A Below Ground Chemical Fight for Phosphate and Habitat - Interactions of Camelina sativa (L.) Crantz with Microorganisms

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Abstract

BACKGROUND and AIMS Glucosinolates and isothiocyanates of young Camelina sativa (L.) Crantz can eliminate microbial species in the soil. It was aimed to demonstrate that only isothiocyanate insensitive microorganisms with phosphate solubilizing activity can be successfully used to improve the plant´s phosphate supply. METHODS We performed rhizotron growth studies and 33P–Imaging to study the uptake of phosphate solubilized from 33P-apatite by inoculated plants and determined the phosphate solubilization capacities of microorganisms by inductively coupled plasma mass spectrometry. Secondary metabolites of Camelina sativa and of the soil fungus Penicillium aurantiogriseum were analyzed by HPLC-MS/MS. Microorganisms and plant seedlings were cultured for growth inhibition studies. Phospholipid fatty acids in soil samples were investigated by gas chromatography and the data analyzed by clustering and principal component analysis. RESULTS 1. The inoculants Trichoderma viride and Pseudomonas laurentiana did not improve the phosphate uptake of Camelina sativa and Abutilon theophrasti. 2. The intrinsic soil microorganisms, including Penicillium aurantiogriseum, the inoculants and the assemblies of root colonizing microorganisms are able to solubilize phosphate from apatite and compete for phosphate. 3. Camelina´s glucosinolates and derived isothiocyanates and, as well secondary metabolites released by P. aurantiogriseum, particularly cyclo-(L-Leu-L-Pro), have the capacity to suppress plant and fungal growth and to destroy a phosphate solubilizing bacterial consortium that colonizes Camelina roots. 4. Loss of young Camelina´s key secondary metabolites, the glucosinolates, is of advantage for fungi, as verified by a green manure experiment. CONCLUSION The results underline functions of secondary metabolites when organisms are under competive conditions for phosphate uptake.

Introduction

Brassica mulches are used for biofumigation to reduce weed growth and crop diseases (Gimsing and Kirkegaard 2009). The method is highlighted as practicable in sustainable agriculture and organic farming, therefore recommended as an alternative practice for controlling primarily pathogenic fungi (Tierens et al. 2001). Species commonly used for biofumigation are cabbage, Indian mustard, rape seed, white mustard and oil radish. The biocidal, in particular fungicidal activity of *Brassica* plants is generated by the hydrolysis of their characteristic secondary metabolites with high allelopathic potential, the glucosinolates, resulting in several breakdown products such as toxic isothiocyanates (ITCs). Another Brassicacea, *Camelina sativa* (L.) Crantz, was recently recognized as a plant eventually suitable for biofumigation (Hu et al. 2019). This species contains three main aliphatic glucosinolates: glucoarabin (9-(methylsulfinyl) nonyl- glucosinolate, GS9), glucocamelinin (10-(methylsulfinyl) decyl-glucosinolate, GS10), and 11-(methylsulfinyl) undecyl-glucosinolate (GS11), (Fahey et al. 2001). Mature *Camelina* plants exhibit an organ-specific accumulation of the glucosinolates, with high contents in the roots and the siliques, while the leaves are almost free of glucosinolates (Czerniawski et al. 2021). The glucosinolate content in *Camelina* shoots decreases with maturation. While 3-day old seedlings contain about 60% of the major glucosinolates originally present in the seeds, only up to 25% are left in 7-day-old

ones (Berhow et al. 2014). In *Camelina* seed meals, the glucosinolate contents varied from 19.6 to 40.3 mmol kg^{-1} dry weight, depending on the accession (Russo and Reggiani, 2017). Thus, the glucosinolate-triggered allelopathic potential is due to glucosinolate/ITC release from seeds and youngest seedlings while that of mature *Camelina* plants may be caused by root exudation and root decay.

The seed meal from *C. sativa*,which contains not only glucosinolates and also their break down products (Czerniawski et al., 2021), impacts soil microbial community structure but inhibits fungal proliferation only temporarily and specifically, while the abundance of *Fusarium* species increased (Hu et al. 2019). Since *Camelina* seed meal did not suppress *Fusarium* wilt (Ren et al. 2018), pathogenic *Fusarium* species might be promoted by biofumigation with *C. sativa*. According to Hu et al. (2019) C. *sativa* seed meal may be therefore suitable only for a specific fungal pathogen control.

Soil amendment with homogenized *Brassica* plant material leads to a high nutritional input. Wang et al. (2014) found an increased soil content of total nitrogen (N), especially as NO_3^-, available phosphorus (P) and potassium (K) after biofumigation with *Brassica napus*. A higher abundance of ammonia-oxidizing bacteria and *Streptomyces spp* after *Brassica napus* seed meal application was detected by Cohen et al. (2005). Thus, the increased availability of nutrients may contribute to shifts in the species composition of the original soil microbial communities, favoring the proliferation of isothiocyanate-insensitive microorganisms at least until these compounds are converted into inactive compounds by detoxification-competent fungi, such as *Sclerotinia sclerotiorum* (Chen et al., 2020) or by likewise competent bacteria, for instance *Pseudomonas syringae* strains (Fan et al. 2011).

Profuse studies evidenced that glucosinolate-containing rotational cover crops and continued monoculturing of *Brassica* crops have a negative impact on maize and soybean, even autotoxicity was observed. Hansen et al. (2019) reported reduced fungal and mycorrhizal abundance and lower grain yield with spring wheat when *Brassica napus* was used as a rotational crop. In another cropping systems experiment with winter wheat and *C. sativa*, Hansen et al. (2020) demonstrated a decline in the total microbial biomass encompassing fungi, mycorrhizae, Gram-positive and negative bacteria. Although triggered by glucosinolate break down products, fungistasis may be more complex (Wang et al. 2014). Presumably, changes in microbial community structure are due to the release not only of plant-derived but also of microbial fungicides that may act in microbe-microbe and plant-microbe interactions. These molecule cocktails could be an important causative principle for fungistasis. The interactions may also influence the availability of macro- and micronutrients for plants, which consequently modulate plant secondary metabolite synthesis. For instance, the biosynthesis of glucosinolates in *Brassica* plants seems to adapt rapidly to changing nutrient conditions. Sulfur (S) and nitrogen deficiency reduce the biosynthesis and phosphate (PO_4^{3-}) limitation affects aliphatic glucosinolate biosynthesis positively or negatively (Hiruma 2019; Kopriva and Gigolashvili, 2016). The glucosinolate content in *C. sativa*, for instance, depends on N and S availability (Jiang et al. 2016). P deficiency increased the accumulation of specific glucosinolates in *Arabidopsis* (Pant et al. 2015), while Frerigmann et al. (2021) reported a decrease of indole glucosinolates. Glucosinolate biosynthesis modulated by phosphate deficiency seems

to strengthen a beneficial root-fungus association with *Arabidopsis* (Trejo-Téllez et al. 2019; Hacquard et al. 2016 a, b). Thus, defined fungicidal isothiocyanates may be differentially abundant under phosphate deprivation, modulating the allelopathic potential, biofumigant properties of *Brassica* species and, perhaps generally, their interactions with fungi.

Microorganisms are essential for P cycling since many of them can solubilize phosphate from inorganic resources (e.g., apatite) which are not bioavailable and, consequently, cannot be used by plants. As shown by Siebers et al. (2018), some of the microorganisms that withstand rapeseed extract treatments, for instance, a *Trichoderma* species, are able to solubilize phosphate from apatite.

In this study, it was first aimed to demonstrate the beneficial effect of two phosphate solubilizing organisms, *Trichoderma viride* combined with *Pseudomonas laurentiana*, on the growth of *C. sativa* (further named also *Camelina*) when cultivated under phosphate deficiency conditions, despite of *Camelina´*s negative effects on some fungi. This study was done in comparison to glucosinolate-free *Abutilon theophrasti* Medik. However, phosphate solubilization was also performed by microorganisms already associated with the roots and by a fungus and co-existing bacteria in the P-deficient arable soil to study phosphate uptake after apatite solubilization.

Guided by these results, we identified the fungus, and some of the accompanying bacteria in the arable soil, and investigated aspects of the entangled allelochemical- and phosphate-driven interferences between *Camelina*, the fungus, and associated and added microorganisms. In the last part we show, that glucosinolate-free shoot material from mature *Camelina* plants has no obvious fungicidal properties, but nevertheless modulates the soil microbial diversity in a soil rich in organic matter.

Materials And Methods

Soils for Rhizotron, Pot and Green Manure Experiments

Rhizotrons were filled with Dikopshof soil. This soil was previously collected from P-depleted plots at the former experimental research station of the University of Bonn at Dikopshof (50° 48" N, 6° 57" E). The plots (pH 6.48) had not received any P-fertilization since 1942 (Kumar et al. 2019; Bauke et al. 2017; Mertens et al. 2008). For other pot experiments, a nutrient-deficient artificially mixed substrate (0-Erde), a standardized substrate type 0 obtained from the Werkverband e.V., Germany was used (pH (CaCl$_2$) 6.1; water holding capacity 83,4%; N < 2 mg·L^{-1} with < 2 mg·L^{-1} NH$_4$-N and < 1 mg·L^{-1} NO$_3$-N; P$_2$O$_5$ 5 mg·L^{-1}; K$_2$O 13 mg·L^{-1}, 19% TOC). Fresh samples of an organic farming species-rich loamy sand (Wiesengut, Siegaue, Hennef, Germany; geographical position 65 m above NN; 7Ê 17' East; 50Ê 48' North) were used. The soil contained 110 mg P/kg, further soil data are given in Siebers et al. (2018).

Plant Material

Abutilon theophrasti Med. seeds were purchased from Herbiseed (Twyford, UK). Seeds of *Camelina sativa* (L.) Crantz were harvested from plants grown as described (Hölzl and Dörmann 2021).

Camelina plants used for soil incorporation were grown in a phytotron (160 µmol m^{-2} s^{-1} light, 25°C and 65% humidity). Seeds were placed in pots filled with the soil used by Hölzl and Dörmann (2021), watered 3 times a week and fertilized once a week. Shoots were harvested when plants reached BBCH scale 64–73.

For extracts, *Camelina* seedlings were grown hydroponically from seeds on cheesecloth under natural conditions. Three and four day-old *Camelina* seedling were harvested, dried between filterpaper and weighed. The seedlings were mortared with quartz sand and water, the homogenate centrifuged at 20000g for 10 min and the supernatant directly added to fungal cultures (extract 1). Other extracts were prepared by homogenization with methanol (1:2,w:v), the slurries filtered and centrifuged at 20.000g for 10 min. The supernatant was removed, aliquoted and the aliquots evaporated to dryness at 60°C for 48h. The dry residue was dissolved either in ddH$_2$O (extract 2), or in 50% methanol for compound identifications. The extracts were checked for the presence of intact glucosinolates using to the UHPLC/MS-MS method described below. All aliquots were stored at -20°C until use. The aqueous extracts were sterilized by filtration (syringe filter Excalibur, pore size 0.22µm, Labomedic, Germany) before adding to culture media.

