Forecrop Effects on Abundance and Diversity of Soil Microorganisms during the Growth of the Subsequent Crop

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Abstract: Plant residues introduced into the soil influence the soil’s physical, chemical, and consequently, biological parameters, which have an influence on plant health and yields. This study was undertaken to evaluate the effects of the cultivation of widespread plant species, faba bean (Vicia faba L., F) and wheat (Triticum aestivum L., W) (as forecrops) grown under conventionally tilled fields, on the diversity of microorganisms and the enzymatic activities of soil during the growth of the subsequent wheat. Bulk soil samples (0–20 cm) were taken three times from two months after residue incorporation into the soil to the maturity of the subsequent wheat crop in order to assess the fungal and bacterial communities (Next Generation Sequencing (NGS), terminal restriction fragments lengths polymorphism (t-RFLP)), ammonia oxidizing archaea (t-RFLP, denaturing gradient gel electrophoresis (DGGE)), and the enzymatic activity of soil. Differences in the genetic structure of ammonia-oxidizing archaea (AOA) were observed for each treatment and sampling term, indicating that plant and weather conditions are the driving force for microorganism selection and adaptation. We observed that the fungal community was more influenced by the forecrop type used than the bacterial community. The activities of the enzymes changed in response to the forecrop and sampling period. A higher number of microorganisms that are associated with plant benefits with respect to nutrients and growth, as well as higher amounts of N in the residues, was noted in faba bean than in wheat soil. This could indicate better growth conditions after faba bean and, consequently, better yield quality and quantity. This may also indicate some protective role of the soil after faba bean against pathogens, which may be connected with lower fungicide requirements. The obtained results lead to a deeper understanding of the microorganism reactions to faba bean and wheat residues during wheat cultivation. In addition, they may be helpful in improving our understanding of subsequent crop yield responses to forecrops.

Keywords: Vicia faba; Triticum aestivum; soil microbiota; crop residue; soil enzymes; ammonia oxidizing archaea; crop rotation

1. Introduction

Among the practices that are used to improve the sustainability of agriculture, legume cultivation (used as a cover crop in intercropping or crop rotation) plays a pivotal role in the enhancement of soil fertility and productivity. Their favorable role in cropping systems mainly includes improving the physical conditions of the soil and biodiversity, the enhancement of soil microbial activities, phosphorus mobilization, and increasing the N status as a result of biological nitrogen fixation [1–3]. The amount of nitrogen derived from the atmosphere (Ndfa) in faba bean plants at maturity (including roots, stems, and stripped pods) reached 67–73%, depending on the growing season [4]. The positive
impact of legume cropping can be further enhanced by associations with arbuscular mycorrhizal fungi (AMF), which improve P and N uptake, nodulation, biomass production, seed yield, and water uptake. It was shown that AMF also reduce the occurrence of soil-borne plant diseases (by reducing disease severity and the colonization of the pathogens and by the production of phytoalexin) [5–8]. Several scientists have reported the effects of legumes on the improvement of different subsequent crop yields and quality, e.g., for wheat, barley, and maize [6,9–12].

Crop rotation with legumes can influence soil biota. Soil microorganisms are important factors influencing soil health and plant production since they play an essential role in decomposing organic matter and cycling nutrients. They may beneficially effect plant growth by producing various metabolically active substances, plant growth regulators, siderophore, and antibiotics. Moreover, they stimulate the mycorrhizal symbiosis, activate plant pathogen resistance, suppress pathogen growth, and alleviate the harmful effects of abiotic stressors like drought and salinity. However, some microorganisms cause plant diseases and reduce crop yield and quality. It has been shown that, after six years of wheat monoculture and wheat rotations with legumes, the bacteria and fungi populations and enzyme activities were higher for the legume-based rotations than for the wheat monoculture or fallow-wheat rotation in soils taken two months after the harvest of the wheat crop, from depths of 0 to 10 cm [13]. Lupwayi et al. [14] revealed that the microbial diversity of the soil (based on functional analyses, including community level physiological profiling) taken from a depth of 0–7.5 cm at the leaf stage of wheat was significantly higher under wheat preceded by legume pea cultivation compared to wheat monoculture. In addition, the effects of legume-based crop rotations were more evident in bulk soil than in the rhizosphere. Similarly, Granzow et al. [15], in a pot experiment with wheat and faba bean plants, detected differences in microbial richness and diversity between intercropping and monoculture. In rice-based crop rotations, legumes significantly influenced bacterial and fungal diversity, as well as the abundance of specific bacterial and fungal taxa [16]. A laboratory study by Alvey et al. [17] revealed differences in the bacterial community structure between cereal with legume rotation and cereal monoculture based on the denaturing gradient gel electrophoresis (DGGE) method. Under field study, Xuan et al. [18] observed that crop rotation systems with mung bean differed significantly in comparison with rice monoculture in relation to the composition, abundance, and diversity of soil bacterial communities. This was shown to have a beneficial effect on rice yield.

The incorporation of crop residues into the soil is connected with changes to its biological activity and microbial abundance. The soil microbial community and structure during plant residue decomposition differs depending on the plant species. It has been shown that, during the decomposition of rye and hairy vetch used as cover crops in corn agroecosystems, the soil bacterial microbiome differed significantly depending on the cover crops used. Additionally, the effect of the plant species used as a cover crop increased with the decomposition period, and the succession of different bacterial groups related to the cover crop species was noted [19]. In the aforementioned study, differences in soil enzymatic activity between treatments were also observed. In another study, during an 11-month period of plant residue (wheat, rape, and alfalfa) decomposition in field conditions, Pascault et al. [20] observed that the composition of the bacterial community was clearly related to the biochemical quality of the residue and degradation stages. However, Scalise et al. [11] noted that, in a field experiment testing grain legumes (pea, faba bean) used in a barley intercropping system, changes were revealed in the bacterial community structure in the succeeding durum wheat using fingerprinting length-heterogeneity polymerase chain reaction (LH-PCR), and the results revealed only a minor role for the cropping treatment on the bacterial community composition. Changes in soil bacterial community composition under legume-based intercropping were observed at the pre-sowing stage, but they disappeared at the harvest stage of the succeeding wheat.

