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To ensure we keep this website safe, please can you confirm you are a human by ticking the box below. If you are unable to complete the above request please contact us using the below link, providing a screenshot of your experience. Export report"…Seed treatments with CP and EMF are further followed by changes in DNA methylation [61], protein expression and numerous enzymatic activities in tissues of growing seedlings [25,34] including proteins that are important for photosynthetic activity and antioxidant defense. Substantial changes in plant associated microbiome due to sunflower seed treatment with CP have been reported [62]. Numerous findings indicate that secondary metabolism is activated in plants growing from the CP-or EMF-treated seeds [30,36,45,63], and that may lead to increased plant fitness and improved growth due to stimulated communication with beneficial microorganisms [59,64]…."Section: Discussionmentioning Exaggerated anticipatory anxiety is common in social anxiety disorder (SAD). Neuroimaging studies have revealed altered neural activity in response to social stimuli in SAD, but fewer studies have examined neural activity during anticipation of feared social stimuli in SAD. The current study examined the time course and magnitude of activity in threat processing brain regions during speech anticipation in socially anxious individuals and healthy controls (HC). Method Participants (SAD n = 58; HC n = 16) underwent functional magnetic resonance imaging (fMRI) during which they completed a 90s control anticipation task and 90s speech anticipation task. 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Substantial changes in plant associated microbiome due to sunflower seed treatment with CP have been reported [62]. Numerous findings indicate that secondary metabolism is activated in plants growing from the CP-or EMF-treated seeds [30,36,45,63], and that may lead to increased plant fitness and improved growth due to stimulated communication with beneficial microorganisms [59,64]…."Section: Discussionmentioning Exaggerated anticipatory anxiety is common in social anxiety disorder (SAD). Neuroimaging studies have revealed altered neural activity in response to social stimuli in SAD, but fewer studies have examined neural activity during anticipation of feared social stimuli in SAD. The current study examined the time course and magnitude of activity in threat processing brain regions during speech anticipation in socially anxious individuals and healthy controls (HC). 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The positive impact of NTP treatment on the acceleration of germination and improvement of root, shoot, and young seedling growth has been demonstrated in previous studies [8,9,[12][13][14]…."Section: Introductionmentioning"…Although treatment with CAP and NTP is common in agricultural applications, most seed studies have concentrated on the notably positive improvements in seed germination, shoot and root growth, and the growth of young seedlings after CAP or NTP treatment [8,9,26,27]. Previous reports referred to the factors influencing germination in relation to different types of plasma (e.g., the atmospheric pressure dielectric barrier charge (DBD) and low-pressure radiofrequency (RF) plasma) and the plasma working gases (e.g., air, oxygen, nitrogen, and argon) [8,25,27]…."Section: Discussionmentioning"…Several reactive oxygen species (ROS) and nitrogen species (RNS) are created in NTP [4]. In food technology, NTP, including CAP, is an innovative technique used to improve the inactivation of pathogens (e.g., Salmonella typhimurium) [5] or spoilage organisms (e.g., Saccharomyces cerevisiae) [3], parasite control [6], seed germination [7,8], and acceleration of seed growth [8,9]. Compared with traditional food techniques, NTP may offer several advantages, including that it can be used at room temperature in a dry environment; is a fast, safe treatment; and there are no chemical residues developed under operating conditions…."Section: IntroductionmentioningSee 1 more Smart Citation Exaggerated anticipatory anxiety is common in social anxiety disorder (SAD). Neuroimaging studies have revealed altered neural activity in response to social stimuli in SAD, but fewer studies have examined neural activity during anticipation of feared social stimuli in SAD. The current study examined the time course and magnitude of activity in threat processing brain regions during speech anticipation in socially anxious individuals and healthy controls (HC). Method Participants (SAD n = 58; HC n = 16) underwent functional magnetic resonance imaging (fMRI) during which they completed a 90s control anticipation task and 