Microorganisms Used for Rhizotron and Pot Experiments

The *Trichoderma viride* F-00612 consortium (collection of D.K. Zabolotny, Institute of Microbiology and Virology, National Academy of Sciences, Ukraine) was grown in liquid YEP medium for 48h and the diluted suspension (OD$_{600}$ = 0.1) used for inoculations in the rhizotron and pot experiments with Dikopshof soil. In contrast to culturing on agar, the *Trichoderma viride* F-00612 consortium loses associated microorganisms, such as *Enterobacter ludwigii* (accession No MH915584), *E. cloacae* (accession No MH915583), *Acinetobacter calcoaceticus* (accession No MH915582), *Bacillus pumilus* (accession No MH915587), *B. subtilis* (accession No MH915585), and *B. safensis* (accession No MH915586), when grown in liquid medium (Voloshchuk et al. 2020). When used as a consortium, culturing was performed on Czapek agar.

Actinomucor elegans (accession KM404167, registered at the DMSZ as strain AbRoF1 used for morphological characterization) established stable associations with *Abutilon* root colonizing microorganisms after re-inoculation (Schulz et al. 2017; Haghi Kia et al. 2014). The resulting consortium contains among others *Pantoea ananatis* (DSM ID 14-714C) and the yeast *Papilliotrema baii* (DSM 100638) as two of the most viable microorganisms on culture plates. These microorganisms are not lost when *Actinomucor elegans* is grown in liquid Czapek medium.

The *Pseudomonas* species used in this study was formerly isolated from *Salvia officinalis* and identified as a strain belonging to the *Pseudomonas putida* complex (E-value: 2e-83, max. identity: 99%, accession No. HM488364). The identity was examined again in 2020 by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig). According to the new identification, the strain (PpSalb ID 20–163 (= HM488364) matches to *Pseudomonas laurentiana* GSL-010 (MG719526.1), 100%

identity), DSMZ ID20-163. For the rhizotron experiments, the bacterium was cultured in LB medium. A suspension of OD_{600} = 0.1 was used for inoculation.

Synthesis of ^{33}P labeled Apatite

Radioactive labelling of apatite was the only method to demonstrate unequivocally the uptake of phosphate, solubilized from labelled apatite, by the seedlings. A three-step apatite synthesis was performed according to Wolff et al. (2018). For the preparation of ^{33}P-$(NH_4)_2HPO_4$, 50 ml of 1M radioactively labeled 1M H_3PO_4 (18.5 MBq ^{33}P) were slowly added into 50 ml 2M NH_3 in H_2O and subsequently stirred for 1h. Ethanolamine (3% w/v) was added as dispersant to prevent aggregation. A freshly prepared 1M $Ca(NO_3)_2$ solution was placed at room temperature in a beaker equipped with a pH meter. The pH was first adjusted to 10 during the following reaction process, a pH of $\geq$ 9 was maintained with aqueous ammonia. Parallel to the radioactive labelling synthesis, a non-radioactive version was synthesized for the pot experiments and analytic product characterization by Raman spectroscopy, using a Bruker RFS 100/S in comparison to formerly unlabeled synthesized apatite, which was characterized by Raman, X-ray diffraction & scanning electron microscopy (Wolff et al. 2018).

Rhizotron Growth Studies and ^{33}P−Imaging

For rhizotron experiments square (120 mm side length), top opened and three-sidewise sealed plastic petri dishes were used (Greiner Bio-One International, Kremsmünster, Österreich). The experiments followed roughly the method described by Bauke et al (2017). The apatite was first ground to a fine powder and then mixed with Dikopshof soil (8:100) in a drum-hop-mixer for several hours. 1g of the homogenized apatite-soil mixture was centrally placed at the bottom of each rhizotron, containing 170 g dry Dikopshof soil.

Roots of 7-day-old seedlings were inoculated with 20 µl *Pseudomonas laurentiana* and *Trichoderma viride* suspensions (see Material and Methods), then placed into the 12 rhizotrons. Non-inoculated plants were used as controls. Rhizotrons were then placed at an angle of 45° in a climate chamber with a day/night-length of 12 h under slowly transition and a light intensity of 320 µmol m^{-2} s^{-1} PAR. Temperatures were set to 22°C and 18°C, respectively, at a relative air humidity of 50%.

Periodic imaging of rhizotrons with suitable imaging plates (200 x 400; DÜRR NDT GmbH & Co. KG, Bietigheim-Bissingen, Germany) using the Bioimager CR35 Bio (Raytest, Straubenhardt, Germany) started one week later. Every time, first a two-step erasing process for resetting the sensitivity to their maximal storage capacity takes place − starting with 30 min under a high energy white light eraser (BAS 100, Fujifilm, Tokyo, Japan), followed by erasing with the Bioimager CR35 Bio immediately before using the plates. The closed, complete rhizotrons were wrapped into protection foil and placed horizontally on a scintillation plate for exposition. Rhizotrons were covered by a foam to prevent irreversible plant damage and weighted with a plastic plate to reach optimal contact between rhizotron/ plant and scintillation foil. The 5h-exposition and subsequent scanning took place in the dark. The sensitive mode with a resolution of 100 µm was chosen as parameter for readout immediately after exposition. In the scanning process,

the photo-stimulated luminescence intensities were measured, receiving a digital autoradiographic image processed by the standard imager software AIDA Image Analyzer 2D (ELYSIARaytest, Straubenhardt, Germany).

The plants were poured after each imaging to prevent scintillation plate damage by moisture. Additionally, the plants were sprayed with water to faster recover the 3-dimensional plant shape after the flat pressure exposition. For parallel monitoring of plant development, simultaneously photos weret taken.

After 3 weeks the experiment was finished by harvesting the plants. Plants were separated from soil, dried and digested (6h, 180°C) with 4 ml 65% HNO_3 in a Loftfield apparatus (Loftfields Analytische Lösungen, Neu Eichenberg, Germany). After dilution and filtration aliquots were mixed with 10 mL scintillation cocktail (ULTIMA Gold XR, PerkinElmer, Solingen, Germany) and the incorporated radioactivity subsequently measured by a Tri-Carb® 3110TR Liquid scintillation counter (PerkinElmer, Solingen, Germany).

Rhizotron Experiment - Re-isolation of Trichoderma viride from Roots

Camelina and Abutilon roots from suspension-inoculated plants were removed from the rhizotron soil after decay of radioactivity and soil particles were carefully picked off prior to placing the roots on Czapek or Sabouraud agar plates. After culturing for 14 days at 25°C in the dark, the plates were checked for Trichoderma viride colonies.

Identification of the Dominant Fungus and Accompanying Bacteria Present in the Dikopshof Soil

Microorganisms present in the Dikopshof soil were isolated by serial dilution as described in Siebers et al. (2018) and cultivated on different media (Sabouraud agar, Pikovskaya solid medium, Czapek agar). Only few microorganisms were detected after 2 weeks. Discrete colonies of a dominant fungus were further cultured on Sabouraud agar. A representative plate was used for identification of an assumed Penicillium species by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig). For identification, the ITS rDNA sequences and morphological markers were used. The species was identified as Penicillium aurantiogriseum Dierckx (isolate 20–165) with a sequence identity of 100% to the reference (GeneBank accession No AF033476).

Single bacterial colonies were picked as template for touchdown-PCR with the bacterial 16S primers (27f AGAGTTTGATCMTGGCTCAG, 1492r(s) CGGTTGTTACGACTT). The PCR products were purified with the NucleoSpin Gel and PCR Clean-up Kit (Macherey& Nagel, Düren, Germany) and sequenced.The bacterial sequences were searched against the NCBI Nucleotide database using BLASTN, leading to the identification of species belonging to the genera Paenibacillus, Rhodococcus, Arthobacter, and

Pseudomonas, (accession numbers: BS1_*Paenibacillus*_sp ON620168; BS2_*Rhodococcus*_sp ON620169; BS4_*Pseudomonas*_sp ON620170; BS5_*Arthrobacter*_sp ON620171).

Setups of Pot Experiments

For the first pot experiment non-sterilized and sterilized Dikopshof soil was used. 50g of the soil was thoroughly mixed with 2g apatite (AppliChem), filled in adequate pots and watered until the soil well moistened. Three *Camelina* or *Abutilon* seedlings (3-days-old) were planted in each pot. Root tips of the seedlings were inoculated with 20 µl *Pseudomonas laurentiana* and *Trichoderma viride* suspensions. The plants were cultured in a phytotron (160 µmol m^{-2} s^{-1} light, 25°C and 65% humidity) until three leaves were developed and cotyledons became yellowish (14–18 days). The plants were watered every second day and fertilized with P- fertilizer at day 8–10 when the first two leaves were unfolded. Shoot length was monitored during the entire culture at days shown in Fig. 3. Each experiment was performed with 15 pots per plant species and was repeated three times.

The second, 21days-pot experiment was performed with 25g 0-soil mixed with 1g apatite, filled in pots and watered as described above. Three *Camelina* or *Abutilon* seeds were placed into each pot. After seven days, 0.5 cm^2 agar plugs covered with mycelia either of the *Penicillium aurantiogriseum*, the *Trichoderma viride* - or the *Actinomucor elegans* consortium were harvested and incorporated to the soil in the following manner: 5 pots – control (no fungus), 5 pots *P. aurantiogriseum*, 5 pots *P. aurantiogriseum* + *Trichoderma viride* consortium, 5 pots- *Trichoderma viride* consortium, 5 pots *Actinomucor elegans*, (all n = 3). The plants were watered every second day and fertilized with −P fertilizer after germination and further every week. The second set up was repeated with P containing fertilizer.

Co-Culture Experiments on Agar Plates and in Liquid Medium

For visualization of phosphate solubilization from apatite, Pikovskaya (PVK) plates were used. The development of transparent halo zones surrounding the microorganism placed on the agar either solely or in combination with other organisms indicate solubilization activity. The tests were performed with cultivable Dikopshof soil microorganisms, collections of *Camelina* root colonizing microorganisms, *P. aurantiogriseum*, *P. olsonii*, *Paenibacillus spec.* from the *Camelina* seed coat, *Pseudomonas spec.* from the *Camelina* seed coat. Co-cultures on PVK agar were performed with nonsterile germinating *Camelina* / *P. aurantiogriseum* for seven days. Cultivable microorganisms from Dikopshof-soil were cultivated on Sabouraud agar. Seedlings from sterilized *Camelina* seeds were co-cultured on MS Phytoagar with *P. aurantiogriseum* until seedlings became decolored.