To date, the dynamics of fungal and bacterial communities in bulk soil after the incorporation of plant residues in field experiments, especially under subsequent crop growth, has been poorly investigated. Thus, the aim of the present study was to investigate the effects of the crop residues of different plants on the soil microbiota and the enzymatic activities of soil during the growth of
subsequent crops in a loamy soil under a conventional tillage system. Two common, globally important crops, which differ with regard to the biochemical composition of the crop residues, were used as a forecrop: faba bean, a grain legume crop, and wheat, a popular cereal. In this study, we evaluated the structure of the fungal, bacterial, and archaeal communities, including ammonia oxidizing archaea, and the enzyme activity. Our investigation focused on the 0–20 cm soil layer, which corresponds to the tilled layer in conventionally tilled systems and is thus within the range of the presence of crop debris. Plant debris incorporated into the soil by shaping soil microbial and physicochemical properties creates conditions for the growth and health of a succeeding crop, which can differ depending on plant species. We undertook this study to improve our understanding of the selective influence of legume and cereal crop residues on the dynamics of microbial and biological activities, which are the essential factors for sustainable crop management and contribute to a better understanding and clarification of the relationship between the forecrop and the soil conditions for the growth of the successive crop.

2. Materials and Methods

2.1. Site and Treatments

The study was performed on a Haplic Luvisol derived from loess in Lublin, Poland (51°15′ N, 22°35′ E). The soil is characterized by clay, silt, and sand contents in the 0–20 cm soil layer of 70 g kg$^{-1}$, 290 g kg$^{-1}$, and 640 g kg$^{-1}$, respectively. The total N content (based on the Kjeldahl method [21]) was 0.75 g kg$^{-1}$; the organic carbon was 8.97 g kg$^{-1}$; and the soil pH had a value of 6.9 ($\text{H}_2\text{O}$).

The available K, P, and Mg contents were at 135, 114, and 39 mg kg$^{-1}$, respectively. K was measured using flame emission spectrometry (FAES) after the wet sulphuric acid digestion of the samples; P was determined using a spectrophotometric method, which involved measuring the intensity of the blue color of phosphate-molybdenum blue; and Mg was determined using flame atomic absorption spectrometry (FAAS). The soil chemical analysis, described above, was based on a 0–20 cm soil profile. The study area was under long-term (35 years) conventional tillage.

In 2017, two crops that are important for agronomy, faba bean (\textit{Vicia faba L.}) (F) and spring wheat (\textit{Triticum aestivum L.}) cultivar Kandela (W), were sown as forecrops in plots of 2 × 3 m, which were arranged in three repetitions and randomly organized. At maturity, the aerial parts of the plants were collected from each plot separately, and the grains were separated from the plant residues. The plant residues (stems and stripped pods for faba bean and straw for wheat) were chopped into approximately 3–4 cm long pieces and were evenly distributed over the surface of the plots that they were collected from.

At the beginning of September, plant residues were incorporated into the soil to a depth of 20 cm. In 2018, the spring wheat (\textit{Triticum aestivum L.}) cultivar Kandela was sown in the field plots planted with faba bean and wheat during the previous growing season. In addition, in order to better explore the effects of the plants, fallow plots (without any plants during the whole analysis period 2017–2018) were set up as a reference (R) (three replicates). The study treatments were as follows: F, where faba bean was planted as a forecrop; W, where wheat was used as a forecrop; and R, the reference soil without plants. The plants were cultivated under conventional systems, and fertilization was applied in both years, according to a soil test performed before establishing the field trail, and it was uniform for all treatments in 2017. In 2018, no fertilization was applied to the reference soil.

Faba bean and wheat straw (forecrops) were analyzed for C and N content, as well as for biomass yield. At maturity, the aboveground parts of the plants were harvested from an area of 0.3 m$^2$, with three replications. The C and N contents in the plants were determined using a CHNS Elemental Analyzer (PerkinElmer, Inc., Waltham, MA, USA). The biomass of the plant residues was similar for faba bean and wheat (Table 1). However, the treatments differed significantly with respect to the N amount incorporated within the residues (faba bean showed 1.7-fold higher values than W), as well as the C and N values, which were about two times higher under wheat than faba bean.
Table 1. Parameters of faba bean and wheat plant residues (forerops) with standard deviations (n = 3).

| Forecrop       | Biomass   | Yield (g m⁻²) | C/N       |
|----------------|-----------|---------------|-----------|
| Faba bean      | 404 (5.9) | 1.88 (0.03) b | 168.6 (2.6) a | 37.5 (0.8) a |
| Wheat          | 459 (47.7)| 1.08 (0.18) a | 192.8 (18.5) a | 76.0 (10.4) b |

N-nitrogen, C-carbon, C/N-carbon to nitrogen ratio. Different letters denote significant differences (p < 0.05).

2.2. Weather Conditions

The weather conditions (rainfall and air temperature) were recorded hourly throughout the experimental period using the meteorological station of the Institute of Agrophysics, Polish Academy of Sciences in Lublin, located within 200 m of the experimental site. The amount of rainfall in 2017 and 2018 was 478.0 and 387.6 mm, respectively, and average yearly air temperatures were 8.3 and 9.7 °C, respectively (Figure 1). The long-term average yearly precipitation and air temperature for this site are as follows: 552 mm and 7.4 °C. With regard to the amount of rainfall during the plant growing season (April-July), it was 157 and 228 mm in 2017 and 2018, respectively, while the long-term average amounted to 247 mm.