90s speech anticipation task. Exaggerated anticipatory anxiety is common in social anxiety disorder (SAD). Neuroimaging studies have revealed altered neural activity in response to social stimuli in SAD, but fewer studies have examined neural activity during anticipation of feared social stimuli in SAD. The current study examined the time course and magnitude of activity in threat processing brain regions during speech anticipation in socially anxious individuals and healthy controls (HC). Method Participants (SAD n = 58; HC n = 16) underwent functional magnetic resonance imaging (fMRI) during which they completed a 90s control anticipation task and 90s speech anticipation task. "…They developed a novel analytical pipeline with the V4 region enabled the best resolution of bacterial communities pro ling up to both family and genus levels. In a recent study, Tamošiūnė et al [29] assessed the effect of cold Plasma treatment on the plant-associated Microbiome using the Ion 16S TM metagenomics kit which seems to be the rst study applying this kit on plantassociated microorganisms. However in this study, authors did not give indication related to the best 16S rRNA hypervariable region that gives a robust identi cation and classi cation of taxa…."Section: Introductionmentioning Exaggerated anticipatory anxiety is common in social anxiety disorder (SAD). Neuroimaging studies have revealed altered neural activity in response to social stimuli in SAD, but fewer studies have examined neural activity during anticipation of feared social stimuli in SAD. The current study examined the time course and magnitude of activity in threat processing brain regions during speech anticipation in socially anxious individuals and healthy controls (HC). Method Participants (SAD n = 58; HC n = 16) underwent functional magnetic resonance imaging (fMRI) during which they completed a 90s control anticipation task and 90s speech anticipation task. Exaggerated anticipatory anxiety is common in social anxiety disorder (SAD). Neuroimaging studies have revealed altered neural activity in response to social stimuli in SAD, but fewer studies have examined neural activity during anticipation of feared social stimuli in SAD. The current study examined the time course and magnitude of activity in threat processing brain regions during speech anticipation in socially anxious individuals and healthy controls (HC). Method Participants (SAD n = 58; HC n = 16) underwent functional magnetic resonance imaging (fMRI) during which they completed a 90s control anticipation task and 90s speech anticipation task. To ensure we keep this website safe, please can you confirm you are a human by ticking the box below. If you are unable to complete the above request please contact us using the below link, providing a screenshot of your experience. Plant seed treatment with high-frequency atmospheric-pressure plasma, also known as cold plasma (CP), has been shown to have a potential to enhance agronomic seed quality by surface decontamination, germination enhancement, and promoting plant growth (reviewed by Ohta (Ohta, 2016). The physical impact of high energy electrons, high frequency electromagnetic and UV radiation could have a direct impact on biological systems; however the main biologically active constituent of CP is a complex mixture of activated radical species produced by dissociation, excitation and ionization of gas atoms and molecules [mainly molecular oxygen (O2), nitrogen (N2) and water (H2O) in air atmosphere] upon collision with high energy electrons and in subsequent chemical reactions (Turner, 2016; Whitehead, 2016). At relatively low gas temperature and electron energies (2-5 eV), which are characteristic to CP, excitation of N2 molecules and dissociation of O2 in air atmosphere leads to accumulation of ozone (O3) (Whitehead, 2016). Higher electron energies lead to the dissociation of N2 and the production of nitrogen oxides (NOx) which inhibit ozone production, and subsequently recombine to form a number of other reactive nitrogen species including oxidant and nitrating agent peroxynitrite anion (ONOO–). Air humidity is also an important factor modulating output of the CP-generated reactive species. In the presence of water vapor, a hydroxyl radical (OH·) is produced in the primary electron collision process involving dissociation of water or by secondary process involving neutralization of ions and by reactions of excited states of O2 and N2 (Whitehead, 2016). Due to the presence of an unpaired electron, OH· radical is highly unstable and could readily oxidize a range of organic compounds (Gligorovski et al., 2015).CP-generated reactive oxygen and nitrogen species (ROS/RNS) are known to have a negative impact on