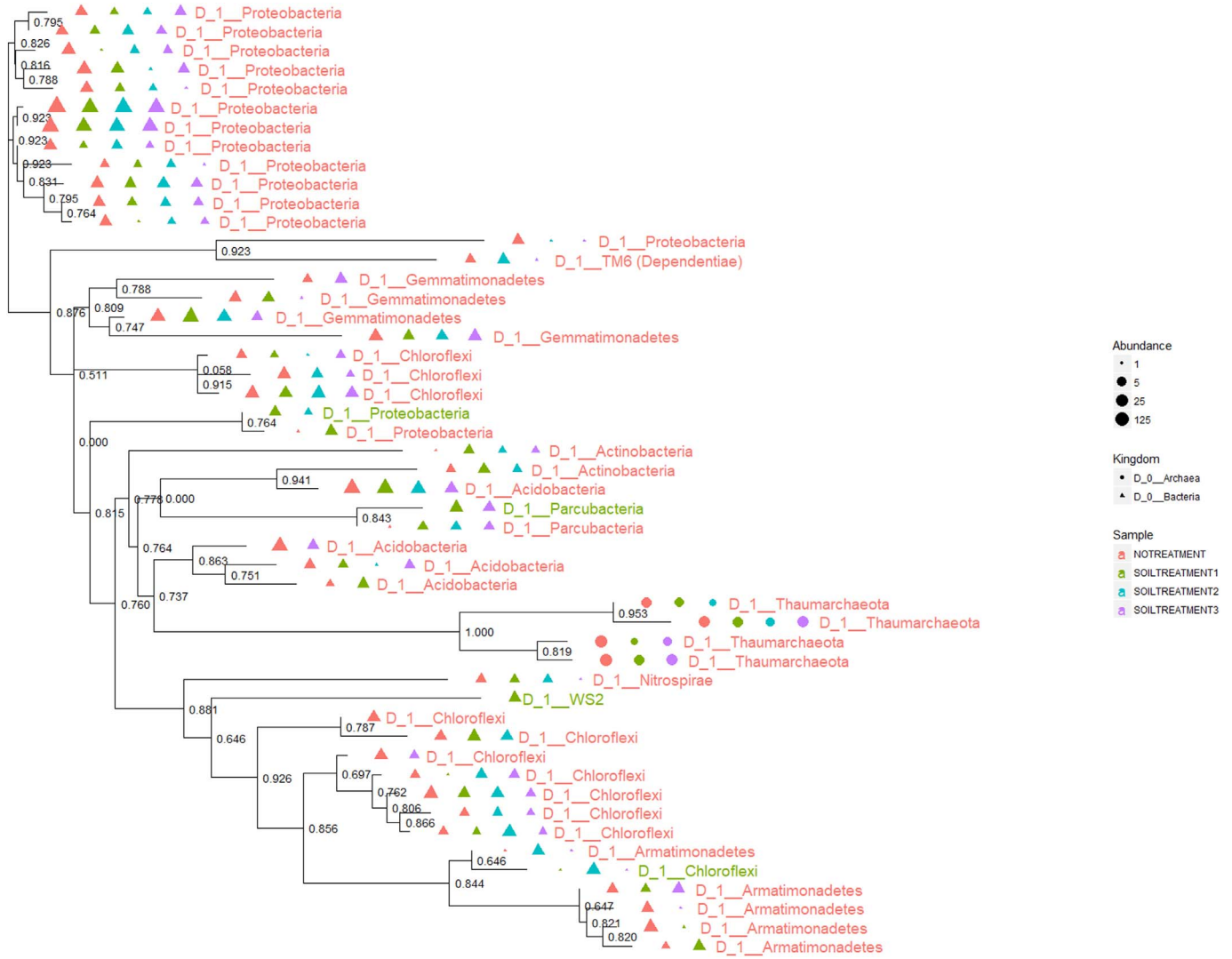

Fig. 2. Phylogenetic analysis considering the relative abundance of the different bacterial phyla in the no-treatment soil and different treatments (Treatment 1 = N-fertilizer + Amino16®, Treatment 2 = Amino16® and Treatment 3: N-fertilizer).

flowering time in order to adapt to abiotic stresses such as drought, cold, heat and salinity [2]. Changes of flowering time (early or late) using rhizosphere microbiome are of utmost importance in plant breeding programs aiming to increase adaptation to the above abiotic stresses. However, agricultural practices and environmental factors can cause alterations in soil microbial communities [3].

Less is known though about vegetable cultivated soil particularly where intensive fertilization is a common agricultural practice. Herein, we report our results of a metagenomic study of Greek soils cultivated with lettuce either with or without the supply of inorganic and currently promoted organic nitrogen fertilizers. Inorganic fertilizers may cause pollution in soil and plants and especially in water causing eutrophication due to nitrogen and phosphorus leach.