Cultures in Liquid Pikovskaya and Czapek-Yeast Medium

The pre-cultures of fungi were done on Sabouraud Agar. 100 mg agar plugs covered with mycelium were placed in flasks containing 250 ml medium under sterile conditions. After placing, the media were supplemented with either 2x10mg sinigrin within 6 days, sterilized aqueous *Camelina* extracts (extract 1)

3 x 2ml within 6 days. The cultures were terminated after 14 days, the mycelium harvested by filtration and placed on filter paper to remove liquid prior to photographic documentation .

Determination of Released Phosphate by inductively coupled plasma mass spectrometry (ICP-MS)

P. aurantiogriseum, Trichderma viride, the *Trichoderma viride* consortium, the *Actinomucor elegans* consortium and the fungus from the *Camelina* seed coat were pre-cultivated on Sabouraud Agar. The collected microorganisms from the root surfaces and all other bacteria were precultured in LB liquid medium. Agar plugs (100 mg)of the fungi and 500µL of the microorganisms grown in LB medium (OD_{600nm} 0.1) were transferred to flasks containing 15 mL PVK (Pikovskaya)medium and cultured for 6 days at 21°C in the dark. All incubations were done in triplicates using material from different cultures of the given species. Subsequently, the cultures were filtered and the filtrates centrifuged at 20.000 rpm for 15 min at 4°C to pellet microbial material and apatite particles. The supernatants were transferred to new tubes and again centrifuged at 20,000 rpm for 10 min. The supernatants of the triplicates were combined, aliquoted (2 mL) and stored at -20°C until analysis. Aliquots of the PVK medium was treated in the same way as the control. Sample preparation for ICP-MS measurements was performed as follows. After centrifugation, the supernatant was transferred into a round-bottomed 15 ml PFA vial (Savillex, Eden Prairie, USA) and placed on a heating plate at 80ºC to be completely dried down in customer-designed laminar flow box in a cleanroom. The dried material was re-dissolved in a mixture of 1 ml 68% ultrapure HNO_3 and 0.5 ml 30% H_2O_2. The closed vial was heated up at 120°C on a heating plate for 1 h to dissolve any organic matter. After digestion, the solution was dried again and then re-dissolved in 1 ml 0.3 M HNO_3 for further dilution before the determination of P concentration started.

The P concentrations were analyzed by quadrupole inductively coupled plasma mass spectrometry (ICP-QMS, Agilent 7900, Agilent, Bremen, Germany). The measurements were performed after two hours of warm-up time to reduce the P background towards 10 ppb (10 µg/L).

Identification of Glucosinolates and Derived Compounds by UHPLC-MS/MS

For the identification, supernatants of the centrifuged culture media and *Camelina* seedling extracts were used. Screening and quantification were carried out using an ultra-high performance liquid chromatography (UHPLC)-electrospray-mass spectrometry (MS) instrument consisting of an ACQUITY UPLC system equipped with a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Eschborn, Germany). The UHPLC was equipped with a cooled autosampler (6°C), binary pump and a Nucleodur C18 Gravity-SB column (150 x 3 mm, 3 µm; Macherey-Nagel, Düren, Germany) thermostated to 25°C. The gradient elution was performed at a flow rate of 1 mL/min with Millipore water (Millipore GmbH, Schwalbach, Germany) with 0.1% formic acid (pH 3.0) as solvent A and acetonitril with 0.1% formic acid as solvent B (VWR International GmbH, Darmstadt, Germany, LC-MS grade) as follow: start with 1% B,

held for one minute, then raising to 100% B in 59 minutes, back to start conditions in further 1 min and held for 4 minutes. The injection volume was 10 µl for each sample.

The mass spectra were recorded with an ESCi source in positive and negative full scan mode. The nebulizer gas was set to 7 bar. The capillary voltage was set to 2.5 kV, the cone voltage to 20 V. Desolvation temperature and source temperature were 600°C and 150°C, respectively. Nitrogen was used as desolvation and cone gas at a flow rate of 1000 and 150 L h^{-1}, respectively. Argon was used as collision gas at a flow of 0.14 mL min^{-1} with a collision energy of 30 eV.

Identification of secondary metabolites released from Penicillium aurantiogriseum by UHPLC/MS-MS

P. aurantiogriseum was cultured for four weeks in Czapek yeast medium (Frisvad et al. 2004). The medium was filtrated, the filtrate centrifuged at 10,000 g for 15 min. The supernatant was extracted with ethyl acetate and the organic and aqueous phases evaporated to dryness. The dried ethyl acetate phase was used for compound identification.

The dry residue was reconstituted with CH_3CN/MeOH (3:1) using vortexing/sonication. After centrifugation the supernatant was filtered using a Socorex® borosilicate glass syringe and 0.2 µm MilliQ Millipore® LCR filters. An aliquot of the filtrate was 10x diluted with MeOH prior to injection (1 µL).

Chromatographic separation was performed on an UltiMate 3000 UHPLC system from Thermo Fischer Scientific (Waltham, MA, USA) equipped with an Acquity BEH C18 column (2.1× 100 mm/1.7 µm, Waters) at a flow rate of 450 µL/min at 30°C. The mobile phase consisted of H_2O + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B). A gradient elution was performed from 5 to 30% B in 8 min, increase to 100% B in 1 min and flushing at 100% B during 2 min. The UHPLC system was connected to a high-resolution QExactive orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with a heated electrospray ion source. Masses were calibrated below < 2 ppm accuracy using the Thermo Fischer Pierce calibration solution. Data were acquired in (+)- and (−)-ESI mode, with a mass range from *m/z* 100 to 800 and 35,000 resolution. MS/MS experiments were performed with a normalized collision energy of 30 eV.

To study fractions with phytotoxic compounds, additional ethyl acetate extracts were fractionated via HPLC using the method described in Schütz et al. (2019). Three fractions were collected: fraction I contained compounds eluting with 15−35% methanol, fraction II those eluting between 36−60% and fraction III compounds eluting between 60−100% methanol. The most hydrophilic fraction was dried and the residue dissolved in 1 mL water. *Camelina* seedlings were placed in cuvettes with 800 µL tap water and 200 µL of the dissolved, aqueous fraction was added, 200 µL tap water was added to the controls. Harmful effects became visible within three days at room temperature and day light conditions.

Cyclo(L-Leu-L-Pro)-Tolerant and Intolerant Microorganisms from Camalia sativa Seed Coats

The fungus associated with *C. sativa* seeds was isolated and cultured on TSB medium.

Colonies of the most abundant bacteria growing on Pikovskaya plates untreated/treated with 200 µl 1 mM cyclo(L-Leu-L-Pro) were picked and used as templates for identification as decribed above. The cyclo(L-Leu-L-Pro) tolerant species was identified as *Paenibacillus polymyxa* (accession No. *Paenibacillus*_sp ON620175). The intolerant yellow colonies are composed of different species, thus presenting a bacterial consortium. One of the species which was most abundant in the young colonies matches several *Pseudomonas* species with identical sequence similarity (accession number: *Pseudomonas*_sp ON620172), whereby *Pseudomonas aeruginosa* could be excluded by PCR analysis using specific primers. Another species that proliferated from aging yellow colonies was identified by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig). A partial 16SrRNA sequence indicates the strain as most likely belonging to *Cytobacillus firmus* (syn. *Bacillus firmus*, ID 22–142; identity 99.9%). Due to the impoverichment of species and their change in abundance during culturing it was not yet possible to comprehensively characterize this complex consortium.

The identification of the fungus associated with *Camelina* seed coats was performed by the DSMZ (isolate ID: 22–51). For identification, the ITS rDNA fragment and the partial β-tubulin gene were used as barcodes for sequencing. The species was identified as *Penicillium olsonii* Bainier & Sartory (strain CBS 232.60 Ex-Typus). The sequences were compared with reference sequences (Genbank, MycoID und INDOOR; *Penicillium olsonii* btub EF652020, KAS6229).

Growth Behavior of the Bacterial Consortium in the Presence of cyclo(L-Leu-L-Pro) and Camelina Glucosinolate Containing Extract

Bacterial consortium cultures were inoculated in LB medium to an OD_{600nm} 0.1, grown at 37° C for 150 min, followed by the addition of a) *cyclo(L-Leu-L-Pro)*, 1.33 mM in methanol; b) *Camelina* extract, 0.1% (v/v) in methanol; c) *cyclo(L-Leu-L-Pro)*, 1.33 mM in methanol and *Camelina* extract, 0.1% (v/v) in methanol. Growth was normalized to OD_{600nm} at which compounds were added to the culture.

Microbial Degradation Capacity of Camelina Glucosinolates

P. aurantiogriseum was cultured on Sabouraud agar until colonies were 5 cm in diameter. 2–3 mg mycelium were removed from the agar and placed into flasks containing 15 mL PVK medium with 300 µL *Camelina* extract 2. The addition of *Camelina* extract 2 was repeated after 3 days of culturing for 6 days at 21°C in the dark. Cultures were grown in triplicates. After 6 days, the cultures were filtrated, the filtrates centrifuged at 20000 g for 10 min and the supernatants combined, resulting in one sample. Further cultures using the same designs were supplemented with 3 units myrosinase (from *Sinapis alba*, Merck, Germany) every two days or with 500 µL of *Camelina* root colonizing microorganisms (RCM), which were prepared as mentioned above. *Camelina* root colonizing microorganisms without the fungus were tested by inoculation of 15 mL PKV medium with 500 µL of the LB precultured assembly for 6 days under the same conditions as described. All samples were aliquoted and stored at -20°C until analysis which were performed by UHPLC-MS/MS as described above.

Green Manure Experiment with Camelina Shoot Material

180 g Wiesengut soil was filled in a 200 mL glass beaker. *Camelina* above-ground plant material (phenological growth stage: anthesis, BBCH scale: 64–73) was crushed with a homogenizer (Philips HR 2870/50 Minimixer) after adding water in a ratio 1:1 (plant/water (w/v)). 50 g of homogenized plant material was added to the soil resulting in a ratio of 0.28 g plant material/g soil) and thoroughly mixed. Beakers were closed with a glass lid and Parafilm and stored in the dark at 21°C. Samples were drawn directly after application (t0), then after 1, 7, 14, 21, 28, and 63 days. Treatments were carried out in three biological replicates.