biological systems and have been used as sterilizing agents to inactivate microorganisms. Disruption of the microbial cell walls, as well as damage to the cell membrane, DNA and protein integrity and function has been reported for free-living and biofilm forming bacteria exposed to CP [reviewed by (Patil et al., 2016)]. Han et al. (Han et al., 2016) have proposed different mechanisms for the inactivation of gram- positive and negative bacteria that are linked to the impairment of the cell wall and the damage of intracellular components, respectively. CP treatment has been shown to effectively disrupt bacterial spores (Hong et al., 2009; Reineke et al., 2015). In addition to reactive chemical species, a negative effect of the UV irradiation on microorganisms and spores has been documented under certain conditions (Moreau et al., 2000; Moisan et al., 2001; Boudam et al., 2006), but its role remains uncertain (Patil et al., 2016). On the other hand, the effects of plasma depend on the dose and the composition of the reactive species generated; and the vitality and activity of plant growth-promoting bacteria enhancing effect following CP treatment has been reported (Ji et al., 2019).An assembly of commensal and pathogenic microorganisms are passed through seeds and these are important for the survival and vigor of the germinated seedlings and plants (Nelson, 2018). Recently, metagenomic analysis of the bacterial 16S rRNA gene or gyrB gene-based species-specific bacterial marker, as well as the fungal internal transcribed spacer, using next-generation sequencing (NGS) has proven to be a powerful technique to study the composition and the dynamics of plant-associated microbial communities (Tamosiune et al., 2019), and seed microbiomes have been investigated extensively (Barret et al., 2015; Klaedtke et al., 2016). Recently, metagenomic analysis revealed a selective effect on the composition of Arabidopsis thaliana seedling and mature plant microbiota by CP treatment of seeds (Tamosiune et al., 2020). In addition, the effect of CP on seed microbiome is supported by studies based on traditional microbiological techniques [reviewed by (Ohta, 2016)]. Efficient inactivation of native microflora upon atmospheric pressure CP treatment was reported for chickpea (Mitra et al., 2014), alfalfa, onion, radish, cress (Butscher et al., 2016a) and lentil seeds (Waskow et al., 2018). Another study used low pressure CP in air or sulfur hexafluoride atmosphere for a variety of cultivated plant seeds and demonstrated that CP effectively inactivates pathogenic strains of Aspergillus spp. and Penicillum spp. depending on seed surface, plasma gas type, plasma treatment time, and the microbial population density (Selcuk et al., 2008). Effective inactivation of an indicator strain of spore-forming Bacillus atrophaeus on Brassica napus seeds was achieved by direct application of dielectric barrier discharge (DBD) plasma (Schnabel et al., 2012). However, Geobacillus stearothermophilus endospore inactivation on wheat grains was less efficient and required extended treatment (Butscher et al., 2016b). This is presumed to result from complex surface structure and geometry of grains, where microorganisms can be sheltered by an uneven surface (Schnabel et al., 2012; Butscher et al., 2016b), and inactivation efficiency is dependent on substrate moisture level and plasma supply settings that determine outcome of treatment with reactive species (Butscher et al., 2016a).Certain CP-generated ROS/RNS, such as superoxide anion (·-O2), NO, hydrogen peroxide (H2O2) or ONOO–, play an important role as signaling messengers in eukaryotic cells (Droge, 2002) and are implicated in the regulation of seed dormancy, germination (Jeevan Kumar et al., 2015) and plant physiology (Del Rio, 2015). Several studies have established that CP treatment of seeds results in a long-term growth enhancement of Arabidopsis (Koga et al., 2015), pea (Stolarik et al., 2015), radish (Kitazaki et al., 2014), soybean (Ling et al., 2014), sunflower (Mildaziene et al., 2019; Zukiene et al., 2019), wheat (Jiafeng et al., 2014), and Norway spruce (Pauzaite et al., 2017). CP treatment significantly increased the length and weight of maize roots (Henselova et al., 2012). Also, improved disease resistance was reported for tomato (Jiang et al., 2014). While the mechanism of CP-generated ROS/RNS action on plants is poorly understood, it is presumed that the response of plants to seed treatment is a consequence of stressor-induced eustress phenomena. Plants can produce more than one phenotype in different environments, and stress factors could have an effect on plant ontogenetic development throughout their life cycles (Dalal et al., 2017; Ibanez et al., 2017). Also, it