![Figure 1. Weather conditions during 2017–2018 and long-term averages for the study site.](image)

2.3. Soil Sampling

The changes in soil microbiota and activity during subsequent plant growth were described on the basis of three sampling periods: 2 months after residue incorporation into the soil in November 2017 (T1); May 2018 at stem elongation (BBCH 31) (T2); and August 2018 at the senescence stage of wheat used as a subsequent crop (BBCH 99) (T3). These sampling periods corresponded to 60, 254, and 331 days after forecrop residue incorporation. The bulk soil (non-rhizosphere) was collected between two adjacent wheat rows. Two months after residue incorporation into the soil, soil samples were collected from the bulk soil, and, in the same way (from bulk soil), soil samples were taken during subsequent wheat growth. The soil was randomly sampled from each plot separately at 5 points from each plot from the layer of 0–20 cm with a sampler 2 cm in diameter and thoroughly mixed into one composite sample (3 composite samples for each treatment). The soil was sieved (0.2 cm mesh) before analysis. One part of the soil samples was stored at 4 °C and used for microbial analysis; another part was stored for a short period at –20 °C for DNA analysis.

The dry weight of the soil (105 °C) was analyzed using a moisture analyzer (RADWAG Max 50/1, Radom, Poland). The dry weights of the soil at T1, T2, and T3 under R were as follows: 16.6%, 12.5%, and 11.7%, respectively. Under F, they were 16.8%, 12.9%, and 11.6%, respectively, and, under W, they were 17.3%, 13.4%, and 12.3%, respectively.
2.4. DNA Isolation and Analysis

Genomic DNA was extracted from 0.5 g of soil using a FastDNA® SPIN Kit for Feces (MP Biomedicals, Solon, OH, USA) according to the manufacturer’s instructions. The amount of DNA was determined by a spectrophotometer (NanoDrop 2000/2000c Thermo Scientific, West Palm Beach, FL, USA) at 260 nm.

2.4.1. Ammonia Oxidizing Archaea (AOA) Population

The abundance of ammonia oxidizing archaea (AOA) based on the ammonia monooxygenase α-subunit (amoA) gene was analyzed with denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment gel polymorphism (t-RFLP) methods. The PCR conditions for this analysis are provided in Table S1. Detailed information about DGGE and t-RFLP analyses has been provided by previous studies [22,23].

2.4.2. Total Archaeal, Bacterial, and Fungal Community

The genetic diversity of the total archaeal, bacterial, and fungal community was assessed using the multiplex approach of the terminal restriction fragment length polymorphism technique (M-tRFLP) [24]. The conditions of the amplification reactions are presented in Table S1. After the PCR, the products of the amplifications were purified with a mixture of two enzymes, thermo sensitive alkaline phosphatase and Exonuclease I (Exo-BAP Mix, EURx, Gdańsk, Poland), under the following conditions: 37 °C for 15 min and then 15 min at 80 °C. Subsequently, the products were purified with Performa® Dye Terminator Removal (DTR) Gel Filtration Cartridges (EdgeBio), according to the protocol of the producer. The first purification stage of PCR amplicons enables the fast and effective purification of samples from primer residues and free nucleotides. The second purification step was performed with Performa DTR Gel Filtration Cartridges (column packed with a gel matrix and optimized to remove deoxynucleoside triphosphates (dNTPs) and other low molecular weight materials). This column also removes fragments up to 15 bases. This two-step purification process provides high quality separation of restriction fragments. The purified DNA products were digested by Hae III restriction enzyme (EURx, Gdańsk, Poland). The 10 µL of restriction mixture contained about 50 ng DNA, 0.6 µL of restriction enzyme (10 U/µL), and 0.6 µL of buffer (ONE buffer, EURx, Gdańsk, Poland) containing 100 µg/mL bovine serum albumin (BSA) (EURx, Gdańsk, Poland), and were incubated at 37 °C for 2 h and inactivated at 80 °C for 20 min. The 1 µL of digestion products were mixed with 9.5 µL of formamide and a fragment length standard GS-600LIZ (Applied Biosystems, Foster City, CA, USA) mixture. The detection of the size and abundances of the restriction fragments was performed with capillary electrophoresis using an automated ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). On the basis of in silico analysis using the TRiFLe program [25], the terminal restriction fragments (t-RFs) were identified using the reference files for archaeal, bacterial, and fungal sequences prepared with the nucleotide database of The National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). The reaction was performed in optimized conditions of the method according to Gryta and Frąc [24]. Despite multiple repetitions of the PCR reaction for the samples from T1, no amplification product was obtained. Therefore, only the results from T2 and T3 were present.

2.4.3. The Structure of the Fungal and Bacterial Population

The soil microbiome (based on the bacterial 16S rDNA gene and the fungal ITS1 fragment) was tested by next generation sequencing (NGS). The PCR conditions of the tested genes and the primers used are provided in Table S1. The fungal community was assessed on the basis of the hypervariable region ITS1, while the bacterial community was based on the hypervariable region V3–V4 of the 16S rDNA gene. Specific primers U341F and U785R for the 16S rDNA gene [26] and ITS1F12 and 5.8S for the ITS1 region [27,28] were used for the amplification and preparation of the library.
The PCR reaction was performed with a Q5 Hot Start High-Fidelity 2X Master Mix, as recommended by the manufacturer (NEB Inc., Ipswich, MA, USA). Sequencing (Illumina MiSeq PE300) was performed with a v2 Illumina kit, and 2 × 250 bp paired–end technology was applied. An automatic preliminary data analysis was performed on MiSeq with software from the MiSeq Reporter (MSR) v2.6. The classification of the species level was performed with the Qiime software using reference sequence databases Greengenes v13_08 and UNITE v7 for bacteria and fungi, respectively. Bacteria and fungi with an abundance of >1% were further analyzed. The ecological guild of the fungal operational taxonomic units (OTUs) was analyzed by FUNGuild [29].

