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Positive Effect of Camelina Intercropping with Legumes on Soil Microbial Diversity by Applying NGS Analysis and Mobile Fluorescence Spectroscopy

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Abstract: Camelina (*Camelina sativa* (L.) Crantz) is a valuable source of essential amino acids, especially sulphur-containing ones, which are generally lacking in leguminous crops, thus representing an alternative source of protein for both humans and farm animals. Rhizosphere soil samples from five experimental plots with mono- and mixed cultivations of three camelina cultivars, including two introduced varieties Cs1.Pro (Luna) and Cs2.Pro (Lenka) and one Bulgarian variety Cs3.Pro (local Bulgarian landrace) with variety 666 of vetch (*Vicia sativa* L.) (Cs3-Vs.Pro) and variety Mir of pea (*Pisum sativum* L.) (Cs3-Ps.Pro), were collected and analysed. The total DNA was isolated from the rhizosphere soils and the presence of the 16S rRNA gene was confirmed by amplification with the universal primer 16SV34. In the present study, the structure of the soil bacterial community in five different plots (Cs1.S.Pro, Cs2.S.Pro, Cs3.S.Pro, Cs3.Vs.S.Pro, and Cs3.Ps.S.Pro) where camelina was grown alone and by being intercropped with pea and vetch was analysed via a metagenomic approach. The number of observed species was highest in the local genotype of the camelina Cs3 grown alone, followed by soil from the intercropped variants Cs3-Vs and CsS-Ps. The soil bacterial communities differed between the sole cultivation of camelina and that grown with joint cultivation with vetch and peas, indicating that legumes considerably affected the growth and development of beneficial microorganisms by aspects such as nitrogen fixing, levels of nitrifying bacteria, and levels of phosphorus-dissolving bacteria, thus helping to provide better plant nutrition. The α-diversity indicated that bacterial communities in the rhizosphere were higher in soils intercropped with vetch and pea. The optical properties of cereals and legumes were determined by their energy structure, which includes both their occupied and free electronic energy levels and the energy levels of the atomic vibrations of the molecules or the crystal lattice.

Keywords: camelina sativa; intercropping with pea and vetch; mobile fluorescence spectroscopy; NGS; soil microbiome

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1. Introduction

The *Camelina sativa* (L.) Crantz is a flowering plant from the *Brassicaceae* family, and it is traditionally grown as an oilseed crop for food feed and industrial uses. This plant is a valuable source of essential amino acids, especially sulphur-containing ones, which are generally lacking in leguminous crops, thus representing an alternative source of protein for both humans and farm animals. Camelina has recently been grown for its potential as a biofuel and biolubricant [1]. The camelina crop has been studied for its extremely high

levels (up to 45%) of omega-3 fatty acids, which is unusual in plant sources [2]. The seeds contain 38 to 43% oil and 27 to 32% protein. Over 50% of the fatty acids in cold-pressed camelina oils are poly-unsaturated: omega-3 fatty acids, alpha-linolenic acid, gamma-tocopherol, and linoleic acid. These oils are also very rich in natural antioxidants, such as tocopherols, which makes them extremely stable and resistant to oxidation and rancidity. *C. sativa* possesses several agronomic properties such as resistance to insects and easy adaptation to any climatic conditions, the only limitation for its cultivation being heavy clay or waterlogged soils, where nitrogen and phosphorus are the most limiting factor for plant growth. Seed yields and oil content are highly variable depending on the environment, but it outperforms rape seed in trials carried out under identical drought conditions [3]. Considering climate change, good drought tolerance has been pointed out as one of the advantages of this crop [4], although drought can have a negative impact, especially during sensitive growth stages [5]. It shows good cold survival as it can germinate at low temperatures and the plants can tolerate freezing [1]. However, this has yet to be fully characterized for all stages of development and varieties. The innovative aspect of the present study is based on growing camelina, which is highly tolerant to drought and cold and can achieve sustainable yields in non-irrigated conditions. The introduction of camelina and cultivation in intercropping conditions raises the following questions about the efficient utilization of natural resources: (i) preventing N-leaching and soil erosion; (ii) the reduction of pest and disease control costs due to higher competitive advantage over weeds and diseases in diversified cropping systems; (iii) promotion of the quality of the seeds of the crops produced to meet the nutritional needs of man and farm animals.

This study is focused on organic farming systems intended to preserve the ecosystem and agricultural land integrity, biodiversity, and food and feed security. The main objective of intercropping is the production of a higher yield per unit area by using resources or ecological processes that would not otherwise be used by a single crop. Intercropping is the practice of growing two or more crops in the same field at the same time. Multi-crop intercropping systems provide ecosystems with benefits derived from their respective independent symbiotic capacity for the nitrogen fixation of legumes and their nitrogen contribution to the next crop, via the remobilization of phosphorus by mycorrhizal and soil microorganisms, resulting in reduced nitrogen input and phosphorus with fertilizers and more rational use of energy, improving biodiversity and overall soil health. Bacteria capable of fixing nitrogen that are also possibly responsible for stimulating plant growth, such as *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter diazotrophicus*, *Azotobacter* sp., *Herbaspirillum* sp., and *Paenibacillus polymyxa*, have been reported to promote the growth of beneficial bacteria and plant growth [6,7]. Chamkhi et al., 2022 reported that the legume species used for the supply of grains fixed 40% of N derived from the atmosphere in intercropped soybean and 30% in the sole crop without fertilizer application [8]. The uptake of phosphate fertilizer was 21% lower in intercrops compared to sole crops for the same yields. Intercropping with legumes significantly improved soil fertility, the rhizobacteria community and diversity, and nutrient availability, which are determinants of increased crop growth [9].