The objective of this study was to investigate how Amino 16 and N-fertilizer application affected the rhizosphere bacterial composition in cultivated lettuce in Greece.

3. Experimental design, materials and methods

Soil samples were collected from experimental fields of the Institute of Plant Breeding and Genetic Resources (ELGO-DEMETER). Samples were collected in March of 2016. Soil sampling was performed according to Souza et al. (2013). All samples corresponded to rhizosphere soil (40°32′19.2″N 22°59′56.5″E) of a lettuce (*Lactuca sativa*) crop located at 65 m above sea level. Nitrogen concentration in the soil samples was measure as follows: NO_3^- extracted from soil with 2M KCl was reduced to NO_2^- by passage through a column of copperized cadmium and the NO_2^- formed was determined colorimetrically by absorbance measurements made at a wavelength of 540 nm [4].

For the purpose of this study, four treatments were used: treatment 1 = N-fertilizer + Amino16®, treatment 2 = Amino16®, treatment 3 = N-fertilizer and treatment 4: crops did not receive N fertilizer or Amino16® and coded as no treatment.

Metagenomic DNA was extracted from 10 g of each soil replicate using the NucleoSpin Soil kit (Macherey-Nagel, Germany), following the manufacturer's procedures. DNA was quantified and purity was verified in a NanoDrop spectrophotometer at 260 and 280 nm (Thermo Scientific, Waltham, MA, USA). DNA purity and quantity were also verified by electrophoresis in 1% agarose gels and samples adjusted to 50 ng/L.

Soil DNA samples were used to prepare paired-end libraries (2 × 300 bp) using 16s rDNA (500 cycles; Illumina, San Diego, CA, USA), for further shotgun metagenomic sequencing in a High Sec 2500 Illumina platform (Illumina, San Diego, CA, USA).

The concentration of NO3-N and the EC was significantly higher in the treatment with N-fertilizer compared with the other treatments

while its concentration was significantly reduced in the combination of N fertilizer with Amino 16® ($P < 0.001$) (Table 1). The other soil characteristics were not affected significantly by the application of the fertilizer or the Amino 16®. Furthermore, no significant changes were observed regarding the yield of lettuce in all treatments (data not shown).

About one million reads where generated for each sample received (~450 k forward and ~450 k reversed reads per case), for both the V2 and V4 regions of the 16S rRNA amplicons. The raw data were filtered and trimmed for quality, using Trim Galore! [5], a wrapper around Cutadapt [6] and FastQC [7], with the default settings for paired reads. The original number of reads, as well as the number of reads after pre-processing is shown in Table 2, indicating high quality reads, with an average retention rate of 97.86% and a minimum of 95.72%.

Attempting to construct contigs using the Trinity [8] suite, almost no butterfly assemblies were reported, mostly due to the low number of reads used, with notable exceptions resulting in very few contigs, mostly for the V4 type of reads, with a notable exception of the Soil Treatment #2 (V4) which reported 9 contigs ranging between 240 and 340 bp. Given this situation, the QIIME [9] pipeline was used in order to construct meaningful Operational Taxonomic Units (OTUs) and produce a taxonomic distribution.

Initially, the reads from each sample were joined using the join_paired_ends.py command of QIIME, producing a number of fragments, ranging from 727 (Soil Treatment #1 V4 sample) to 2622 (Soil No Treatment V2 sample), with an average of 1536 fragments per sample. The resulting fragments were then processed towards finding the optimal number of open reference sequences, resulting in 10,478 independent sequences. Ultimately, these sequences where clustered into 445 OTUs which were taxonomically annotated using the SILVA rRNA database (https://www.arb-silva.de/).

The application of either N-fertilizer or Amino 16® in soil, cultivated with lettuce plants, reduced the relative abundance of Proteobacteria and Soilbacteriales in each treatment (Fig. 2). The effect of soil improvement on the lower taxonomical level was not uniform, but the relative abundance of the Gemmatimonadetes, increased in the combination treatment (N-fertilizer and Amino 16®). Soil improvement, however, reduced the relative abundance of a wide range of bacterial groups in some treatments but had a limited effect in others (Figs. 1 and 2) However, in each treatment, application of Amino 16® reduced the relative abundance of the Acidobacteria. It is also interesting to mention that we found bacteria belonging to Nitrospiraceae which are reduced especially in treatment 2 at the same time archea are also reduced significantly in treatments 1, 2 and 3.

OTUs representing the core microbiome of soil were calculated separately for each of the treatments and then summarized (Fig. 2). Differences in the relative abundance of those OTUs between the 4 treatments exist for several Proteobacteria and Acidobacteria OTUs, which occurred in a higher proportion in the no treatment microbiome compared to the other treatments.

Hence, additional studies are needed in order to enhance our knowledge about the effect of different soil treatments on the soil microbiota with the aim of preserving health and quality of a soil under a specific treatment and at the same time improve plant fitness.

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