2.5. Biochemical Analysis of Soil

A soil activity assessment included enzymes that play an essential role in the cycles of C, such as dehydrogenase, cellulase, and β-glucosidase. N transformation was assessed with urease, while P transformation was conducted using measurements of acid phosphomonoesterase activity. Table 2 shows some basic information concerning these analyses. The activity of dehydrogenase was determined by assessment of the 2,3,5 triphenyl-tetrazolium chloride (TTC) reduction to triphenyl formazan (TPF) after soil incubation for 96 h at 30 °C. β-glucosidase was estimated by measuring the p-nitrophenol released after the incubation of the soil with p-nitrophenol glucoside (PNG) solution at 37 °C for 1 h. Urease activity was determined using urea as a substrate and determining the release of ammonia after incubation for 18 h at 37 °C. Cellulase activity was based on evaluation of the released reducing sugars by soil incubated (for 24 h at 50 °C) with carboxymethyl cellulose (CMC) sodium salt solution. Acid phosphomonoesterase was assessed by incubating soil samples with p-nitrophenyl phosphate (PNP) (for 1 h at 37 °C) and then measuring the p-nitrophenol concentration. All analyses were performed in three replicates, and the activities were calculated based on the dry (105 °C) weight of the soil.

Table 2. Methods used for the assessment of enzyme activities.

| Enzyme             | Substrate                                          | Reference                                               |
|--------------------|----------------------------------------------------|---------------------------------------------------------|
| Dehydrogenase      | 2,3,5-triphenyltetrazolium chloride (TTC)          | Thalmann [30], modified by Alef [31]                   |
| β-glucosidase      | 4-Nitrophenyl β-D-glucopyranoside (PNG)           | Alef and Nannipieri [32]                               |
| Urease             | urea                                               | Zantua and Brenner [33]                                |
| Cellulase          | carboxymethylcellulose (CMC) sodium salt          | Schinner and von Mersi [34]                            |
| Acid phosphomonoesterase | p-nitrophenyl phosphate (PNP)                     | Tabatabai and Brenner [35]                             |

2.6. Statistical Analysis

Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA, 2011) was used for statistical analyses. A GLM Univariate Analysis was applied, and the two effects (sampling time and plant treatment) were tested as independent variables. The t test was applied to make a comparison between treatments.

DGGE patterns were rated by the presence (1) or absence (0) of individual bands. A dendrogram was obtained from the binary data describing the banding patterns, and it was obtained through the use of an unweighted pair group mean average (UPGMA; Dice coefficient of similarity).

3. Results

3.1. Forecrops and Sampling Time Result in Changes in Genetic Diversity of Ammonia-Oxidizing Archaea (AOA) Community

3.1.1. Differences in the AOA Community Structure (DGGE Method) Were Observed for Forecrop Treatment and Sampling Time

The amount of isolated DNA from the soil was highly differentiated depending on the treatment and time (Table 3). The lowest amount of DNA was isolated from the reference soil, irrespective of
time, and, at T2 and T3, F showed a greater amount of DNA than W. It should be noted that, at T1, a higher amount of DNA was noted for wheat compared to faba bean.

Table 3. Concentration of DNA (ng µL⁻¹) after extraction. T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively.

| Sampling Term | Treatment |
|---------------|-----------|
|               | Faba Bean | Wheat | Reference Soil |
| T1            | 89        | 137   | 56           |
| T2            | 150       | 142   | 111          |
| T3            | 114       | 110   | 68           |

The DGGE fingerprints based on a bands pattern indicated changes in the ammonia oxidizing archaea community in soils under different plants. Shifts in the archaeal composition at different times of sample collection were observed for the three treatments of faba bean, wheat, and R. The similarities between the DGGE patterns obtained from the fingerprints of the various soil samples were calculated and visualized as a clustered tree to evaluate the changes within the ammonia oxidizing archaea community under different plants used as forecrops and in the reference soil without plants (Figure S1). Clear differences in the AOA community structure were observed for each treatment and sampling period, which indicates that plant and weather conditions are the driving force for microorganism selection and adaptation. However, the dendrogram derived from the DGGE patterns indicates that the fingerprint of the ammonia oxidizing archaea in the soil under wheat is clustered together with the profile of the community from the reference soil without plants in two sampling times: T2 and T3. While the recovered DGGE fingerprints from faba bean at T2 and T3 created a separate cluster, the resulting dendrogram showed that archaeal communities in the first sampling period were not grouped, and separate tree branches for faba bean, wheat, and R were observed.

t-RFLP fingerprinting showed that the forecrop influenced the population of the ammonia-oxidizing archaea (AOA) community (Figure 2a,b). Soil samples taken two months after residue incorporation into the soil showed different t-RFLP patterns than the soils from the other times, irrespective of restriction enzymes. Using a rare-cutting restriction enzyme (Csp6I), two main fragments were obtained at T1: 52 bp (with an abundance of 37–70%, depending on the treatment) and 189 bp (Figure 2a). Digestion with the AluI enzyme (Figure 2b) revealed one main fragment of 253 bp (85–88%) which was only observed at T1, and other specific fragments for this sampling period (57, 58, 149, 1and 63 bp). Apart from T1, in general, no dominant fragment was observed under all three treatments. Generally, the faba bean treatment has been shown to have a greater number of fragments than other treatments at all sampling times after AluI digestion. AluI digestion resulted in some fragments that were observed in wheat treatment (51, 183, and 275 bp) and that were absent in faba bean treatment. For both restriction enzymes, differences in the terminal restriction fragment (T-Rf) profiles were clearly apparent with regard to the sampling period and treatments.

3.1.2. Relative Abundance of Total Archaeal, Bacterial and Fungal Community Was Modified by Forecrops

The differences between the soil samples in the total archaeal, bacterial, and fungal community structure were observed mainly in the relative abundance and, to a lesser extent, in the presence of specific T-Rfs (Figure S2). A similar number of T-Rfs fragments was noted for all treatments with regard to archaea (from 29 to 32 fragments, depending on the treatments), bacteria (9–12 fragments) and fungi (7–10 fragments). However, significant differences between the treatments were noted with respect to the Shannon index, especially for fungi and bacteria (Table S2).
Aeromonas were more pronounced at T3 than at T2 (Table S3). Fusarium At T1 and T3, a greater number of these fungi was under faba bean soil than wheat, and the opposite situation was noted at T2. Taking into consideration the whole sampling period, fungal abundance at all sampling periods. Relatively higher differences between plants were observed at T2 and T3 than at T1. The effect of the plant species increased with time.