is important to consider that stressor-induced plant physiology changes might affect plant colonization by seed and environment derived microorganisms leading to changes in plant-associated microbial community, and such changes may have an impact on plant growth.Our earlier study using a low-pressure capacitively coupled CP device revealed that protein expression changes in common sunflower (Helianthus annuus L.) seedlings induced by pre-sowing plasma treatment of seeds were very similar to the effect exerted by electromagnetic field treatment, suggesting that electromagnetic field constituent of CP may induce the observed changes on plant physiology (Mildaziene et al., 2019). DBD devices operate at the ambient atmosphere and plasma discharge generates more complex and enriched ensemble of reactive O2 and N2 species of which many could have an impact on biological systems, such as antimicrobial effect or interference with signal transduction in seed tissues. Therefore the objectives of this study were to a) assess the effect of pre-sowing seed treatments with DBD plasma on development of common sunflower, b) to study the influence on composition of plant-associated bacteria, and c) to assess the change in protein expression pattern in roots. The effects of pre-sowing seed treatments on the main features of organ development, such as root length and leaf and inflorescence size, were assessed throughout the life-span of sunflower plants. As plasma treatment could have direct or indirect effect on plant-associated microbiome and its role in plasma stressor-induced plant response has been poorly understood, a metagenomic analysis of 16S rRNA gene was used to analyze changes in the composition of plant-associated bacteria in seedlings of sunflower upon growth-stimulating treatment with CP. Proteomics analysis was used to study changes in protein expression patterns in roots of emerging seedlings to uncover protein expression patterns that might be linked to the direct plant response to CP and/or changes in the composition of plant-associated microbiome.Materials and MethodsPre-Sowing Seed Treatment With CPSeeds of the common sunflower confectionery variety 'Nykrségi fekete' harvested in 2016 were received from the Institutes for Agricultural Research and Educational Farm, University of Debrecen (Hungary). Seeds were stored at 4–10°C and were used for experiments carried out in 2018-19.Seed treatment was carried out using a dielectric barrier discharge (DBD) device described by Koga et al., (2015). CP source (40 mm × 44 mm) constructed from 20 ceramic-coated stainless steel electrodes was used, and eight seeds per treatment were placed on a glass plate in an area of 20 mm × 30 mm in size that was expected to have a homogenous distribution of the discharge (estimated based on pH changes in a 96-well plate filled with bromophenol blue solution). The distance from the seed surface to the source of CP was maintained at approx. 3 mm. The discharge voltage, frequency and power were 7.0 kV, 14.4 kHz, and 4.64 W, respectively. Irradiation with CP was carried out at room temperature, and relative humidity was maintained at 40%–60%. To maintain seed surface temperature below 45°C, repetitive 2 min irradiation with 1 min rest intervals was applied up to 4 times to achieve cumulative CP irradiation of 2 min (CP2), 4 min (CP4), or 8 min (CP8). After CP treatment, seeds were stored in plastic bags for 1 week at 25°C in the dark. The control seeds were stored under the same conditions. Treatments for all experimental conditions were replicated 3 times.Measurement of Seedling and Plant Morphological ParametersFor morphology analysis at an early growth stage, seeds were sown in unsterile peat substrate and seedlings were maintained under controlled growth conditions in a climatic chamber under 16 h photoperiod at 22°C. Four days after seedling emergence, they were removed from substrate, roots were washed to remove the substrate, wiped with a paper towel, and the length of the roots and hypocotyls was estimated using image analysis software ImageJ (Schneider et al., 2012). During the first 2 weeks of growth, seedling height, length, and width of cotyledons and the first leaf was directly measured at 2-day intervals starting 1 week after seedling emergence.For mature plant morphology analysis, plants were grown under field conditions. Plants from the three experimental groups and three replicates were randomly distributed as blocks of 8–10 plants in four separate rows. After 3 weeks, leaf length and width were measured and a cumulative value of leaf size was estimated based on the relative size of the first five leaves compared to the control group. The size of inflorescences was assessed after 3 