The soil microbiome is the subject of research investigating the processes that counteract soil, the influence of abiotic and biotic factors, and methods of improving soil fertility. Plants influence soil biotic properties, and these changes in turn reflect on the growth, production, and survival of plant species [10]. A small percentage of microorganisms inhabiting natural ecosystems can be cultivated in the laboratory, in which case, traditional microbiological analysis methods cannot be useful. Through these, the dynamics of mixed crops, which are related to relevant biochemical changes in the soil, cannot be revealed. Developments in molecular biology have led to the development of techniques to ascertain soil microbial diversity [11].

Fluorescence spectral analysis allows non-invasive analysis and treatment in a short time, with high sensitivity, and without disturbing the integrity of the biological object. The optical properties of cereals and legumes are determined by their energy structure, which includes both the occupied and free electronic energy levels, as well as the energy

levels of the atomic vibrations of the molecules or the crystal lattice. The optical parameters and spectral properties also change as a function of temperature, pressure, external electric, and magnetic fields, etc., which allows the obtaining of essential information for the optimization of the yield and the content of polyunsaturated fatty acids (PUFA) [12].

2. Materials and Methods

2.1. Field Experiments

For soil microbiome analysis, soil samples were taken according to the methodology used in the experimental fields at the Agroecological Centre of the Agricultural University of Plovdiv (Plovdiv, Bulgaria, Yagodovo field, (Latitude: 42°08′60.00′′ N Longitude: 24°44′59.99′′ E), where camelina is grown, over two experimental years under the SCOOP project. Field trials were conducted on a certified organic field. The soil type was Mollic fulvisols—FAO—with a low humus content of 3.7% and a neutral pH. A randomized complete block design was used for conducting a small plot experiment on the effects of an intercropping system of camelina and protein crops, compared to sole crops of the same species. Three genotypes of *Camelina sativa*—the Polish winter varieties Luna (Cs1.Pro) and Lenka (Cs2.Pro) and a local Bulgarian landrace (Cs3.Pro)—were grown in two successive years: 2022 and 2023, in a pure stand and combined with fodder pea (*Pisum sativum* L.) and Cs3.Pro sand vetch (*Vicia sativa* L.) Cs.Vs.Pro. In this study, we present the results from the autumn cultivation in 2022 on small plots of 10 m^2 (1.4 × 7.7 m) (Supplementary Figure S1). The sowing was executed with a plot seeder Wintersteiger AG, with 800 germinating seeds/m^2. Each variant of the sole crop or its combination was investigated over three replications. Fertilization with solid fertilizers that are approved for organic farming was carried out before soil cultivation, with 30 kg/ha of active substance nitrogen.

2.2. Soil Samples and DNA Extraction

For the study, average samples were collected and analysed from five experimental plots with independent or mixed cultivation of three camelina varieties with pea variety Mir and vetiver variety 666. The soil was collected from the rhizosphere zone to the roots of 10 plants of each variant, randomly chosen. Rhizosphere soils were stored in sterile containers at −20 °C. The soil samples were cleaned and prepared in the laboratory at the Department of Microbiology and Environmental Biotechnology, Agricultural University of Plovdiv, and prepared for metagenomic analysis.

The DNA extraction method for rhizosphere soils with mono-cultivation of camelina varieties Cs1.S.Pro, Cs2.S.Pro, and Cs3.S and intercropped with vetch Cs3.Vs.S.Pro and pea Cs3.Ps.S.Pro was carried out using an isolation kit (Himedia, Thane, India), as defined by the mode-feed method of Nair et al. (2014) [13]. At A260/280 nm, the isolated DNA was quantified via the utilization of a Quantus fluorometer (Promega, Madison, WI, USA), and stored at −20 °C before being processed and sent to Novogene (Cambridge, UK) for NGS (HiSeq, Illumina, San Diego, CA, USA).

2.3. Metagenomic Sequencing and Analysis of Data

The amplification of PCR products for the 16S region with 16SV34 primers was successful for the five samples. The amplicon was sequenced on the Illumina paired-end platform to generate 250 bp paired-end raw reads (Raw PE), and then merged and pretreated to obtain Clean Tags [14]. The chimeric sequences in the Clean Tags were detected and removed to get the Effective Tags, which can be used for subsequent analysis, as was published by [14,15].