As shown by TRiFle analysis, the differences between bacteria community between treatments were more pronounced at T3 than at T2 (Table S3). Aeromonas (T-Rf 113) and Anabaenopsis (T-Rf 257) abundance was greater in faba bean than in wheat soil at T3, and a reverse trend was noted for Klebsiella, Serratia, and Halobacillus. Fungi Scopulariopsis, Yamadazyma, and Arthrinium (T-Rf 180) were specific to faba bean (T2), and Nodulisporium, Mollisia, Sarocladium, and Gaeumannomyces, which correspond to T-RF 200, were only detected under wheat (at T3). Taking into consideration the whole sampling period, Erysiphe, Sarcodon, Trochila, Yurkova, Boletus, Pannaria, Klyveromyces, Venturia (T-Rf 113), Aspergillus, and Kodamae (T-Rf 439) were more abundant in faba bean, while Corticium (T-Rf 174), Leptosphaeria, Cyberlindnera, Lobulomyces, Ceratobasidium, Aleria, Saccharomyces (T-Rf 170), and Conocybe (T-Rf 300) dominated in wheat.

3.2. Forecrops AFFECTED Fungal and Bacterial Community Composition and Abundance

3.2.1. Faba Bean as Forecrop Promotes Higher Abundance of Potentially Beneficial Fungi than Wheat

The fungal community was composed of five dominant phyla with a relative abundance >1%. The fungi belonging to Ascomycota prevailed for all treatments, irrespective of the sampling time (Figure S3). Interestingly, in soil where faba bean was cultivated as a forecrop, Ascomycota showed a greater number of OTUs than for wheat (in the range of 28–47%) at T1 and T3. Basidiomycota and Mortierellomycota were less abundant in the soil (11–23%). Other phyla (Mucoromycota and Rozellomycota) were observed in low abundance for all treatments (<3%). Large differences were noted between wheat and faba bean in the abundance of fungi. Fungi belonging to nearly all of the phyla identified decreased in population for wheat as compared with faba bean at T1 and T3 (22–54%) and increased at T2 in the range of 20–77%. The reference soil generally revealed the lowest fungal abundance at all sampling periods. Relatively higher differences between plants were observed at T2 and T3 than at T1. The effect of the plant species increased with time.

The composition of fungi in the soil at the genus level was substantially changed by the forecrop used (Figure 3). Irrespective of the sampling time, the most abundant fungi in all analyzed soils belonged to Mortierella (from 2382 to 8180 OTUs, depending on the treatment and time). At T1 and T3, these fungi decreased in the following sequence: faba bean, wheat, and R soil. Additionally, Trichoderma, Acremonium, and Solisiccocuszyma were present in the greatest abundance among the dominant fungi. At T1 and T3, a greater number of these fungi was under faba bean soil than wheat, and the opposite situation was noted at T2. Taking into consideration the whole analysis period, a greater abundance of Minimedusa, Eucaphaeria, Pseudogynnoascus, Clonostachys, and Mortierella was observed under faba bean than under wheat, while, under wheat, the more dominant phyla were Penicillium and Exophiala. As for Fusarium, it was at a similar level at T1 and T2 for both plant treatments, and, at T3, it was 25-fold...
more abundant in faba bean than in wheat. Among the Mortierella genus, the most abundant was M. alpine, and, among Penicillium P. subrubescens, P. atrovenetum dominated. The Acremonium genus was mainly represented by A. furcatum and Solicoccozyma by S. terricola, while Trichoderma was represented by T. stellatum and T. martial (Table S4).

Figure 3. Number of operational taxonomic units (OTUs) of the dominant fungal genera, as affected by forecrop. OTUs less than 2% were not analyzed. T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively.

The biodiversity indices (Shannon, Simpson, and Chao1) were higher in faba bean than wheat at T1 and T3 and lower at T2 (Table S5). Generally, the Shannon and Simpson indices under R were the lowest, and Chao1 was the highest among the treatments. Regarding beta-diversity, on average, 72% of species were common to all treatments, irrespective of time. A similar fungal species richness was noted for all sampling periods, from 396 to 467 (Table 4). In general, the number of species increased in the order of faba bean, wheat, and R at all sampling periods. With regard to the sampling periods, these parameters decreased over time. The number of specific species at T1 and T3 was the greatest for the faba bean treatment.

Table 4. Beta-diversity of fungal and bacterial OTUs as influenced by forecrop.

| Term | The Total Richness for All Treatments | The Number of Species in Group | The Number of Species Common for All Treatments | % of Species Common for Both Treatments | The Number of Species Specific for Treatment |
|------|--------------------------------------|-------------------------------|-----------------------------------------------|----------------------------------------|--------------------------------------------|
|      |                                      | F    | W    | R    | F    | W    | R    |                                  | F    | W    | R    |                                  |
| Fungi|                                      |      |      |      |      |      |      |                                  |      |      |      |                                  |
| T1   | 467                                  | 257  | 261  | 283  | 178  | 69   | 73   | 59                          | 33   |      |      |                                  |
| T2   | 431                                  | 226  | 249  | 276  | 170  | 75   | 43   | 65                          | 45   |      |      |                                  |
| T3   | 396                                  | 219  | 213  | 242  | 158  | 72   | 62   | 51                          | 29   |      |      |                                  |
| Bacteria|                                   |      |      |      |      |      |      |                                  |      |      |      |                                  |
| T1   | 44                                   | 39   | 42   | 34   | 28   | 72   | 0    | 1                          | 0    |      |      |                                  |
| T2   | 44                                   | 42   | 39   | 33   | 30   | 71   | 2    | 1                          | 1    |      |      |                                  |
| T3   | 44                                   | 37   | 42   | 29   | 23   | 62   | 0    | 2                          | 1    |      |      |                                  |

T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of subsequent the wheat crop, respectively. F, W, and R denote faba bean, wheat, and reference soil, respectively.
3.2.2. Changes in Fungal Trophic Mode and Guilds as Affected by Forecrops

The share of fungal OTUs for the study treatments grouped with respect to trophic modes are presented in Figure S4. It was found that, in all soils, saprotrophs dominated (33–42%, depending on treatments), and, at T1 and T2, they were the most abundant under faba bean. Saprotroph-symbiotroph (15–21%, depending on treatment), pathotroph-saprotroph-symbiotroph (12–18%), and pathotrophs (11–12%) were also abundant.