months.Bacterial 16S RNA Metagenomic AnalysisMetagenomic analysis included samples of seedlings from the control and CP4 treated experimental groups. Samples of root and cotyledons were collected 4 days after seedling emergence and combined samples of leaves and cotyledons were collected after 2 weeks of cultivation under controlled growth conditions. Samples were flash frozen in liquid nitrogen, ground to fine powder and stored at -70°C.Two different bacterial DNA extraction methods were employed and six unique libraries were created. Samples were prepared using the standard bacterial DNA purification method based on application of cetyltrimethylammonium bromide (CTAB) (Ding et al., 2013) (DNA extraction method 1) or extraction using sodium dodecyl sulfate (SDS) detergent (Wang et al., 2008) (DNA extraction method 2). All DNA extractions were performed according to the author instructions, with the exception of starter quantities adjusted to 2 g, and the final elution performed in 20 µl of nuclease free water.DNA sequencing analysis was carried out using semiconductor-based Ion Torrent sequencing technology (Thermo-Fisher Scientific, USA). DNA library preparation procedure followed the 16S Metagenomic Sequencing Library Preparation Protocol (Thermo-Fisher Scientific, USA). The hypervariable regions of 16S rRNA gene were amplified by two separate PCR reactions and V2–4–8, V3,6–7 specific primer sets of the Ion 16S Metagenomics Kit (Thermo-Fisher Scientific, USA). Cycling conditions were as follows: 25 cycles of 30 s denaturation at 95°C, 30 s annealing at 56°C and 20 s extension at 72°C. To confirm successful amplification of 16S rRNA DNA samples, negative (water) and positive (E. coli DNA) controls were used. Equal volumes of V2–4–8 and V3,6–7 amplification reactions were combined. Final DNA library was made from 50 ng of combined amplicons with the Ion Plus Fragment Library Kit and Ion Xpress Barcodes Adapters, 1–16 (Thermo-Fisher Scientific, USA) following the manufacturer's recommendations. The quality and concentration of the libraries were determined by the MCE-202 MultiNA DNA analyzer (Shimadzu, Japan). Adapter-ligated and nick-repaired DNA was purified using 1.4 volumes of Agencourt AMPure beads (Beckman Coulter, USA) and eluted in Tris-EDTA buffer. The accurate library concentration for template preparation was estimated by qPCR and Ion Library TaqMan Quantitation Kit (Thermo-Fisher Scientific, USA). Each sample was adjusted to 10 pM. Equal volumes of all samples were combined and emulsion PCR was carried out using Ion OneTouch 2 System and Ion PGM Hi-Q View OT2 Kit (Thermo-Fisher Scientific, USA). The amplified clonal libraries were enriched using Ion PGM Enrichment Beads on Ion OneTouch ES instrument (Thermo-Fisher Scientific, USA). The enrichment efficiency was assessed using Ion Sphere Quality Control Kit (Thermo-Fisher Scientific, USA). Prepared template spheres were loaded on Ion 316 v.2 chip and sequencing was performed on Ion Personal Genome Machine (PGM) system using Ion PGM Hi-Q Sequencing Kit (Thermo-Fisher Scientific, USA).Base calling and run demultiplexing were performed by Torrent Suite v.5.0.5 (Thermo-Fisher Scientific, USA) with default parameters. Sequencing data was processed using 16S Metagenomic workflow of the Ion Reporter Software v.5.10.5.0 (Thermo-Fisher Scientific, USA). Reads were trimmed by primers at both ends. Threshold for unique reads was set to 10. Taxonomic identification was performed using MicroSEQ 16S Reference Library v.2013.1 and Greengenes v.13.5 databases. Threshold value for percentage identity for genus and species ID was 97%.Sunflower Seedling Proteome Analysis Using Two-Dimensional ElectrophoresisFor proteome analysis of roots of emerging seedlings, the experimental design and sample preparation was the same as that for the bacterial metagenomic analysis. Total cell protein was prepared using phenol extraction and ammonium acetate precipitation, as described previously (Isaacson et al., 2006). Internal standards were prepared from a pooled mixture of all protein extracts. Protein separation and detection was performed using a differential gel electrophoresis procedure as described previously (Tamosiune et al., 2018). Sample aliquots of 50 µg were labeled with Cy3 and Cy5 fluorescent dyes, and the internal standard was labeled with Cy2 dye (Lumiprobe, USA). For the preparative gel, 500 µg of unlabeled internal standard was mixed with 50 µg of Cy2 labeled internal standard. Isoelectric focusing was performed on 24 cm IPG strips with a linear gradient of pH 4–7 using Ettan IPGphor (GE Healthcare, USA). Further, the proteins were separated on 1-mm thick 10-16% polyacrylamide gradient gels using Ettan DALTsix (GE Healthcare, USA). Gels were