Metagenomic sequencing was carried out at Novogene (Cambridge, UK). Library preparation was performed with a Nextera DNA Flex kit (Illumina), following standard procedure. The amplicon was sequenced on the Illumina paired-end platform to generate 377 bp paired-end raw reads (Raw PE), and then merged and pretreated to obtain clean tags. The data were processed by QIIME software, v.1.9.1 (http://qiime.org/ accessed on 15 February 2014), as published by [16]. The first stage of the 16S rRNA gene analy-

sis involved quality control of the sequences to exclude from the analysis those with a length of fewer than 200 nucleotides, with a quality score of less than 25, with misread sequences of primers and multiplex identifiers, extensive homopolymer repeats (more than 8 nucleotides), and unidentified nucleotides. The chimeric sequences in the clean tags were removed to obtain effective tags, which were used for subsequent analysis [17–19]. The OTUs (operational taxonomic unit) were selected at more than 97% similarity. ACE and Chao indices and Shannon and Simpson diversity indices were calculated using the QIIME v. 1.9.1 [20]. Alpha diversity metrics summarize the structure of an ecological community by measuring the number of taxonomic groups together with the abundances of the groups, as was published by Willis (2019) [21]. Alpha diversity was analysed by the utilization of six indices, including Observed-species and Shannon indices, as calculated using QIIME (Version 1.9.1) and displayed with R software (Version 2.15.3) [16,22]. The heatmap based on the Weighted Unifrac and Unweighted Unifrac distances was analysed with R software (Version 2.15.3). The same R software was used to find the differences in the dominant taxa between the three groups of samples at each taxonomic rank; the top 10 taxa with the average abundance of the three groups of samples at each taxonomic rank were selected to generate a ternary plot. OTU comparisons were performed using the Venn diagram package (R software v.4.0.3) [22]. In our 16S information analysis process, the input file is the beta diversity distance matrix, which is the difference value matrix composed of the OTU abundance of two samples. After the statistical processing of the data, p values < 0.05 were considered statistically significant [23].

The BioProject accession number provided for these SRA data is PRJNA1106797 with submission ID: SUB14407308, submitted on 28-04-2024 The BioSample accessions are SAMN41150171, SAMN41150172, SAMN41150173, SAMN41150174, and SAMN41150175. SRA records will be accessible with the following link: https://www.ncbi.nlm.nih.gov/sra/PRJNA1106797 released on 1 May 2024.

Beta diversity, a comparative analysis of the microbial community composition, was carried out according to the taxonomy annotation results of all the samples and the abundance information of feature sequences [24]. The differences between different samples (groups) were found through Beta diversity index inter-group difference analysis, via the utilisation of the multivariate statistical method of Non-Metric Multidimensional Scaling (NMDS) [25]. For this analysis, samples with the closest distance are clustered together to form a new node. The average distance between the most abundant and other samples is calculated as the two closest samples.

2.4. Fluorescence Spectroscopy Setup

The mobile experimental setup used by fluorescence spectroscopy includes the following components as is shown in Figure 1:

- A laser diode (LED) with an emission radiation of 245 nm, with a supply voltage in the range of 3 V. It is housed in a hermetically sealed TO39 metal housing. The emitter has a voltage drop of 1.9 to 2.4 V and a current consumption of 0.02 A. The minimum value of their reverse voltage is—6 V.
- A rod lens of the achromatic doublet type. It is composed of two bonded lenses with different Schott and Corning dispersion coefficients, with an anti-reflective coating. The radii of the two lenses are selected so that the chromatic aberration of one lens compensates that of the other. The tolerance of the diameter of the forming optics is −0.005 mm.
- The multimode optical fiber is FG200LEA. It has a core diameter of 200 μm and a step index of refraction.
- A CMOS detector with a sensitivity ranging from 200 nm to 1100 nm. Its resolution is $\delta\lambda = 5$. The profile of the detector sensor projections along the X and Y axes is also designed for very small amounts of data, unlike many widely used sensors.

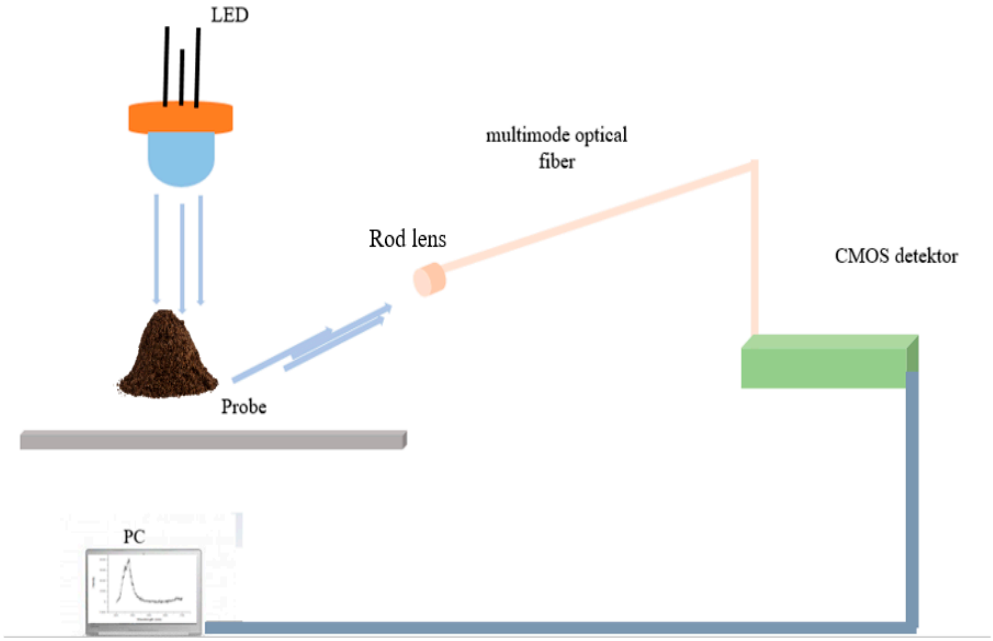

Figure 1. Mobile experimental installation used by fluorescence spectroscopy.