PLFA Analysis

The microbial community structure was described by analysis of the phospholipid fatty acid composition (PLFA) in the soil. Lipid were extracted according to Bligh and Dyer (1959), and analyzed as described in Kruse et al. (2015). Lipids were fractionated according to their polarity by solid phase extraction (Gasulla et al. 2013). Soil samples were dried for 24 h at 105°C in the oven to determine the soil moisture content and dry weight. For PLFA analysis,he acyl groups of the separated lipids were cleaved and converted into their methyl esters by methanolysis (FAMEs), (Browse et al. 1986). For quantification, an internal standard (100 μL of tridecanoic acid, 50 μg/mL in methanol) was used. All chemicals were of analytical grade. In total 28 PLFAs were identified in the soil samples. Specific PLFAs (or combinations thereof) were assigned to certain groups of organisms as described in Siebers et al. (2017). For plant material and other eukaryotes such as algae, PLFA 18:3 was used as marker. FAMEs were analyzed using an Agilent 7890 gas chromatograph with Supelco SP-2380 capillary column and a flame ionization detector (Siebers et al. 2017).

Statistics

Statistical analysis of growth and phosphate data was performed with PRISM 9.0. Significant differences were calculated by use of the Student's t-test. Variables were subjected to one-way analysis of variance (ANOVA). Normality was tested according to Anderson-Darling, DÁgostino & Pearson, Shapiro-Wilk and Kolmogorow Smirnov. Student's t-tests were performed in addition and P-values provided in results. Results are presented in the figures as means ± standard deviation .

To evaluate the effects of green manure application on microorganisms we used clustering and a clustered heatmap to reveal hierarchical clusters in data matrices (Engle et al., 2017). To this end, we used heatmap and factoextra packages in R. In addition to the clustering and clustered heatmap, we also performed principal component analysis (PCA) and then both the observations and the original variables were illustrated in the principal component space (Gabriel, 1971). In a biplot, closely aligned variables are positively correlated with each other where the stronger the correlation is when the larger the arrows are. Negatively correlated variables are aligned in opposite directions and the strength of the correlation is

again measured by the magnitude of the arrows. Non-correlated variables are typically shown by arrows that are aligned in 90 degrees to each other.

Results

Growth Studies

Rhizotron Experiments - Uptake of Phosphate Solubilized from ^{33}P-Apatite

Since a *Trichoderma* species was identified as a P solubilizing fungus insensitive to rapeseed glucosinolate break down products (Siebers et al. 2018), the uptake of solubilized ^{33}P-labeled phosphate by *C. sativa* and *A. theophrasti* in the presence and absence of *Trichoderma viride* and *Pseudomonas laurentiana* was studied. *P. laurentiana* was included, because the bacterium is known to possess plant-growth-promoting and phosphate solubilizing properties (Rafikova et al. 2020; Huang et al. 2010). Both species were applied as suspensions to the root tips. ^{33}P-apatite was mixed with P-depleted Dikopshof-soil, placed into rhizotrons and one 4-day-old seedling per rhizotron was planted (Fig. 1a).

^{33}P−imaging revealed first radioactivity in leaves of *Abutilon* and a lower amount in *Camelina* seedlings at 8 to 9 days after planting. Radioactive ^{33}P incorporation increased continuously until the shoots were harvested after 27 days, indicating microbial solubilization of ^{33}P from the applied apatite (Fig. 1a). The development of many *Camelina* seedlings was retarded in the presence of *T. viride* and *P. laurentiana* during the early growth stage, others died few days after planting. However, most of the surviving *Camelina* plants caught up with the early growth retardation until harvest. Since any attempt to re-isolate *T. viride* from suspension-inoculated *C. sativa* and *A. theophrasti* roots after the rhizotron experiments with Dikopshof soil failed, it is likely that the fungus was not able to establish an association with the plants. Quantification of absorbed ^{33}P however indicated that applications of *T.viride* and *P. laurentiana* did not result in a significant increase of uptake compared with equally developed control plants. *Abutilon* seedlings exhibited, in tendency, an even lower uptake in the presence of the two microorganisms (Fig. 1b).

Pot Experiment - Growth of Camelina and Abutilon seedlings on Dikopshof -soil

These results pointed to active phosphate-solubilizing microorganisms in the Dikopshof soil that render inoculations of the root tips with *T. viride* and *P. laurentiana* suspensions ineffective. As an alternative explanation, phosphate-solubilizing microorganisms others than *T. viride* and *P. laurentiana* might already have colonized the roots or additional unknown, interfering factors might exist. Therefore, pot experiments with the two plant species using non-sterilized and sterilized Dikopshof-soil supplemented

with unlabeled apatite were performed. The experiment was started with 3-day-old seedlings of *Abutilon* and *Camelina*.

Growth monitoring disclosed the existence of interfering organisms, which were eliminated by sterilization.

With *C. sativa* in non-sterilized soil, the development of the seedlings was first significantly stimulated in the presence of *T. viride* and *P. laurentiana* in contrast to the rhizotron experiment, but later, the difference between inoculated and non-inoculated seedlings was abolished (Fig. 2a). With sterilized soil, we found again a stimulatory but not significant effect by inoculation between day 3 and 7 after planting. The stimulation turned significant during day 10 and 15. As also found with *Abutilon* to some degree, shoot growth of the non-inoculated *Camelina* plants was retarded in the sterilized soil (Fig. 2b). The results, shown in Fig. 3b and c, provide clues that soil microorganisms may contribute to phosphate solubilization and their elimination by sterilization reduces the amount of solubilized phosphate. The *A. elegans* consortium stimulated the growth of *C. sativa* significantly after day 13 (Fig. 2c) in sterilized soil, but it is questionable whether the *A. elegans* consortium contributes considerably to the supply of the plants with phosphate (see below). Since the fungus releases high amounts of the indole-3-acetic acid (IAA) precursor tryptophan (Haghi Kia et al. 2014), growth stimulation could be due to microbial auxin production and perhaps less to phosphate solubilization, but this was not further addressed.

With the non-sterilized soil, *Abutilon* showed no difference in the growth during the first week but further development tended to be suppressed when the seedlings were inoculated with *T. viride* and *P. laurentiana* suspensions (Fig. 2d). In contrast, with the sterilized soil, a significant growth stimulation was found in presence of the two microorganisms from day 10 to day 15 after planting (Fig. 2e). In the sterilized soil without the inoculants, shoot growth was reduced during this period, also in comparison to the non-sterile sets. In addition, we tested a suspension prepared from a formerly isolated *Actinomucor elegans* consortium, naturally found on *Abutilon* roots. This suspension stimulated the growth significantly in sterilized soil between day 4 and 11 after planting (Fig. 2f). Thus, the stimulation occurred much earlier than observed with *T. viride* and *P. laurentiana*.

These findings led to the assumption that microorganisms already associated with the roots considerably contribute to phosphate solubilization and interfere with soil microorganisms and the ones used for inoculation.

Penicillium aurantiogriseum and Bacteria from Dikopshof Soil

We started a search for microorganisms existing in the Dikopshof-soil, which influence plant growth under P−/P + conditions and which were eliminated or highly reduced by sterilization. The soil harbored only a few microorganisms cultivable on Sabouraud agar, among them a fungus which overgrew accompanying microorganisms during progressing cultures (Fig. S4). Some of the accompanying

bacteria were identified as belonging to the genera *Paenibacillus*, *Rhodococcus*, *Arthrobacter* and *Pseudomonas*. The fungus was identified as *Penicillium aurantiogriseum.*

Co-culture of Camelina and Abutilon seedlings in 0-Soil Pot Experiments

To gain more insights in the effects of *P. aurantiogriseum*, another series of pot experiments was performed with older seedlings growing in sterilized 0-soil, supplemented with apatite. Seeds were directly germinated in the pots and after 7 days, agar plugs covered with mycelia of *P. aurantiogriseum*, the *T. viride* or *A. elegans* consortia, respectively, were placed into the soil. The consortia were used because they host numerous bacterial species which may support *T. viride* and *A. elegans* consortia in suppressing *P. aurantiogriseum* (compare material and methods). For a simpler design, the inoculation with the *P. laurentiana* was omitted.

In the arrangements with only *P. aurantiogriseum*, 60–70% of *Camelina* plants died (Fig. 3). When plugs of the *T. viride* consortium were added to *P. aurantiogriseum*, the development of *Camelina* was similar to the controls (no fungus) and to plants in pots containing only plugs with the *T. viride* consortium. Addition of the *A. elegans* consortium plugs was stimulatory to the growth of *Camelina* seedlings. Contrarily, *Abutilon* was not affected by the *P. aurantiogriseum*, nor had the combination *P. aurantiogriseum*/ *T. viride* consortium or sole *T. viride* consortium any effect on growth when compared to the control without fungus (Fig. 3).

The growth behavior was compared to arrangements with sterilized 0-soil supplied with phosphate containing fertilizer. Phosphate supply resulted in a different growth behavior of *Camelina* in the presence of *P. aurantiogriseum.* The fungus had now no negative effect on *Camelina* growth. Addition of the *T. viride* consortium or in combination with *P. aurantiogriseum* was without effect and growth was similar to the control (no fungus), whereas addition of the *A. elegans* consortium was stimulatory as observed already in the experiments with the Dikopshof soil. *Abutilon* showed again no differences between control (no fungus) and the combined addition of *P. aurantiogriseum* / *T. viride* consortia, *T. viride* / *A. elegans* consortia, but now few seedlings died when exposed to *P. aurantiogriseum* only. Thus with *P. aurantiogriseum*, sufficient P supply diminished competitive interactions with *Camelina*, but seem to imbalance interactions with individual *Abutilon* plants.

Microorganisms Colonizing the Camelina Root

Another *Penicillium species, Penicillium olsoni*, was found on *Camelina* seed coats and surrounded some of the growing seedlings (Fig. S4). The roots of many *Camelina* seedlings were heavily covered with yellow colonies, representing a bacterial consortium composed of several species (Fig. 6). One of the species that grew from disintegrating colonies on agar plates was identified to be most likely a *Cytobacillus firmus* strain, another species presents a yet unidentified *Pseudomonas* species, while *P. aeruginosa* could be excluded. Because the bacterial consortium was unstable when cultured and lost viability over time, it was not possible to dissect the additional species, much less the entire species

composition, by the used methods. Another root colonizing species was identified as *Paenibacillus polymyxa.*

Evaluation of the Microorganisms Capacity for Phosphate Solubilization

The ability of the microorganisms for phosphate solubilization was evaluated by inductively coupled plasma mass spectrometry. The Dikopshof soil microorganisms *Pseudomonas* spec., *Rhodococcus* spec., *Arthobacter spec.*, *Paenibacillus* spec., the total microbial collection from root surfaces of *Camelina* and *Abutilon* seedlings, single organisms from *Camelina* seed coat/root (*P. olsonii*, *Paenibacillus polymyxa*), *P. aurantiogriseum*, *T. viride* suspension, the *T. viride* consortium, *P. laurentiana* suspension, the *A. elegans* consortium and the consortium member *Pantoea ananatis* were compared for their ability to release phosphate from apatite. ICP-MS analysis confirmed the high ability of *P. aurantiogriseum* to solubilize phosphate (Fig. 4).