scanned using a fluorescence scanner FLA 9000 (GE Healthcare, USA). Relative protein quantification was performed using DeCyder 2-D Differential Analysis Software, v.7.0 (GE Healthcare, USA), and the Biological Variation Analysis module was used to match protein spots of four biological repeats and experimental groups.Preparative gel was fixed in 50% methanol and 10% acetic acid. Protein spots were excised manually and subjected to protein digestion with trypsin, according to a method described previously (Shevchenko et al., 2006). Protein digests were loaded and desalted on a 100 µm × 20 mm Acclaim PepMap C18 trap column and separated on a 75 µm × 150 mm Acclaim PepMap C18 column using an Ultimate3000 RSLC system (Thermo-Scientific, USA), coupled to a Maxis G4 Q-TOF mass spectrometer detector with a Captive Spray nano-electrospray ionization source (Bruker Daltonics, Germany). Peptide identification was performed using the MASCOT server (Matrix Science, USA) against Helianthus annuus L., genome database v.1.0 (Badouin et al., 2017). Threshold value for the identification of proteins was a Mascot score of >50 and at least two peptides.For protein annotation, NCBI Protein database was queried using Blast2GO software (Conesa et al., 2005). Arabidopsis homologues were identified by BLAST search against TAIR10 gene models at the Sunflower Genome Database (. Protein interactions were assessed using the String database (Szklarczyk et al., 2015).Statistical Data AnalysisThe seed treatment and seedling cultivation experiments were carried out 4–6 times to confirm reproducibility, and samples used for metagenomic and proteome analysis were collected from at least three separate experiments. Means of morphological parameters were compared between the experimental groups using one-way analysis of variance function of Prism v. 3 (GraphPad Software Inc.) and significance of differences compared to the control were identified using Tukey post-hoc analysis ($p < 0.05$). Data are presented as mean of at least 3 independent experiments and standard error of the mean.For the 16S rRNA analysis, operational taxonomic units (OTU) abundance information was normalized to the sample with the fewest sequences and alpha diversity and beta diversity were subsequently performed. Four indices were applied to analyze Alpha diversity: observed-species, Chao1, Shannon and Simpson indices. These metrics reflected diversity and richness of the individual samples and were plotted using IonRerporter v.5.10.5.0 software. Beta diversity was used to compare diversity of bacterial communities among the six experimental groups. The bacterial richness at genus level was quantified using principal coordinate analysis (PCoA) and abundance data using Bray-Curtis metrics (IonRerporter v.5.10.5.0), and the relative bacterial species abundance was visualized using heatmap software (.The ANOVA analysis of the DeCyder software was used to identify statistically significant ($p < 0.01$) differences in protein abundance between the control and CP4 experimental groups using four biological repeats. A threshold value of at least a 1.2-fold difference in protein abundance was used.ResultsEffect of Plasma Treatment on Sunflower Seedling and Mature Plant MorphologyTo establish an optimum CP treatment duration, sunflower seeds were placed at 3 mm distance from the DBD plasma source and irradiated for 2, 4, or 8 min cumulative duration. For the morphology analysis, 150–220 seedlings from 6 independent experiments were used for each experimental group. Meanwhile, during the 2 weeks of seedling growth, the height of the seedlings remained similar in all experimental groups (Supporting material Figures S1 and S2), the effect of CP treatment emerged as a difference in size of cotyledons and first true leaf (Figure 1). The most significant difference in length of cotyledons was reached at day 10 for the CP 4 min group which had an 11.6% ± 2.1% higher mean value compared to the control. This difference was lost when the cotyledons reached their maximum length after 2 weeks. On the contrary, the first true leaf dimensions of the CP4 group remained consistently larger during the experiment. At the end of the experiment, the mean value of leaf length and width was 14.6% ± 2.1% and 14.3% ± 2.0% larger compared to the control, respectively. It is notable that shorter (2 min) or longer (8 min) treatment duration resulted in a complete loss of the growth-promoting effect, but without a significant adverse effect on plant morphology. Very similar length of hypocotyls for control and treated experimental groups suggests that germination timing was not affected by CP treatment. Figure 1 Effect of plasma treatment on sunflower seedling cotyledon (A, B) and