The sample is irradiated by the LED, after which it fluoresces. The emission signal is registered at 45 °C by the rod lens, which transmits it through the optical fibre to the detector.

The three unique advantages of this scheme are:

- Inclusion of the Rod lens in the construction of the system due to its increased light transmission efficiency, achieved by almost completely filling the air gaps between the individual lenses.
- Unique design of optical fibre coupling from a headquarters lens in a duralumin housing. In this way, the optimal design for compiling with optical fibres and forming images with laser diodes with low levels of intense losses is achieved.
- Registering of the emission signal at 45 °C.

3. Results and Discussion

3.1. Relative Abundance

The rarefaction and rank abundance curves shown in Figure 2 are widely used for indicating the biodiversity of samples. A rarefaction curve is created by selecting randomly a certain amount of sequencing data from the samples, and then counting the number of the species they represent. The observed species richness varied between the five soil samples. The rarefaction curves in Figure 1 showed some of this variation in the microbial abundance of the soil samples, particularly between Cs2.S.Pro, Cs3.S.Pro, and Cs2.S.Pro. The Rarefaction curves can directly reflect the rationality of the sequencing data volume and indirectly reflect the richness of the microbial community in the samples. If the curve is steep, this means that a lot of the species remain to be discovered.

Soil bacteria play an essential role in the cycling of organic matter in nature, soil formation, and soil fertility. Simultaneously, various secondary metabolites produced by soil bacteria during their life processes are also crucial to the soil ecological environment [26]. For a better visualization of the taxa with a higher relative abundance and their proportion in different classification levels for each soil sample, the most abundant 10 taxa at the phylum level were selected to form the distribution histogram of the relative abundance of taxa. Most major bacterial phyla such as Actinobacteria and Proteobacteria were present in Cs3.S.Pro, with nearly 20–60% of the total sequences identified as members of these phyla (Figure 3, in red and blue colour). Firmicutes are the most abundant in the rhizosphere soil

of the introduced camelina variety Cs1 and were decreased in the other soil samples. It is well known that intercropping can significantly affect soil properties and the composition of the microbial community. In our study, the dominant bacteria at the phylum level were Actinobacteria, Proteobacteria, Gemmatimonadota, Bacteroidota, Firmicutes, and Acidobacteriota, accounting for approximately 92% of the total abundance of the bacterial community. Despite differences in relative abundance, Actinobacteria, Proteobacteria, Acidobacteria, and Chloroflexi were also the dominant bacteria found by Chu et al. (2016) and Zhou et al. (2020) [27,28]. Actinobacteriota constitutes one of the largest bacterial phyla in many types of soil ecosystems [29]. They often live as plant commensals [30], nitrogen-fixing symbionts in soil [31,32], and are also a significant source of extracellular enzymes and secondary metabolites [33]. From the data, it is clear that intercropping significantly decreased the Firmicute abundance from 18.5% to 1.8% in the Sc2.S.Pro and Sc3.S.Pro and the intercropped plots Cs3.Vs.SPro and Cs3.Ps.SPro. In contrast, Actinobacteria and Acidobacteria abundance increased compared to the Cs1.S.Pro soil (Figure 3). Similar results have been obtained in intercropping experiments in which the bacterial biomass and activity in mono-cropping differed from those detected in intercropped systems [34].