Fungi were also analyzed with respect to the predicted functions in soil (guilds). Assigned OTUs with an abundance of less than 1% share between 33–42% of the fungi, depending on the treatment (Figure 4). It was found that undefined saprotrophs dominated (24–43%, depending on treatment). The most numerous of the undefined saprotrophs were taxa Hypocreales, which was noted in much larger numbers in faba bean than in wheat in all sampling periods, and taxa Eurotiales, which was, in turn, more numerous in W. Endophyte-litter saprotroph-soil saprotroph-undefined saprotroph was the second group among the dominant fungi, and Mortierellaceae was the representative taxon of this functional group. The average values from all sampling periods were similar for faba bean and wheat and much lower in R for Mortierellaceae. Fungi belonging to animal pathogen-undefined saprotroph, plant pathogen, animal pathogen and endophyte groups were also abundant. Endophytes were represented by taxa Lecythophora and Trichoderma martiale and were present in lower numbers in R. Plant pathogens were represented by Mycosphaerella, Plectosphaerella, and Clonostachys, which were present in decreasing numbers as follows: faba bean, wheat, and R.

3.2.3. Forecrops Shift Bacterial Community in Soil

The bacterial community was composed of 10 dominant phyla. In all sampling periods, Actinobacteria and Proteobacteria were the most abundant in the soil (Figure S5). Actinobacteria decreased in the order of faba bean, wheat, and R (except T1). Another phylum that dominated in the soil was Acidobacteria, and it was in most cases the lowest in faba bean and the greatest in R. Other phyla were less abundant, and they occurred in an abundance lower than 8%. It should be noted that the use of wheat as a forecrop lead to a greater number of OTU for most phyla than under faba bean at T1 and T3, and the opposite situation was noted at T2. The differences between the plant species were especially evident at T1 and T2, in particular for Nitrospirae and Bacteroidetes. It is noteworthy that, for each treatment, with the reduction of bacterial abundance, an increase in fungal abundance was observed (Figures S3 and S5).

Irrespective of the treatments, the soil was dominated by the bacteria genera Rhodoplanes, DA101, Mycobacterium, Kaistobacter, and Candidatus Solibacter (Figure 5). The differences between faba bean and wheat at the genus level were most visible at T1, when faba bean revealed a lower abundance of Candidatus Koribacter, Actinoplanes, and Candidatus Solibacter (by 191, 162, and 97%, respectively) than wheat. At T3, Actinoplanes and Candidatus Koribacter were more numerous in wheat than in faba bean (by 257 and 88%, respectively).

The Shannon biodiversity index for bacteria increased as follows: F, W, and R. Furthermore Simpson’s index did not show any variability between treatments during the whole sampling period. The effect of the treatments on Chao1 changed due to the sampling period (Table S5).

When it comes to beta-diversity, most of the identified species were common for all treatments (62–72%) (Table 4). The total richness, as well as the number of species, including specific species, showed a low variability between treatments. Taking into account all of the sampling periods, the most abundant species in the soils were Paenibacillus chondroitinus, Bacillus flexus, Sphingomonas wittichii, Actinoallomururus triomotensis, and Edaphobacter modestum (Table S6).
Figure 4. Fungal OTUs grouped in terms of guilds as affected by forecrop. OTUs with relative abundance greater than 1% were analyzed. T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively.
The preceding crop significantly influenced the enzymatic activities in the soil (Table 5). Dehydrogenase, which represents the intracellular activity of microorganisms and β-glucosidase, both connected with C cycling and showed a similar response to study treatments. At T1, it was more active ($p > 0.05$) in wheat than for other treatments, and, later, at T2 in faba bean than in wheat and R. During the whole period, cellulase showed a higher activity in plots where wheat was cultivated as the previous crop compared with faba bean. Similarly, acid phosphomonoesterase functioned more intensively in wheat than in faba bean. Soil urease increased in faba bean in relation to wheat at the time of maturity of the subsequent wheat. Generally, the reference soil showed the lowest enzyme activity at all sampling periods. However, acid phosphomonoesterase and cellulase showed relatively high degrees of activity at T1 and T2. All enzymes showed significant variability with time; cellulase and β-glucosidase showed similar changes with time for each treatment.

### Table 5. Changes in soil activity during vegetative period as influenced by forecrop.

| Enzyme (mg PNP kg$^{-1}$) | Term | Faba Bean | Wheat | Reference | $p$ Values | $T \times F$ |
|--------------------------|------|-----------|-------|-----------|------------|-------------|
| Dehydrogenase (µg TPF g$^{-1}$ d$^{-1}$) | T1   | 26.5 aB  | 31.5 aA | 18.3 cA    | 0.000      | 0.000 0.000 |
|                           | T2   | 35.4 aA  | 28.3 bA | 21.1 cA    |            |               |
|                           | T3   | 21.0 aC  | 18.4 aB | 8.2 bB     |            |               |
| β-Glucosidase (mg PNP kg$^{-1}$ h$^{-1}$) | T1   | 206 bB   | 231 aA | 174 cA     | 0.000      | 0.000 0.000 |
|                           | T2   | 228 aB   | 195 bB | 158 cA     |            |               |
|                           | T3   | 232 aA   | 233 aA | 170 bA     |            |               |
| Urease (mg N-NH$_4$ kg$^{-1}$ h$^{-1}$) | T1   | 2.6 aB   | 2.6 aB | 1.9 bA     | 0.000      | 0.000 0.004 |
|                           | T2   | 3.0 aA   | 3.3 aA | 2.2 aA     |            |               |
|                           | T3   | 2.5 aB   | 2.1 bC | 1.9 bA     |            |               |
| Cellulase (µg glucose g$^{-1}$ d$^{-1}$) | T1   | 41.1 cB  | 123.0 eA | 71.4 bA    | 0.000      | 0.000 0.000 |
|                           | T2   | 54.8 bB  | 77.5 bB | 55.2 bB    |            |               |
|                           | T3   | 68.2 bA  | 88.2 bA | 31.8 cC    |            |               |
| Acid phosphomonoesterase (mg PNP kg$^{-1}$ h$^{-1}$) | T1   | 27.1 bB  | 34.0 aB | 34.3 aB    | 0.000      | 0.000 0.000 |
|                           | T2   | 33.8 bA  | 38.2 aA | 39.7 aA    |            |               |
|                           | T3   | 29.0 abB | 31.3 aC | 28.4 bC    |            |               |