first leaf (C, D) length (A, C), and width (B, D). Data from six independent experiments is presented as mean and standard error of the mean. Color of the symbols represent significant differences of the mean values compared to the control (green – $p < 0.05$, red – $p < 0.01$).Further, the long-term effect of CP-induced leaf growth stimulation was investigated in field experiments with plants grown to maturity. The size of the five true leaves was measured after 2 months of cultivation. Taking into consideration leaf size variations depending on age, a relative value was assessed for length and width of each consecutive leaf as compared to the control, and the derived cumulative values are shown in Table 1. In agreement with the results obtained in the experiments with sunflower seedlings, an average leaf length and width dimensions were significantly larger compared to the control (8.1% ± 1.8% and 9.9% ± 2.5%, respectively) upon the CP 4 min treatment, and the stimulating effect was abolished by the longer treatment duration. Furthermore, plants of the CP 4 min group yielded 12.7% ± 4.1% larger capitulum. Meanwhile, the mean value of plant height remained within a margin of error among the three experimental groups. Table 1 Effect of CP treatment on sunflower mature plant morphology.Since the extraction of roots of mature plants grown in field or expanded seedlings grown in peat substrate could have an adverse effect on the precision of root measurements, the effect of CP treatment on root development was assessed at an early stage of seedling development (4 days after seedling emergence) while the main root could be easily extracted intact (Supporting material Figure S1). Roots of the seedlings germinated from the CP 4 min treated seeds were 8.7% ± 2.7% ($p=0.014$) longer [6.5 ± 0.15 (n=133) and 7.1 ± 0.17 (n=143) cm for the control and treated seedlings, respectively]. In agreement with the experiments described above, no significant effect on hypocotyl length [1.9 ± 0.04 (n=136) and 1.9 ± 0.03 (n=146) cm for the control and treated group, respectively] was detected. Size of the cotyledons was not examined as it closely corresponded to seed size at this growth stage.CP Treatment-Induced Changes in Plant-Associated Bacteria Community StructureTo investigate bacterial metagenomic diversity, six DNA pools were extracted from the control and CP-treated roots and cotyledons of the emerging seedlings, and leaves of 2-week-old seedlings. The samples were subjected to 16S rRNA metagenomic analysis using Ion Torrent sequencing platform. Sequencing and read mapping statistics are presented in Supporting material Table S1. For the six samples, 5,165,808 raw reads were generated in total. Following demultiplexing, quality trimming and chimera removal of initial sequence reads, from 404,580 to 879,930 sequences were collected for each sample (3,509,611 in total) of high-quality sequence with a read length from 235 to 243 bp.After rarefaction at a depth of 29,710 sequences per sample, a total of 487 distinct OTUs were obtained with 97% similarity cutoff for all samples. The detected OTUs were assigned into 13 phyla, 27 classes, 55 orders, 113 families, and 158 genera. At the phylum level, Firmicutes was dominant in all samples (Supporting material Figure S3), though it was more prevalent in CP-treated samples as compared to the control. In consecutive order of abundance, other phyla included Proteobacteria, Actinobacteria and Bacteriodetes, and these had a similar distribution among the samples. An exception was the class of Gammaproteobacteria which was observed only in root (control and treated) and control cotyledon samples.At a family level, OTUs were allocated to 113 different taxa; however, depending on the sample, 71% to 83% of the OTUs were assigned to three dominant families of Bacillaceae, Rhodobacteraceae and Moraxellaceae, and within these families only a small fraction (less than 1%) of the OTUs were assigned to the genus level. On the contrary, 66%–86% of the OTUs allocated to the remaining 110 families were assigned to a lower taxonomic level. This indicates that the majority of the sequences assigned to the three dominant families had low similarity to the microbial database used for read mapping and could include a significant proportion of plastid or mitochondrial sequences that are absent in the database and would be erroneously assigned to the related bacterial families. Following this line of reasoning and considering that the three dominant families were similarly abundant among the samples, this would be of low interest to pursue; therefore only OTUs assigned at the genus level were used for further analysis of microbial diversity.To compare the richness and diversity