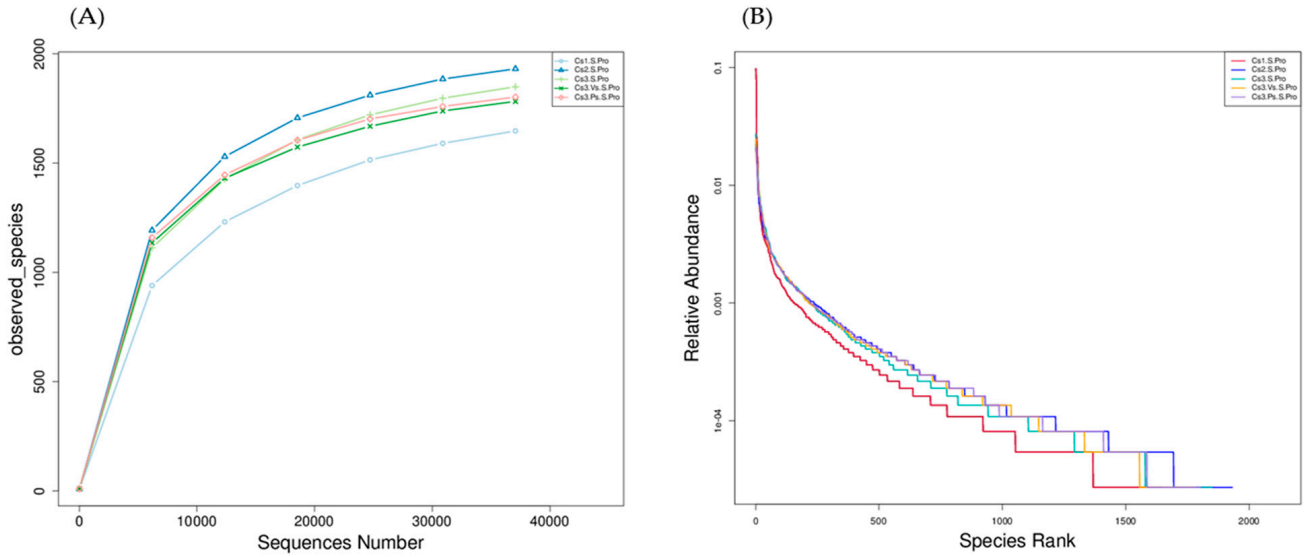

Figure 2. (**A**) Number of observed species (97% OTUs) from 16S amplicons for each of the five soil samples. (**B**) Rank Abundance curves of alpha diversity.

3.2. Abundance Heatmap Cluster of Different Microbial Classes

According to the abundance information of 35 genera of all the samples, the heatmap was drawn to check whether the samples with similar processing were clustered, while the similarity of and differences in the samples can also be observed. *Bacteroidia*, *Gammaproteobacteria*, *Bacilli*, and *Chlostridia* predominated in the Cs1.S.Pro soil (Figure 3). *Thermoanaerobaculia*, *Veruccomicrobiae*, and *Holophagea* were the most abundant in the Cs2.S.Pro soil. *Methanosarcinia*, *Anaerolineae*, and *Planctomycetes* dominated in the soil with the Cs3 cultivar of camelina when grown alone. An intercropping system with vetch stimulated the growth and distribution of *Nitrospira, Nitrososphaeria*, and *Blastocatellia*, involved in the oxidation of ammonia to nitrate via nitrite (Figure 4).

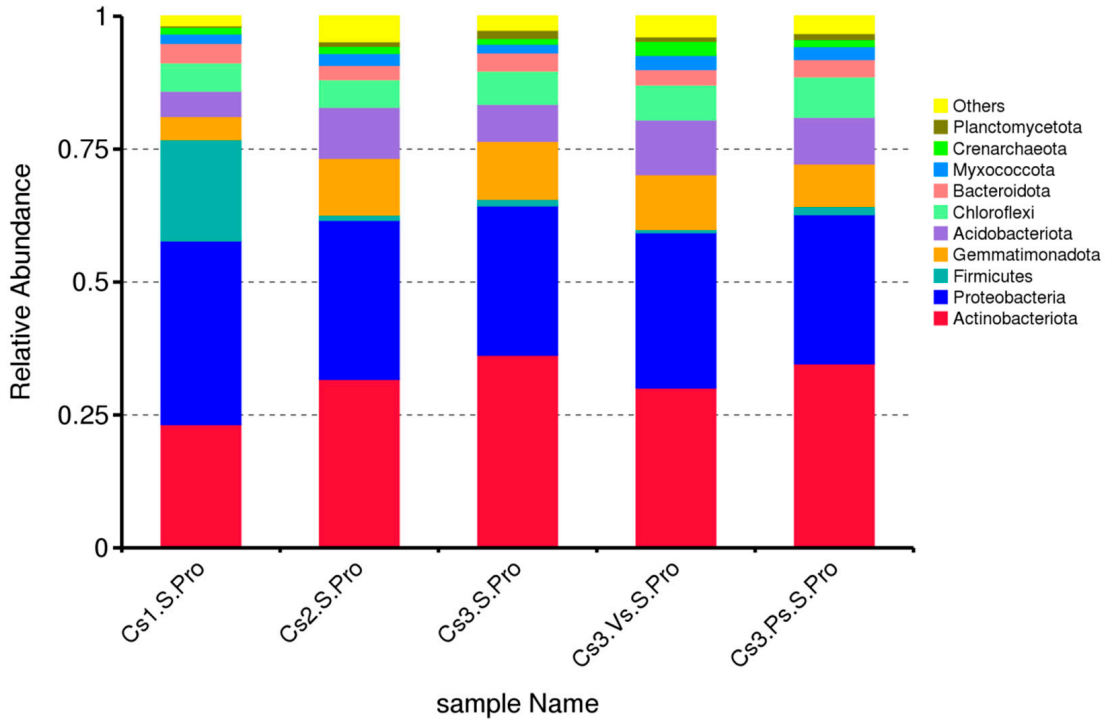

Figure 3. A histogram of the relative abundance of taxa at the phylum level.