T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively. TPF: triphenyl formazan; PNP: p-nitrophenyl phosphate. Different small letters for forecrop treatments within terms and big letters for sampling terms within forecrop treatments indicate significant differences ($p < 0.05$).
4. Discussion

In this study, the dynamics of the soil microbiome and activity in relation to different crop residue (faba bean and wheat) incorporation during the subsequent wheat-growing period were characterized. It was found that genetic structure of ammonia-oxidizing archaea, the abundance of fungi and bacteria taxa, and soil enzymes were influenced both by the forecrops used and by the sampling time.

Soil microorganisms affect the functioning of soil ecosystems. They influence nutrient cycling and regulate soil and plant health and, consequently, the size and quality of the crop. It is widely known that the introduction of organic material (e.g., plant residues) leads to a higher number of microorganisms and activity by supplying C for energy and cell proliferation [36]. The results presented in this paper indicate that the changes in the communities from different microbe groups are related to the use of various forecrops. However, these communities share common bacterial members. Moreover, we observed that fungi were much more influenced by the choice of forecrop than were bacteria, which shows that fungi are more sensitive to the type of plant residues applied.

In this study, we noted the large difference between the treatments in relation to the abundance of some dominant fungi. Through an analysis of the whole experimental period, after the use of faba bean as a forecrop, the soil showed about twice the amount of *Clonostachys* as compared with wheat as a forecrop (Figure 3). Although *Clonostachys* has been classified as a plant pathogen by FUNGuild, many reports have indicated its beneficial role for plants. Certain species of mycoparasitic fungi from this genus (e.g., *Clonostachys rosea*) are used to control diseases caused by a wide range of plant pathogenic fungi, including *Fusarium culmorum*, *Fusarium graminearum*, and *Alternaria* spp. [37,38]. Some species of *Fusarium* are well-known wheat pathogens [39]. However, some *Fusarium* representatives may promote plant growth or may be used in the role of biocontrol as beneficial endophytic fungi [40,41]. In our study, *Fusarium* was at a similar level under both forecrop treatments at T1 and T2 and was more prevalent in faba bean at the senescence stage (Figure 3).

Among dominant soil fungi, we noted other fungi with antagonistic properties against plant pathogens and fungi that can beneficially affect plant growth. *Penicillium*, *Trichoderma*, *Mortierella*, *Minimedusa*, and *Mucor* were observed under all treatments, and they were present in the lowest number in the reference soil (Figure 3). The greatest differences between plant treatments (faba bean and wheat) were shown for *Mortierella*, *Penicillium*, and *Minimedusa*. It was stated previously that inoculation with *Mortierella* sp. increased urease activity in the bulk soil and in rhizosphere soil in relation to an uninoculated control and showed P-solubilizing activity in bulk and rhizosphere soils in combined inoculation with AMF [42]. In our study, the greatest difference between faba bean and wheat in terms of *Mortierella* abundance was at T3, when it was 54% more abundant under faba bean than wheat, and this finding was connected with significant changes in urease activity. This may be connected with higher N content in faba bean than in wheat treatment. The contribution of *Mortierella* to the N and P cycles should have a favorable effect on plant productivity. Moreover, the *Minimedusa polyspora* species that possesses antifungal properties against *F. oxysporum* [43] and *Acremonium furcatum*, which produces antimicrobial metabolites [44], was also more frequently observed in faba bean than wheat.

However, *Penicillium* and *Mucor*, which are fungi known for phosphate solubilization, as well as plant growth regulation and siderophore production [45,46], occurred more frequently in wheat than in faba bean soil (on average 63% and 32%, respectively). However, fungi that were potentially beneficial to plant growth activities (*Penicillium*, *Trichoderma*, *Mortierella*, and *Minimedusa*) were more abundant in faba bean than in wheat soil. AMF, which showed beneficial effects for soil status and plant yield, were not detected in our soil samples. They belong to the phylum Glomeromycota, which was identified in the soils but in low abundance (<1%). This low abundance may be connected with the fact that analyses were performed in the bulk soil. AMF were detected in higher abundance in the rhizosphere or roots than in the bulk soil [47–49].

In this study, we also detected differences between treatments with regard to the abundance of bacteria with antagonistic or plant growth promoting capacities. Among the dominant bacteria, *Arthrobacter* was identified, which is a mycoantagonistic fungi against *P. debaryanum* and
F. oxysporum [50], as well as Streptomyces, which is widely used due to its nitrogen fixing ability, phosphate and potassium solubilization, phytohormone production, and antifungal activity [51]. These fungi were more abundant in soil after faba bean than after wheat cultivation.

In treatments with faba bean as a forecrop, an increased abundance of the phylum Nitrospirae and its genus Nitrospira was identified in comparison with wheat during all analysis period (Figure S5 and 5). Members of this group play pivotal roles in nitrification as aerobic nitrite-oxidizing microorganisms [52]. By increasing the ratio of nitrate ions to ammonium in the soil, these bacteria have a positive effect on plant growth, since nitrate ions are more easily absorbed by plants. Its higher abundance in faba bean soil may be connected with a greater N amount with faba bean than with wheat residues. In study soil, R. leguminosarum (symbiotic bacteria for faba bean) were identified, however, they were detected at comparable levels in faba bean and wheat treatments and two times less in reference soil (Table S6). This could indicate that the experimental soil is rather rich in this indigenous rhizobial strains.