However, the microbial assembly collected from *Camelina* root bathing suspensions had a similar capacity, while the assembly from *Abutilon* roots even outperformed the one of *P. aurantiogriseum*. The *T. viride* consortium and *T. viride* from liquid culture reached only 20–25%, *P. laurentiana* about a third of the amount solubilized by *P. aurantiogriseum*, whereas with the *A. elegans* consortium almost no free phosphate could be measured. Since the Zygomycete develops, in contrast to *T. viride*, a cotton-like, fluffy mycelium, it is possible that phosphate is trapped within the nexus of the hyphae and could not be captured for the ICP-MS measurements. This explanation is supported by the relative high phosphate solubilization found with the *P. ananatis* isolate from the *A. elegans* consortium (Fig. 4). On PVK agar, solubilization of apatite bound phosphate by the *A. elegans* consortium could not be unequivocally detected by transparent areas in the agar, due to the nature of the mycelium. *P. olsonii* from *Camelina* seed coats solubilized phosphate in similar quantities as measured for *P. aurantiogriseum*, especially when *Camelina* extract 2 (sterilized aqueous *Camelina* extract prepared from dried methanolic extracts redissolved in water) was added. The solubilization of *P. aurantiogriseum* was dimished in the presence of the *Camelina* extract. *P. polymyxa* solubilized more phosphate than *P laurentiana* and *T. viride*. Phosphate solubilization activity of the root colonizing microbial assemblies, the fungi *P. aurantiogriseum* and *P. olsonii* and further microorganisms could be visualized by culturing on PVK agar plates (Fig. S1). The yellow bacterial consortium, which could not be used for ICP-MS as culturing of the intact consortium was not possible in liquid PVK and other media for three days, exhibited the strongest solubitization on PVK agar plates (Fig. 6).

The evaluation of phosphate solubilization confirmed the presence of phosphate solubilizing microorganisms in the Dikopshof soil, the presence of P solubilizing microorganisms associated with the root surface of both plant species and with the seeds of *Camelina*.

Hints For Below Ground Chemical Fights

Camelina´s Antifungal Weapon: Glucosinolate Breakdown by Soil Bacteria

Since *Abutilon* was not inhibited by *P. aurantiogriseum* under P-deficiency and, in turn, the fungus showed no reduced growth when exposed to *Abutilon* extracts (data not shown), the deadly effect of *P. aurantiogriseum* on *Camelina* in the P deficient 0-soil is thought to be linked with *Camelina* glucosinolates. Therefore, further work focused on *Camelina*.

It is not known how *P. aurantiogriseum* copes with glucosinolates and their break down products. Therefore, differently prepared glucosinolate containing extracts from 3-day-old *Camelina* seedlings were tested for their effects on the growth of *P. aurantiogriseum.* As determined by UHPLC-MS/MS analysis, the sterilized aqueous *Camelina* extract prepared from dried methanolic extracts which were redissolved in water (extract 2) contained large amounts of the major glucosinolates glucoarabin, glucocamelinin and 11-(methylsulfinyl)undecyl-glucosinolates (Fig. 5; Fig. S3). When measured immediately after preparation, the freshly prepared aqueous extracts (extract 1) had a similar content of glucosinolates, which dropped during the next hour. The glucosinolates are by far the dominant secondary metabolites in *Camelina* extracts. *Camelina* also contains a number of phenolic acids and flavonoids in lower amounts which are all wide spread in higher plants, thus not specific for *Camelina*. The major phenolic secondary metabolites in *Camelina* extracts are quinic acid, chlorogenic acid, sinapoylglucoside, catechin- and epicatechinglucoside, different quercetinglycosides and quercetin. Quercetinglycosides are characteristic phenolic compounds in *Camelina* (Berhow et al. 2014). They had seemingly no inhibitory effect on *P. aurantiogriseum*. UPLC-MS/MS analyses indicated that the phenolic compounds were not or only in traces detectable in the incubation media with *P. aurantiogriseum.* They could be metabolized or otherwise eliminated, e.g. by polymerization. The identified phenolic compounds are listed in Table S1.

When exposed to extract 2 in PVK medium, *P. aurantiogriseum* did not degrade the glucosinolates and grew almost like the controls. Thus, *P. aurantiogriseum* is either unable or avoid to produce ITCs from *Camelina* glucosinolates by own enzymes, which is an advantage for the fungus since the intact glucosinolates are not toxic. Addition of myrosinase to extract 2 led to a break down of *Camelina* glucosinolates. When *P. aurantiogriseum* mycelium was treated with these myrosinase-supplemented extracts, the fungus did not grow at all (Fig. 5). Also, spores did not germinate. Prolonged culture of the mycelium in PVK medium led to high amounts of gluconic acid, a compound which is not or only found in traces in the *Camelina* extracts (Table S1).

For further evaluation of the glucosinolate degradation capacity, the fungus was treated with several other glucosinolates. Sinigrin was the only tested compound which was degraded by *P. aurantiogriseum* yielding allylisothiocyanate, but growth inhibition by allylisothiocyanate occurred only in PKV and not in Czapek/yeast extract medium. Neither glucotropaeolin, progoitrin and its derivative goitrin were degraded nor affected the growth of the fungus (Fig. S2; Fig. S4).

The finding that *P. aurantiogriseum* does not degrade *Camelina* glucosinolates entailed experiments to examine soil and root colonizing bacteria for their ability to degrade the glucosinolates. The four soil bacteria belonging to the genera *Paenibacillus spec.* (BS1-DS), *Arthrobacter spec.* (BS5-DS), *Rhodococcus spec.* (BS2-DS), *Pseudomonas spec.* (BS4-DS), all efficient in phosphate solubilization (Fig. 5), were exposed to extract 2 for 3 days in PKV medium. LC-MS analyses of the culture media disclosed different capacities of glucosinolate degradation. The *Pseudomonas*, *Arthrobacter* and the *Rhodococcus* species showed highest degradation capacity, while the *Paenibacillus* species degrade less than 60% of the glucosinolates (Fig. 5).

The chromatogram shows the retention times of glucoarabin (1), glucocamelinin (2) and undecyl-glucosinolate (3).

The picture below the chromatogram illustrates the strong inhibited growth of *P. aurantiogriseum* when incubated in liquid PKV medium with myrosinase containing *Camelina* extract 2 (right) in comparison to the control without myrosinase (left).

There was no clear preference for the degradation of a specific *Camelina* glucosinolate. The microorganisms collected from the *Camelina* root surface by bathing were also examined for glucosinolate degradation under phosphate deficiency, but with negative results. The members of the assembly able to grow in PKV medium did not degrade *Camelina* glucosinolates and addition of *P. aurantiogriseum* did not elicit the degradation.

Penicillium aurantiogriseum´s Toxic Secondary Metabolite Cocktail Attacks Camelina and it´s Root Colonizing Bacterial Consortium

The competition of root colonizing bacteria for phosphate and the release of glucosinolates by *Camelina* led us address the question if toxins released by *P. aurantiogriseum* eliminate or suppress the growth of *Camelina* under phosphate deficiency. When 7-day-old *Camelina* seedlings grown from surface sterilized seeds were co-cultured with *P. aurantiogriseum* on MS Phytoagar plates, the seedlings were overgrown, shoots lost chlorophyll and finally died almost discolored (Fig. 6a, b, d).

The fungus produces a variety of bioactive metabolites, which are released to the surrounding environment. Their characterization was achieved by extraction of the *P. aurantiogriseum* culture medium with ethyl acetate and UHPLC-HR-MS (Fig. 7, Table S2). Data processing with Metaboscape® allowed the identification of nine compounds of which five have been described to be produced by *P. aurantiogriseum* (Lund and Frisvad 1994): Patuline and penicillic acid, both known for their phytotoxicity, as well as the mycotoxines verrrucosidin, aurantiamine, and auranthine. Patulin has also been described to exist in soil and mulches (Ismaiel and Papenbrock 2015). In addition, we found the antibiotic diketopiperazines cyclo(Phe-Pro), the dipeptide cyclo(Leu-Pro) which was most abundant and maculosin (cyclo (Pro-Tyr), furthermore the bicyclofarnesol derivative fuegin, a sesquiterpene. The diketopiperzines were not yet recognized as compounds synthesized by *P. aurantiogriseum*).

To study the toxic effects on *Camelina* seedlings, compounds of the EtOAc extract of Czapek yeast medium were fractionated by HPLC, dried and the residues dissolved in H_2O. From the three collected fractions, only fraction I containing hydrophilic compounds was deleterious for 4-d-old *Camelina* seedlings. They died by drying out within 48h although being placed in cuvettes filled with tap water (Fig. 6c), in contrast to applications of fraction II and III. The identification of the responsible compound is still in progress. Phytotoxic penicillic acid is not responsible for the drying out, but it reduced seedlings growth within three days, while cyclo(Leu-Pro) has no visible effect on the growth of *Camelina* (data not shown).

When germinating *Camelina* seeds were placed on PVK agar plates and immediately treated with cyclo(L-Leu-L-Pro), the yellow colored bacterial colonies, which were dominant on control plates without cyclo (L-Leu-L-Pro) were eliminated (Fig. 6), in contrast to *Paenibacillus polymyxa.* Growth experiments in the LB medium revealed that the bacterial consortium was not inhibited by cyclo(L-Leu-L-Pro) and aqueous *Camelina* extract when applied separately, when incubated for 1 day. However, a combination of cyclo(L-Leu-L-Pro) and low concentration (0.1% w/v) of aqueous *Camelina* extract inhibited the growth significantly (p < 0.001) down to about 50% (Fig. 6).

These experiments demonstrate that interactions of *Camelina* together with its associated microorganisms on the one side and *P. aurantiogriseum* on the other side are featured by the release of allelopathic secondary metabolites used to suppress competitors. Suppression of the bacterial consortium should considerably reduce phosphate solubilization within the rhizosphere as the consortium forms biofilms at the surface of *Camelina* roots (Fig. 6h, Fig. S4). The results led to the assumption that *Camelina* glucosinolates, glucosinolate degrading soil bacteria and cyclo(L-Leu-L-Pro) from *P. aurantiogriseum* have the potential to reduce the microbial biodiversity on *Camelina* roots when acting in concert.