In the co-cultivation of camelina with peas, out of 30 bacterial classes that have the greatest influence on plant biomass degradation in the soil microbiome, were Actinobacteria, Rubrobacteria, Acidobactreria, KD4-96, Entotheonellia, and Chloroflexia. In contrast to the intercropping system with vetch, in the camelina–pea soil, the abundance of species from the phyla *Nirtosphaeria* and *Nitrospira* was low. *Nitrospira* can utilize simple organic substrates, and they can survive in soil conditions where organic carbon is available and the paucity of nitrogen limits the *Nitrosomonadaceae*. When nitrogen fertilizer is applied, they may be less able to take advantage of the increased ammonia concentration, and their levels may decrease in the soil. Those results correspond to the findings of Li et al., 2019 [35]. There, they observed less pathogenic *Bacilli* and *Clostridia* in the Cs3.Ps.S.Pro soil compared to a sole cultivation of camelina. Thus, the application of camelina–pea intercropping has a beneficial role in suppressing soil-borne plant pathogens. Ji et al., 2022 reported that the yield of pepper was positively correlated with the contents and the relative abundance of *Bacillus* and *Clostridium* in soil and was negatively correlated with the NO_3-concertation in soil [36].

From our results, soil bacterial communities varied in intercropping soil types with peas and vetch (Figure 4). Soil environmental factors are another factor explaining these soil bacterial community variations. Soil pH was most likely the primary factor determining differences in ammonia-oxidizing bacteria composition between different plots. Intercropping with legume plants improves the utilization of nutrient resources and the growth performance of intercrops [37]. Legume-based intercropping systems are known to increase microbial diversity and improve soil functioning, where nutrients become more available for plant roots [38]. Burle et al. (1997) reported that legume-intercropping systems have beneficial effects on soil nutrient availability [39].

Figure 4. Taxonomic abundance cluster heatmap at the class level.

3.3. Alpha Diversity

Six alpha diversity indices were applied to compare soil microbial community differences between the five different soil types (Table 1). The Good's coverage indices in soil were higher than 0.99, indicating that the sequenced results were sufficient to reveal the

true diversity. Observed species were higher in the soil with co-cultivation of camelina and vetch Cs3.Vs.S.Pro (1931 species) and significantly lower in the soil with mono-cultivation of the introduced variety Cs1. The indices of Sannan and Simpson were higher in soils when the local variety Cs3 grew alone and was intercropped with peas and vetch (Table 1). 16S rRNA gene sequencing revealed that the soil bacterial composition and diversity among the single and intercropped soils differed according to the variation in the community structure. The alpha diversity index showed significant differences in the alpha diversity of soil bacterial communities when camelina was grown with peas and vetch (Table 1).

Table 1. Alpha diversity according to six indices.

Soil Sample	Observed_Species	Shannon	Simpson	Chao1	ACE	Goods_Coverage	PD_Whole_Tree
Cs1.S.Pro	1647	7.791	0.975	1770.6	1828.321	0.992	134.844
Cs2.S.Pro	1782	8.982	0.996	1896.5	1901.636	0.994	138.570
Cs3.S.Pro	1849	9.094	0.995	1976.1	2008.721	0.993	146.101
Cs3.Vs.S.Pro	1931	9.226	0.996	2037.4	2051.597	0.994	148.461
Cs3.Ps.S.Pro	1802	9.150	0.996	1934.4	1910.993	0.994	139.961

3.4. Beta Diversity Analysis

Beta Diversity was analysed according to the taxonomy annotation results of all the samples and the abundance information of the feature sequences; the feature sequence information of the same classification was combined to obtain the species abundance information table (Profiling Table). At the same time, the phylogenetic relationship between feature sequences was used to calculate the unweighted unifrac distance [40,41]. The unifrac distance was determined to calculate the distance between samples by using the evolutionary information between the microbial sequences in each sample. The weighted unifrac distance (unweighted unifrac) was further constructed using the abundance information of the feature sequence [40]. Finally, the differences between different samples (groups) were found through beta diversity index inter-group difference analysis using Non-Metric Multidimensional Scaling (NMDS).

The weighted Unifrac distance and Unweighted Unifrac distance between single microbial diversity in soil with the cultivation of camelina vs. intercropping diversity were selected to measure the dissimilarity coefficient between pairwise samples, which is a widely used phylogenetic measurement method in microbial community sequencing projects. The heatmap based on the Weighted Unifrac and Unweighted Unifrac distances is plotted in Figure 5.

3.5. Non-Metric Multidimensional Scaling (NMDS)

Non-metric multidimensional scaling analysis is a ranking method applicable to ecological research [25]. A total of 1647 to 1931 OTUs were found to be present in the samples; 235 were identified, using the KW filter, to have significantly different abundances between the single cultivated camelina varieties and varieties intercropped with peas and vetch. The result of the NMDS analysis, based on the sequencing results (OTUs), is in Figure 6. The NMDS based on Bray–Curtis dissimilarities was conducted to reflect the soil bacterial beta diversity of the rhizosphere soil with camelina compared to soil with co-cultivation of camelina with legumes. The results showed that OTUs were clustered within the inter-cropping system of Cs3.Vs.S.Pro and Cs3.Ps.S.Pro (Figure 5), indicating a clear separation of soil bacterial communities with single cultivations of camelina (Figure 6), which showed distance (stress = 0.0). This result indicated that the three camelina varieties Luna, Lenka, and local Bulgarian landrace, when solely cultivated, congregated in different rhizosphere microbial communities.