A higher number of fungi and bacteria with activities beneficial to plant growth in faba bean soil than in wheat soil could indicate better growth conditions for plants after faba bean and, consequently, better yield quality and quantity. This may also indicate some protective role for the soil after faba bean against subsequent wheat pathogens, which may be connected with lower fungicide requirements. This beneficial influence of faba bean residues on soil conditions with a resulting improvement in plant yield was proved in this study since the biomass of the subsequent aboveground wheat parts (grains with straw) was statistically higher for faba bean than for wheat (945 and 838 g m$^{-2}$, respectively, unpublished data).

In this study, we showed that, generally, the Actinobacteria, Bacteroidetes, and Firmicutes were present in lower abundance in the reference soil during the whole analysis period in comparison with soils enriched with plant residues (Figure S5). Many other studies have revealed that these copiotrophic bacteria are abundant in soil that is rich in easily decomposable organic matter [53,54]. The opposite situation was noted for phyla Acidobacteria, Gemmatimonadetes, Planctomycetes, and Verrucomicrobia, which are oligotrophic (nutrient-poor) and were abundant especially in R soil. These results are consistent with other observations [55,56]. A negative correlation between the C level and the Acidobacteria community was observed by Fierer et al. [53].

The plant residues used in our experiment differ greatly with respect to C and N content and biomass. Cellulose and hemicelluloses are the main compounds of plant straw. Although these compounds were not determined in our study, a study by Petersson et al. [57] showed high differences in the concentration of these compounds between cereal (winter rye) and legume straw. The study proved that faba bean showed a lower amount of both compounds than rye. These differences in residue composition were clearly reflected in our study in soil cellulase activity, which was greater in wheat than in faba bean (Table 5). The dynamics in soil cellulase activity depend on many factors, such as the availability of the substrate, the amount of enzyme, and the environmental conditions (e.g., pH and temperature). The release of soluble sugars from incorporated substrates is rate-limiting for the whole hydrolysis process [58], causing a decrease in cellulase activity. Acid phosphomonoesterase was generally more active after wheat than faba bean cultivation, which may be connected with the lower available P concentration in the soil after wheat than faba bean plant cultivation. This could be partially explained by the difference between treatments in Penicillium and Mucor abundance (Figure 3), which showed phosphate solubilization activity. It was also shown that legume contained more P than wheat. Nuruzzaman et al. [59] observed that faba bean took up 78% more P from the soil when compared with wheat.

The differences in soil activity and microbiome between faba bean and wheat could be further enhanced by biologically active substances in the plant residues. Pods of faba bean are rich in phenolic compounds [60], which are important chemicals engaged in diverse processes between plants and microbes: legume-rhizobia and arbuscular mycorrhizal symbioses and plant defense against pathogens [61]. As shown in an earlier study, flavonoids positively affect soil rhizosphere activity [62].
In this experiment, the forecrop influence on properties of bulk soil was studied. However, the response of plant rhizosphere should be also taken into account in understanding forecrop effects on the succeeding crop. Differences in microbial diversity in the bulk soil between treatments with legume and wheat (as preceding crops) were noted earlier [14]. The study by Chang et al. [63] proved that differences in crop productivity within an agricultural field in the same season were associated with bulk soil microbiome composition. Based on bulk soil (collected at pre-planting and 60 days after soybean planting), Yang et al. [64] observed that wheat straw return led to changes in the taxonomic and functional traits of the soil bacterial and fungal communities. Legume–barley intercropping significantly affected the microbial activity of the soil (bulk soil and soil particles loosely adhering to the root systems) during the following cropping season with durum wheat [11]. Further research is needed to assess the effects of different doses of plant residues of different plant species, microbiome responses in different soil layers, and the response of subsequent plant yields and health, including infection by soil-borne pathogens, under different weather conditions.

In conclusion, this study was helpful in developing a deeper understanding of the response of the soil microbial factors to the application of residues from different plant species (faba bean and wheat). The results showed that the type of plant residue applied shapes the diversity and abundance of the fungal community to a greater extent than the bacterial community in bulk soil, as well as shaping enzyme activity during subsequent wheat cropping. It was found that the use of faba bean as a forecrop creates somewhat better soil conditions for the growth and health of a succeeding crop than wheat, due to the more abundant fungi and bacteria with potentially beneficial activities.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/12/1971/s1, Figure S1: Dendrogram obtained by unweighted pair group mean average (UPGMA; Dice coefficient of similarity) for soil under subsequent crop (wheat) depending on forecrop type (faba bean and wheat) and referenced soil. T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively; Figure S2: Abundance of t-RFLP fragments of total archaea (a), bacteria (b), and fungi (c) in soil. T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively; Figure S3: Distribution of the dominant fungal phyla detected in soils after faba bean and wheat cultivation and in reference soil. OTUs less than 1% were grouped in other. T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively; Figure S4: Fungal OTUs of trophic modes as affected by forecrop. T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively; Figure S5: Number of OTUs of the dominant bacterial phyla as affected by forecrop, OTUs less than 1% were grouped in other. T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively; Table S1: Summary of primers and PCR conditions for DNA amplification; Table S2: Shannon–Weaver diversity index and the number of fragments estimated by a t-RFLP peaks profile for total archaea, bacteria, and fungi. T2 and T3 denote: stem elongation and the senescence stage of the subsequent wheat crop, respectively. Different small letters for forecrop treatments within terms and big letters for sampling terms within forecrop treatments indicate significant differences (p < 0.05); Table S3: Archaeal, bacterial, and fungal genus in soil. Prediction was performed as in silico analysis using the TRiFLe package; Table S4: Number of OTUs of identified fungi species in study treatments; Table S5: Biodiversity indices for bacterial (16S rDNA) and fungal (ITS1) populations depending on forecrop. T1, T2, and T3 denote: two months after residue incorporation into the soil, stem elongation, and the senescence stage of the subsequent wheat crop, respectively; Table S6: Number of OTUs of identified bacteria species in study treatments.

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