of bacterial species in individual samples, alpha diversity indexes of Simpson, Shannon, Chao1 estimator and the observed species were used (Supporting material Table S2). A number of the observed OTUs varied from 41 to 114 and the highest number of OTUs was observed in roots and leaves of the control plants, whereas the CP4 treated cotyledons had the lowest number of OTUs. Rarefaction curves demonstrated that OTU abundance was diverse among the different samples (Supporting material Figure S4), and the saturating number of OTUs indicated that bacterial communities were sufficiently sampled.Based on the alpha diversity indexes (Supporting material Table S2), the control leaves and roots had similar microbial richness, which was the highest among all samples. The richness was reduced in leaf samples after CP treatment (approximately two-fold for the Chao1 and 4.5-fold for H' and S) but only slightly affected in the roots. Meanwhile, the microbial richness of cotyledons was the lowest among the control samples, and it was further reduced after CP treatment but the effect was lower compared to leaf samples (approximately 1.5-fold for all the indexes).The Bray–Curtis dissimilarity matrix of beta diversity was calculated to differentiate the bacterial communities at the genus level among the control and CP-treated sunflower samples (Figure 2). The first four components comprised a larger than 98.5% variation. The first and second axes include the largest variability (65.86% in total) and mainly reflect differences between microbiome of sunflower seedlings germinated from the plasma-treated seeds (CP, solid line) and control seedlings (C, dashed line). For the remaining two axes that include 32.66% of variability, the control and CP-treated data points are projected more closely and the differences related to distinct tissues used in the analysis are more prominent. There was little association between the experimental groups representing different plant organs or the effect of CP treatment. Almost half (43.8%) of the detected variation was included in the first component representing a strong clustering of the control leaf and cotyledon groups, with the CP-treated root experimental group as well as CP-treated leaf and cotyledon groups at the extremes of the axis. The two extremes of the second component (22.1% variation) represented an association between control root and leaf with CP-treated cotyledon experimental groups and the control cotyledons and CP-treated root and leaf samples. Figure 2 Variability within the data set of 16S rRNA metagenomic analysis projected in a set of four uncorrelated axes of the principal coordinate analysis (PCoA) carried out using Bray-Curtis dissimilarity index. Proportion of variance for each dimension is indicated on top. For each axis, objects ordinated closer to one another are more similar than those ordinated further away.The differences detected by PCoA were illustrated by the Petal map based on distribution of the OTUs (Supporting material Figure S5). The analysis revealed that the control and CP treated samples of different parts of sunflower share or has distinct bacterial OTUs. The diagram revealed 29 core OTUs including Mycobacterium, Rhodococcus, Nocardioides, Solimonas, Curtobacterium, Candidatus Portiera, and Pseudomonas as the most common genera. Interestingly, the number of unique OTUs for the control leaves of sunflower contrastingly differed from those treated with CP and the number of OTUs in CP-treated cotyledons were also extremely low.Differences in bacterial abundance at the genus level are shown as a heatmap in Supporting material Table S5. Among the 158 bacterial genera identified across all samples, the distribution of the 29 most common (>0.5% of the total abundance) genera was summarized in Figure 3. Major variation was observed for the Mycobacterium genus that was greatly enriched in the leaf (28% of total sequences) and cotyledon (4.3%) samples upon CP treatment, meanwhile a considerable proportion (4%) of the sequences assigned to the genus in the control root samples was reduced approximately ten-fold after CP treatment. Other notable differences observed after CP treatment included a large relative increase in the abundance of Solimonas and Rhodococcus spp. in roots, Curtobacterium spp. in cotyledons, and a decrease in abundance of the dominant (>1% of total sequences) Solimonas, Pseudonocardia, and Rhodococcus spp. in cotyledons as well as a decrease in the abundance of Pseudomonas, Nocardioides, Rhodococcus, and Candidatus Portiera spp. in leaves. Figure 3 Bacterial composition at a genus-level for the 16S rRNA gene in control (C) and cold plasma treated (CP) sunflower samples. "Others" group includes minor genus with