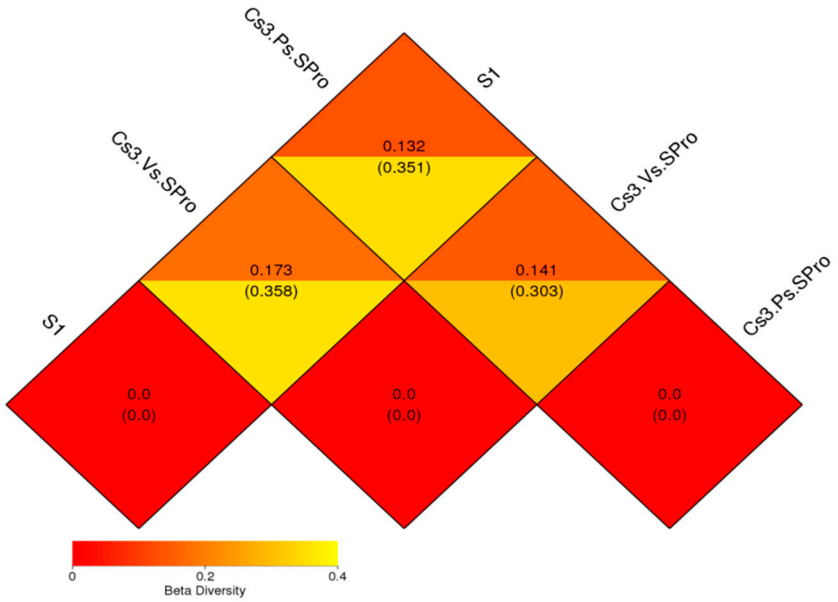

Figure 5. Beta diversity heatmap.

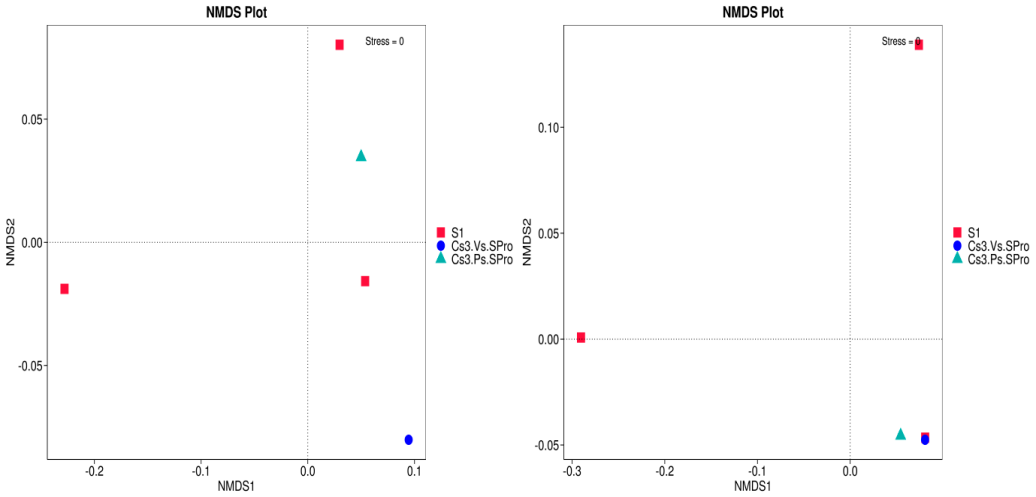

Figure 6. NMDS based on Weighted Unifrac distance (left) and Unweighted Unifrac distance (right). Each data point in the graph stands for a sample. The distance between data points reflects the extent of the variation. Samples belonging to the same group are in the same colour. When the value of Stress factor is 0, it is considered that the NMDS is reliable to some extent.

3.6. Mobile Fluorescence Spectroscopy

The optical properties of a soil are determined by its energy structure, which includes both the occupied and free electronic energy levels as well as the energy levels of the atomic vibrations of the molecules or the crystal lattice. The possible transitions between these energy levels, as a function of photon energy, are specific to the soil in which certain genotypes are grown, resulting in spectra and optical properties unique to it. The soils in which the camelina was grown contained particles smaller than the wavelength of visible light. Particles in the turbid medium, such as the soil in this study, act as independent light sources, emitting incoherently and causing the samples to visibly fluoresce.

A correlation was clearly observed in the value of the emission wavelengths of the soil samples from self-grown plants co-cultured with peas and vetch. In the self-grown plants, the highest intensity was Cs3 (Figure 7). These results are directly proportional to the results of the abundance heatmap cluster of different microbial classes and the Non-Metric Multidimensional Scaling (NMDS). The corrections in the spectral distribution were carried

out on the different rhizosphere microbial communities of the three varieties of Camelina Luna, Lenka, and Local Bulgarian landrace, which were cultivated independently.