Green Manure Experiment - Loss of Camelina Glucosinolates Save Fungi and Dynamics of the Microbial Biodiversity

To support the results presented above, an experiment was performed with homogenized *Camelina* shoots in the anthesis stage to verify the loss of negative impacts on fungi when glucosinolate-free shoot material is incorporated into the soil. The incubation was performed with an organic farming soil rich in organic matter, which had not been used for *Brassica* cultivation for four years (Siebers et al., 2018).

Lipid-fingerprinting was used to estimate the dynamic microbial biodiversity with a focus on fungi. As shown in Fig. 8, the phospholipid-derived fatty acid (PLFA) profiles changed significantly upon application of the plant material. We recorded a dramatic increase in eukaryotic and microbial fatty acid markers in comparison to the untreated soil shortly after adding the shoot material which was further intensified during the next day (t1), indicating 1), that the incorporated shoot material contained active associated fungi and bacteria and 2), that the input of nutrients may have led to activation and proliferation of soil microorganisms which were previously in an inactive state (Blagodatskaya and

Kuzyakov 2013). While 15:0 anteiso, a marker for Gram (+) bacteria, was abundant in the untreated soil. A considerable different profile of PLFAs was established after application of the shoot material at d0 with the highest increase of the branched fatty acid 16:0 14-Me, probably a stress response to of some bacterial groups to phenolics released from the *Camelina* shoot material (De Carvalho and Caramujo 2018). The stress marker 17:0cyclo of Gram (-) bacteria was increased, 18:3 showed a strong increase and the fungal marker 18:2ω6,9c also increased. The 15:0 anteiso marker disappeared and did not recover at any time point of the experiment. During the following weeks, the PLFA profile changed less dramatically, characterized by continuous increases of 16:0 (d0-d63), and a continuous decrease of 18:3 from d1 to d63. An increase of the fungal marker 18:2ω6,9c occurred from d1 to d28, followed by a decrease to d63. Even after 63 days, the original fatty acid composition of the untreated soil, which was characterized by markers of Gram (+) bacteria, was not reestablished. The arbuscular mycorrhizal fungi PLFA marker 16:1ω5c was low (Veum et al. 2019), and was not heavily affected by the shoot material.

The treatment enhanced the abundance of the cyclopropane fatty acid 19:0cyclo presenting a stress marker of Gram (-) bacteria after 63d. Several other fatty acids increased slightly after 63d, namely 16:1ω9, 18:0; 18:1ω7c, 18:1ω7, and 18:1ω9, which may indicate a starting alteration or remodulation in the community structure, perhaps pointing to a completed decay of the shoot material and underpinning that certain groups of bacteria had suffered from the treatment. In summary, the application of *Camelina* shoot material dramatically enriched the soil with fatty acids originated from plant associated bacteria and fungi and from the plant itself, but only for about four weeks. The remaining enrichment decreased during the next weeks, while some long-term alterations were maintained, for instance a higher abundance of the fungal marker.

Pearson correlations among examined variables showed that, except for the t0 and untreated soil PLFA fatty acid profiles, the remaining variables are highly correlated to each other, showing Pearson correlation values higher than 0.67 to 0.99 (Fig. S5). Two main groups of fatty acids could be identified by cluster analysis. Group 1 encompasses prokaryotic fatty acid 15:0 anteiso (Gram (+) bacteria, untreated soil) and 16:0 14Me

(t0) in outer positions - and the mycorrhizal fungal marker. Group 2 is composed of the two eukaryotic fatty acid marker 18:2ω6,9 (fungal marker, plant endophytes) and 18:3 (plants) and the fatty acid 16:0 present in eukaryotic and prokaryotic organisms (compare cluster plots shown in Fig. 9 and cluster dendrogram shown in the heat map (Fig. 8).

In the next step principal component analysis was conducted (Fig. 10, Fig. S5-S8). The eigenvalues depict that PC1 and PC2 explains more than 83% of the variation within the data which is enough to exclude further components from analysis (Fig. S6), square cosine or squared coordinates (cos2) of the fatty acids over PC1 and PC2 are shown in Figure S6. The cos2 values underline the quality of the representation of the fatty acids on the factor map (PC1 and PC2). The cos2 values are higher for cluster 1 than for cluster 2, which indicates that these fatty acids are presented by PC1 and PC2 with enough quality (Fig S6). The contribution (in percent) of the examined variables (untreat, t0, ...t63 = same of the

samples) on PC1 and PC2 are illustrated in Fig. S8. All variables show a contribution of 5 to 15 percent on PC1 and PC2 which seems to be enough to keep them in the analysis.

The biplot (Fig. 10) simplifies the information between examined variables and PC1 and PC2. As seen from the biplot, x1d, x7d, x14d, x21d, x28d, and x63d are positively correlated with PC1 and each other. The right-aligned arrows indicate these positive correlations. Nearly similar lengths of arrows indicate that these variables are correlated with PC1 with nearly similar strength. The angles between arrows and PC1 axis also show that these variables are highly correlated with each other except for x63d which is already proved by previously provided heatmap of Pearson correlations between variables. Contrary to PC1, untreat and t0 variables are correlated with PC2. An upward alignment of the arrow in the case of t0 shows that t0 is positively correlated with PC2 while a downward alignment of arrow in the case of untreat variable shows that untreat variable is negatively correlated with PC2. This is also proved by calculating the correlations between examined variables and PC1 and PC2 (Table S3).

Discussion

Phosphate solubilitation from Apatite - Competition among organisms

Our study provides an impression how root colonizing microorganisms compete with soil inhabiting ones for solubilized phosphate, whereby plant and microbial secondary metabolites have important functions in the affected interactions.

In a recent review, Raymond et al. (2020) discussed the contradictory effects of phosphate solubilizing microorganisms when used as biofertilisers in agriculture. The authors assumed that the microorganisms, which differ in their requirements for P, have not the capacity to solubilize sufficient phosphate to improve plant growth and crop yields under field conditions. However, Amy et al. (2022) highlighted the ability of plants to collect microorganisms with strategies for phosphate solubilization that are complementary to their own ones, which was thought to exacerbate competition with soil microorganisms. Microorganisms have evolved several strategies to solubilize rock phosphate minerals, while the use of organic acids like gluconic acid is wide-spread (Tanuwidjaja et al. 2021). Also *P. aurantiogriseum* seems to use gluconic acid for apatite solubilization, since this compond was heavily produced during culture in PVK medium. The results obtained with *Camelina* and *Abutilon* in the rhizotron experiments may underline the suggestion that plants specially select microorganism for integration in their microbioms, which could be a reason for the failed reisolation of *T. viride* from the root surfaces and the high phosphate solubilization activity of microbial collections from roots of both plant species. These findings are supported by high phosphate solubilization capacities of the isolated and identified microorganisms from roots.

Camelina glucosinolates strongly favor ITC insensitive microorganisms. Under P deficiency, P solubilizing insensitive soil microorganisms have therefore an advantage but their conflict with root colonizing ones

is of disadvantage for the plant. Inoculating young seedlings with *Trichoderma viride* and *Pseudomonas laurentiana* seems to complicate the situation as root microbiomes get imbalanced because they are confronted with two further competive species in addition to the soil microorganisms. Consequently, in the rhizotron experiment with the young seedlings, the added *T. viride* and *P. laurentiana* suspensions could not improve the plants phosphate supply. The positive effects of the two microorganisms became only obvious in the pot experiment with sterilized Dikopshof soil, where soil microorganisms were eliminated or severly reduced.

As stated by Pang et al. (2021), the importance of secondary metabolites in molecular communication between interacting organisms is still underestimated, as well as the functions of changed secondary metabolite accumulations under nutrient deficiency. P-limitation is known to influence the biosynthesis of secondary metabolisms in many species. In *Arabidopsis* and other plants, the levels of many polyphenols are higher under P- limitation (Pant et al. 2015). Low phosphate conditions change the secondary metabolite biosynthesis in bacteria (Romano et al. 2015; Henriksen et al. 2022; Barreiro and Martínez-Castro 2019), and the influence of nutrients on fungal secondary metabolite production is well known (Van der Molen et al. 2013). Chevrette et al. (2022) conclude from their studies with a model microbial community that secondary metabolites are crucial for shaping interaction networks. We hypothesize that altered production of secondary metabolites under P deficiency may completely remodel such networks.

Secondary Metabolite Arsenals for Belowground Chemical Fight

With the older seedlings used in the 0-soil pot set ups, the application of large amounts of the *T. viride* consortium had not only a growth promoting effect, but also abolished the destructive influence of *P. aurantiogriseum*, highly abundant in the soil in these experiments. We assume that, aside from the release of plant growth promoting compounds, the positive effect is less due to root colonization and phosphate solubilization but more the result of suppressing *P. aurantiogriseum* in the soil. *T. viride* is known to produce many fungicidal secondary metabolites (Sood et al. 2020, see also Figure S 3) able to suppress *P. aurantiogriseum*. Also, some of the Dikopshof soil bacteria obviously release myrosinase-like enzymes or arylsulfatases that led to the glucosinolate-break down products which are deleterious for the fungus. Arylsulfatase activity assumed to be originated from microorganisms has been found within the rhizosphere, for instance of *Sinapis album, Lolium perenne, Triticum aestivum* and *Brassica napus* (Knauf et al. 2003). *Aspergillus flavus* converts certain glucosinolates to nitriles by arylsulfatase and a β-thio-glucosidase (Galletti et al. 2008). This pathway could be realized also by other microorganisms, but seemingly not by *P. aurantiogriseum* or the *Camelina* root colonizing microorganisms under the conditions used in this study.

P. aurantiogriseum synthesizes an arsenal of more than thirty antibiotics. Aside from the compounds identified in this study, pseurotin, terrestric acid and citrinin have been found (Frisvad et al., 2004). In addition to patulin and penicillic acid, at least two further compounds, citrinin and terrestric acid, are phytotoxic.