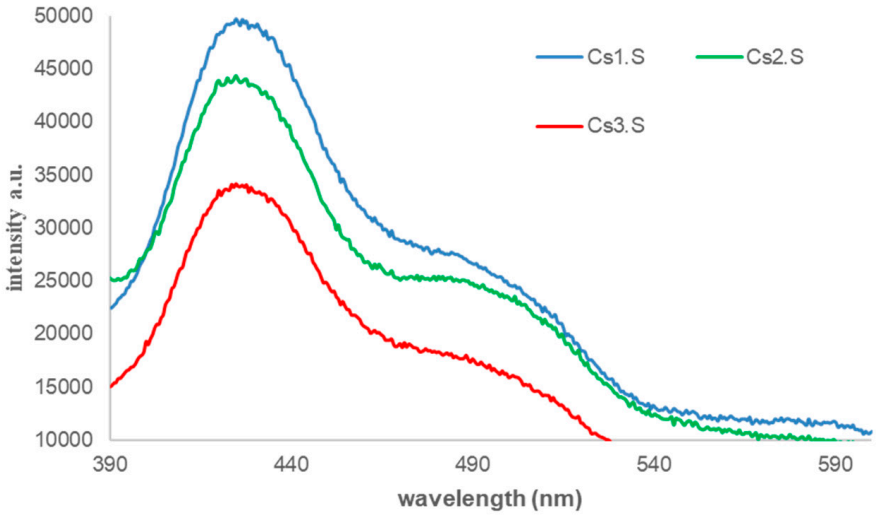

Figure 7. Difference spectral distribution in Cs1.S, Cs2.S, and Cs3.S (self-grown plants).

The signal intensity of the intercropped samples was higher than that of the self-grown plants. The sample with the highest signal intensity was the sample grown in combination with vetch (Figure 8). The results are directly proportional to the results of the alpha diversity and abundance heatmap cluster of different microbial classes. Correlations in spectral distributions in single and intercalated soils are based on the diffuse bacterial composition and diversity of soils, being directly proportional to variations in community structure.

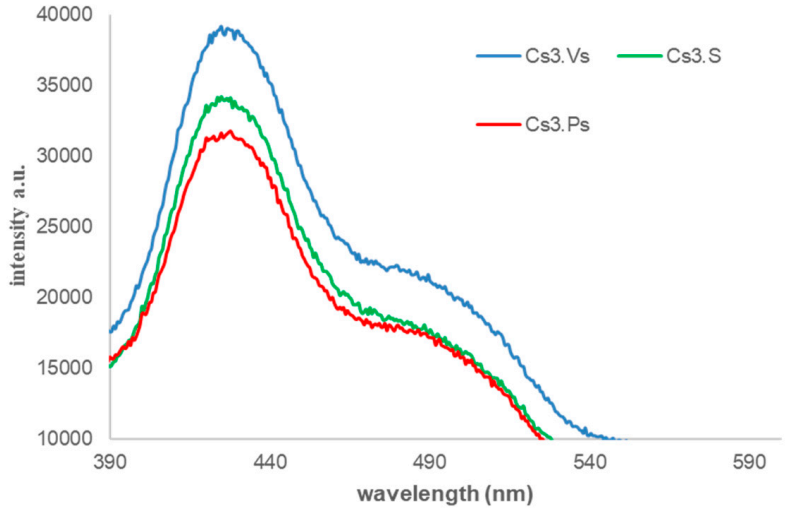

Figure 8. Difference spectral distribution in Cs3.S, Cs3.Vs, and Cs3.Ps (intercropped samples).

4. Conclusions

The current study indicated that intercropping camelina with peas and camelina with vetch in the first year of experimentation changed the microbial community structure and altered the soil nutrient content. Significant differences were found in the bacterial community structure between the five monosystems and the different intercropping soil samples. These differences showed legumes' influence on the camelina's bacterial structure. Results suggest that peas and vetch could play an important role in maintaining agricultural ecosystem stability and improving crop growth. Changes positively affected soil microbial community structure and crop yield, with plant pathogen problems associated with

monocrops. The intercropping systems assayed (Sc3.Vs.S.Pro and Sc3.Ps.S.Pro) increased the camelina oil content and yield compared to the local camelina (Sc3 BG landrace) due to the better nutrition of the plants (unpublished data). The results from the NMDS assessing the beta diversity among soils revealed that the soil bacterial communities in soils with intercropping diverged significantly from the soils with single camelina cultivation. A systematic engineering approach for the alignment (optical tuning) of a dedicated mobile fluorescence spectroscopy applied research facility was found to be applicable in the characterization of the soil. It was proven that mobile fluorescence spectroscopy will support local analyses of soils in which camelina is grown alone, as well as those in combined cultivation of camelina with fodder peas and vetch.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14199046/s1, Supplementary Figure S1. (A) A randomized complete block design was used for setting a small plot experiment; (B) Cs1 (Luna), Cs2 (Lenka), and Cs3 (Bulgarian landrace); (C) Cs3 intercropped with pea (*Pisum sativum* L.); (D) Cs3 intercropped with vetch (*Vicia sativa* L.).

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