The more than 200 known diketopiperazines, isolated from bacteria, fungi and actinomycetes, differ in their bioactivity. Cyclo(L-Leu-L-Pro) from *P. aurantiogriseum* culture medium is also produced by several bacteria, for instance *Streptomyces fungicidicus* (Li et al. 2006), *Pseudomonas putida* (De Carvalho and Abraham 2012) and by some other fungi. Cyclo(L-Leu-L-Pro) suppresses biofilm formation of some Gram (+) bacteria and has nematocidal activities (Zhai et al. 2019; Gowrishankar et al. 2016). Interestingly, the compound induces disease resistance in *Arabidopsis thaliana* (Noh et al. 2017). However, compound mixtures may damage defined competitors. As shown with the bacterial consortium colonizing *Camelina* roots, glucosinolates and cyclo (L-Leu-L-Pro) act in concert, resulting in deleterious consequences for the consortium. The new effectiveness is helpful for the fungus to reduce or even eliminate the competitive bacterial consortium that improve phosphate availability for the plant, thus finally the plant is also damaged.

Cyclo(L-Phe-L-Pro), found in the *P. aurantiogriseum* medium, is synthesized by many fungi and bacteria such as plant colonizing *Lactiplantibacillus plantarum* or *Bacillus amyloliquefaciens* (Defoirdt, 2019). The compound has also antifungal properties (Mishra et al. 2017; Yu et al. 2021). The third diketopiperazine found in *P. aurantiogriseum* media, cyclo(L-Pro-L-Tyr), was more recently described to possess auxin-like properties able to promote plant growth (Ortiz-Castro et al. 2011). In older studies, cyclo(L-Pro-L-Tyr), also named maculosin, was found to act as a phytotoxin (Stierle et al. 1988). The differences of these findings might be due to dosage dependencies. In addition to the mentioned toxins, *P. aurantiogriseum* might possess additional tools to damage plants. For instance, proteomic analyses revealed that emitted low molecular weight (< 45 Da) volatiles reduce the amount of aquaporin proteins, the iron carrier IRT1 and apoplastic peroxidases in *Arabidopsis thaliana* (García-Gómez et al. 2020).

Presently, it is unclear whether the quality and quantity of the released compounds, and the composition of the entire compound cocktail, is influenced by neighboring plants and/or their associated microorganisms. The compound composition might be even modulated in a way that plant growth promotion is achieved. It is also unknown whether *Camelina* has a higher sensitivity to the toxins when growing under P deficiency.

Our study gives strong hints for crucial roles of secondary metabolites in the fight for phosphate between microorganisms and plants. Consequently, when the glucosinolate contents drops in aging *Camelina*, the effects of the plant on soil microorganisms changes and the fungicidal effects of the fungi are lost, as ascertained by the green manure study.

Green Manure Experiment

The dynamics in the fatty acid profiles argue for an input of plant associated microorganisms rather than for an excessive development of hyphae from fungal spores and proliferation of other microorganisms already existing in the soil. The analyses disclosed the lost potential of mature *Camelina* shoot material to suppress fungi, concluded from the high amount of the fungal marker 18:2ω6,9c after introduction of the plant material into the soil. Based on the conducted lipid-fingerprinting analysis, five different groups of fatty acids could be classified according to their reaction to green manure application: group 1

contains the 15:0 antesio marker which is abundant only in the untreated soils; group 2 contains the 16:0 14-Me (iso) marker which appears in soils at the time of plant material application and diminishes from soils by less than 1 day after application; group 3 contains 18:3 and 18:2ω6,9c markers whose abundance increases after a couple of days, then it decreases after some days. Group 4 contains the 16:0 marker whose abundance constantly increases after the application of the plant material, and finally, group 5 contains the remaining markers that show no clear reaction to green manure applications. Green manure from mature, almost glucosinolate-free *Camelina* shoots incorporated into the soil alters soil microbiomes, presumably due to microorganisms associated with the plant material and possibly in combination with other released secondary metabolites existing in the plant material. Here, in particular the high amounts of quercetin derivatives in *Camelina* could have modulating functions (Bag et al. 2022). The modifying influence of green manures from different plants on soil bacterial communities has been shown in several studies, for instance by Le Blanc (2022), Dong et al. (2021) and Longa et al. (2017).

As a conclusion, the results indicate that microorganisms already associated with the roots considerably contribute to phosphate solubilization, arising conflicts with soil microorganisms and the ones used for inoculation under phosphate deficiency conditions. As shown with *P. aurantiogriseum* and young *C. sativa* seedlings, secondary metabolites have a pivotal role in damaging or even killing of competing organisms and of those which release compounds toxic for the fungus. Such functions of secondary metabolites are presently underestimated in agriculture. The presented study primarily highlights the complicated and multi-faceted interactions between plants and microorganisms involving secondary metabolites, providing an idea of the importance of secondary metabolites in the establishing of alliances and manipulation of accompanied organisms, which is remodeled under phosphate deficiency and when key secondary metabolites are no longer synthesized. Also, this study stimulate the reevaluation of the current opinion that allelopathy and competition for resources have to be separately contemplated. Disentangling of the highly complex interactions, if at all possible, needs further intensive studies.

Declarations

All listed authors have approved the manuscript before submission, including the names and order of authors.

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Author Contributions:

Conception and design: Margot Schulz, Diana Hofmann. Material preparation, data collection and analysis were performed by Diana Hofmann, Margot Schulz, Björn Thiele, Mehdi Rahmati, Vadim Schütz, Seungwoo Jeong, Laurent Bigler, Bei Wu, Frederico Held, Nikolina Babic, Joachim Hamacher, Georg Hölzl. The first draft of the manuscript was written by Margot Schulz and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures
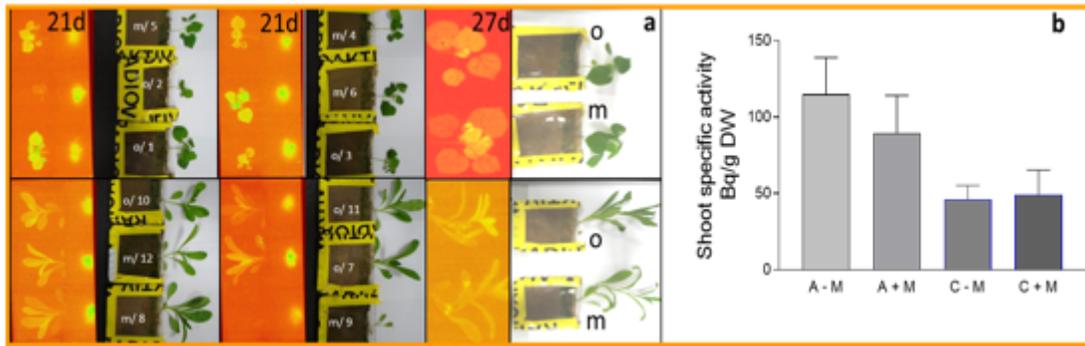
Figure 1

a Representative uptake experiment of radiolabeled P from ^{33}P-apatite by *C. sativa* and *A. theophrasti* seedlings after 21 and 27 days. o: without inoculants, m: inoculated. The inoculations had no clear influence on phosphate uptake (n=6 per plant species). Growth differences were abolished with most of the seedlings after 27 days. **1b** Uptake of [33]P (specific activity Bq/g dry weight,) from solubilized ^{33}P-apatite. A-M, A+M: *Abutilon* seedlings inoculated with and without *T. viride* suspension and *P. laurentiana*; C-M, C+M: *Camelina* seedlings inoculated and without these microorganisms. Differences in ^{33}P uptake were not significant. n=3/species and treatment (t- test).
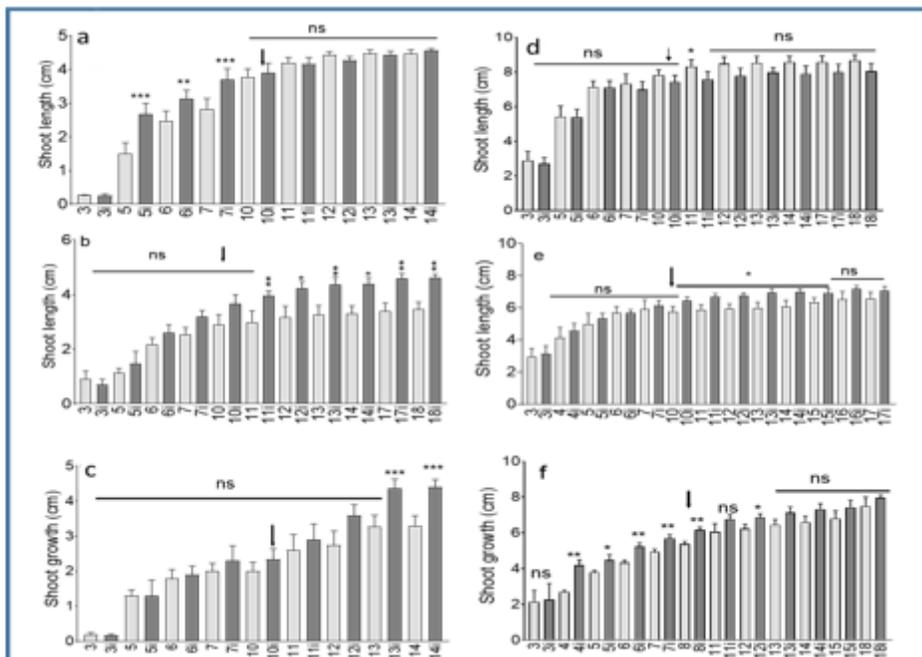
Figure 2

Pot experiment with non-sterile and sterilized apatite containing Dikopshof soil and seedlings of *Camelina* and *Abutilon*. X axis: age (days) of not-inoculated seedlings (gray bars) and inoculated ones (i,

black bars), (inoculi: *T. viride, P. laurentiana* suspensions). The arrows mark the day of P-free fertilizer application. *Camelina*: (**a**) non-sterile soil; (**b**) sterilized soil; (**c**) sterilized soil+ *A. elegans* consortium and without the consortium. *Abutilon*: (**d**) non sterilized soil; (**e**) sterilized soil; (**f**) sterilized soil + *A. elegans*consortium and without the consortium. T-test; *, p<0.05; **, p<0.001; ***, 0.001; ns, non significant.
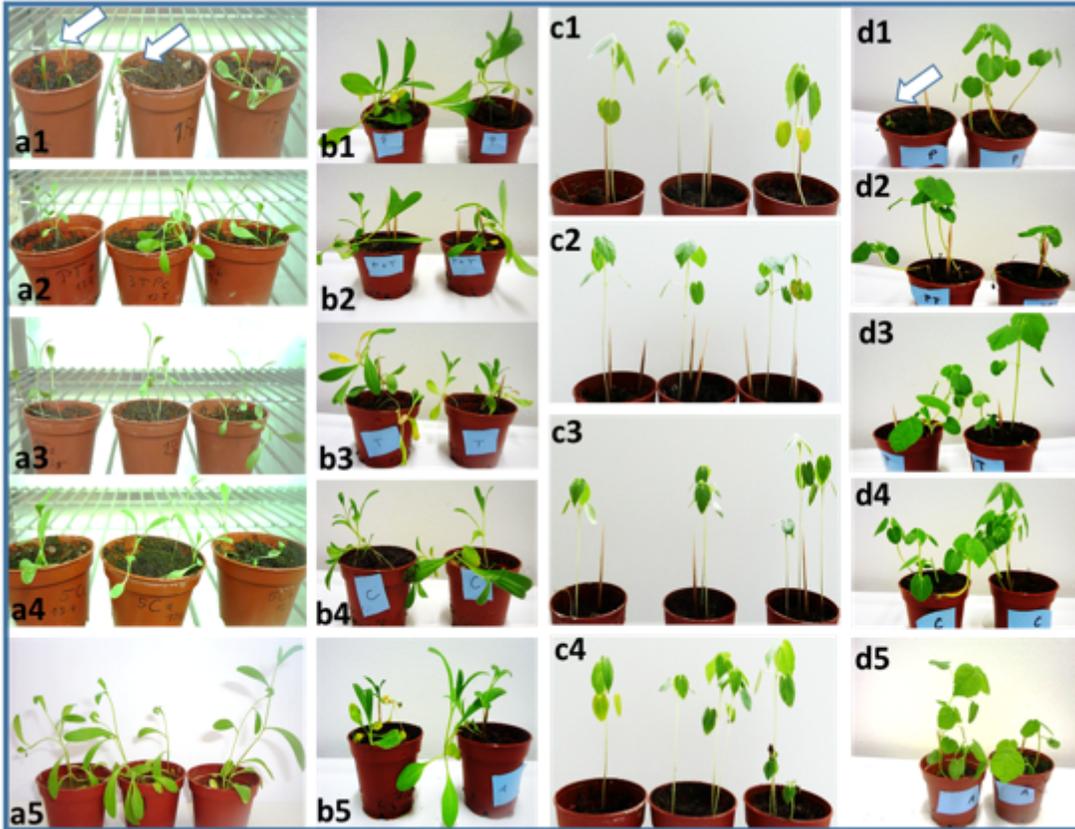
Figure 3

Pot experiments with sterilized 0-soil supplemented with mycelia of *P. aurantiogriseum* (a1, b1, c1, d1), *P. aurantiogriseum* + the *T. viride* consortia (a2, b2, c2, d2), *T. viride*consortium (a3, b3, c3, d3) and *A. elegans* consortia (a5, b5, d5). a: *Camelina* in 0-soil + apatite; b: *Camelina* + phosphate containing fertilizer; c: *Abutilon* in 0-soil+ apatite; d: *Abutilon* +phosphate containing fertilizer. Controls a4, b4, c4, d4 were without addition of fungi to the soil. White arrows point to dying plants.
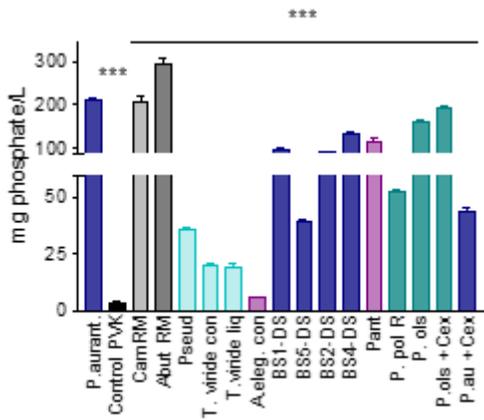
Figure 4

Inductively coupled plasma mass spectrometry analyses of PVK medium and the media after culturing *P. aurantiogriseum*, *Camelina* root surface microorganisms (Cam RM) which included an unstable bacterial consortium (see below), *Abutilon* root surface microorganisms (Abut RM), *P. laurentiana* (Pseud), *T. viride*consortium (T. viride con), *T. viride* grown previously in liquid culture (T. viride liq) and *A. elegans* consortium (A. eleg. con). *Paenibacillus spec.* (BS1-DS), *Arthrobacter spec.* (BS5-DS), *Rhodococcus spec.* (BS2-DS), *Pseudomonas spec.* (BS4-DS), *P. ananatis* isolate from *A. elegans* consortium (Pant), *Paenibacillus polymyxa* from *Camelina* roots (P. pol R), *Penicillium olsonii* from *Camelina* seeds (P. ols), + Cex: *Camelina* extract added. Dark blue: microorganisms from Dikopshof soil, light blue: added microorganisms (rhizotron); mauve: *P. ananatis* isolate, belonging to the *A. elegans* consortium; grey: root associated microorganisms from *Camelina*; dark grey: root associated microorganisms from *Abutilon*; petrol: microorganisms associated with *Camelina*seed coats. All culture mediums contain significantly more free phosphate than the PVK medium. n=3 with 3 technical replicates; significance compared to the control ***: p≤ 0.001 (t-test). For Dikopshof soil microorganisms, see also Fig. S1.

Degradation % left	Glucoarabin (1)		Glucocamelinin (2)		Undecyl-glucosinolate (3)	
Compound Structure						
Sample (3 combined cultures)	Area average	%	Area average	%	Area average	%
RCM+Cam	1132104	93	2140467	84	407898	78
P.au. +RMC+Cam	1023913	84	1954021	76.4	421651	80
P.au. +Cam +M	Traces	>0.001	Traces	>0.001	Traces	>0.001
Pseudomonas	101048	8.3	186799	7.3	29824	5.7
Paenibacillus	796584	65	1737681	68	350476	67
Rhodococcus	171133	14	350916	13.7	6380	12
Arthrobacter	184911	15	356284	14	55519	10

Figure 5

UHPLC-MS analyses of culture media (PKV) supplemented with *Camelina* extract 2 (Cam). P. au.: *P. aurantiogriseum*; RCM: *Camelina* root associated microorganisms; M: myrosinase; *Pseudomonas, Paenibacillus, Rhodococcus, Arthrobacter* species are isolates from Dikopshof soil. Triplicates of the different culture supernatants were combined to one sample, technical replicates: n=3. The amounts of the three glucosinolates left after three days of culturing is given in % of the control (100%, calculated from peak areas).

The chromatogram shows the retention times of glucoarabin (1), glucocamelinin (2) and undecyl-glucosinolate (3).
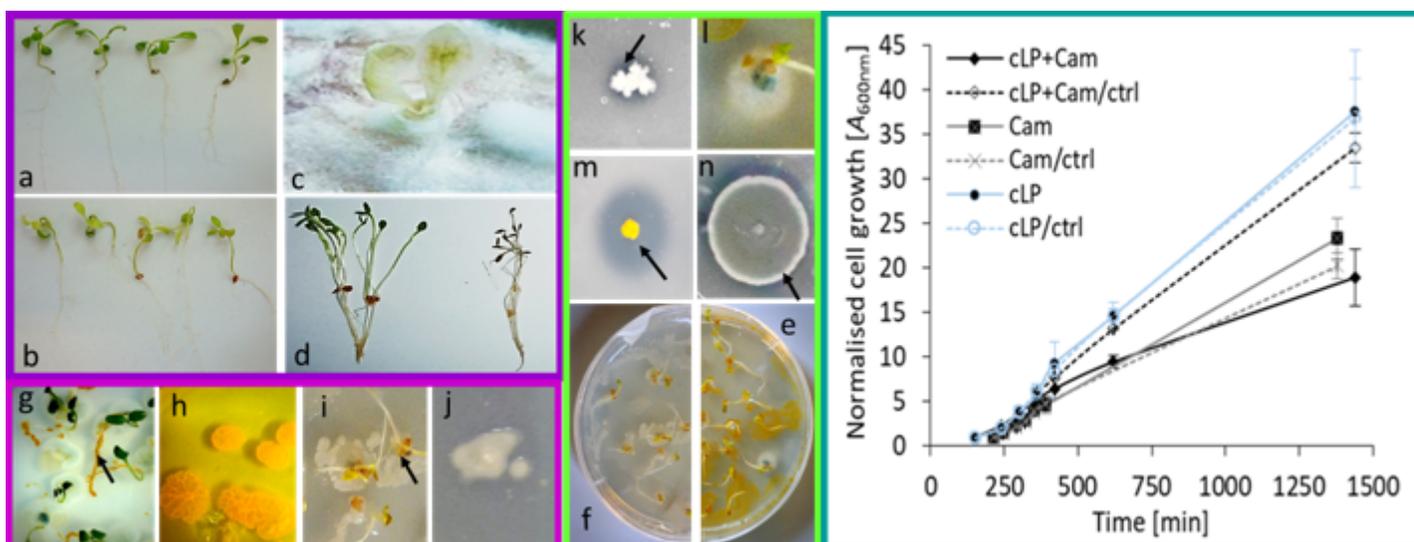
Figure 6

Picture panel left side. (**a**) Control, *Camelina* seedlings grown on MS Phytoagar without *P. aurantiogriseum.* (**b,c,d**)The fungus destroys *Camelina sativa* seedlings by different strategies. (**b**) *Camelina* seedlings grown on MS Phytoagar with the fungus get yellowish. (**c**) Sterilized *Camelina*seedlings co-cultured on MS Phytoagar with *P. aurantiogriseum* became decolored. (**d**) left: *Camelina*seedlings exposed to a hydrophilic fraction containing *P. aurantiogriseum*secondary products dried out, control right.

Phosphate solubilization and effect of cyclo L-Leu-L-Pro. (**e**) Control, germinating *Camelina* seeds on PVK agar with developing colonies of a bacterial consortium (yellow, enlargement of colonies shown in (**h**), a low number of *P. polymyxa* colonies (one colony enlarged in **j**) and seed coat colonizing *Penicillium olsonii,* enlarged in (**l**). (**f**) Germinating *Camelina* seeds on PVK agar treated with 200µl 1mM cyclo(L-Leu-L-Pro): the consortium disappeared, colonies of *P. polymyxa* developed dominance.

The consortium (**m**)*, Paenibacillus polymyxa* (**k**) and *P. olsonii* (**n**) are able to solubilize phosphate from apatite, as indicated by the halo zone (arrows).

Biofilm on roots. (**g**) illustrates the biofilm formation of the consortium on *Camelina* roots (arrow), (**h**) single colonies of the consortium; (**i**) shows that no colonies of the consortium grew in presence of the dipeptide. The arrow points to empty seed coats.

Graph right side: Suppressive effect of cyclo(L-Leu-L-Pro) (cLP) in combination with *Camelina*extract on the growth of the bacterial consortium. Dashed lines represent controls (ctrl) containing the respective amount of methanol. Data are means ± standard deviations of four biological replicates.
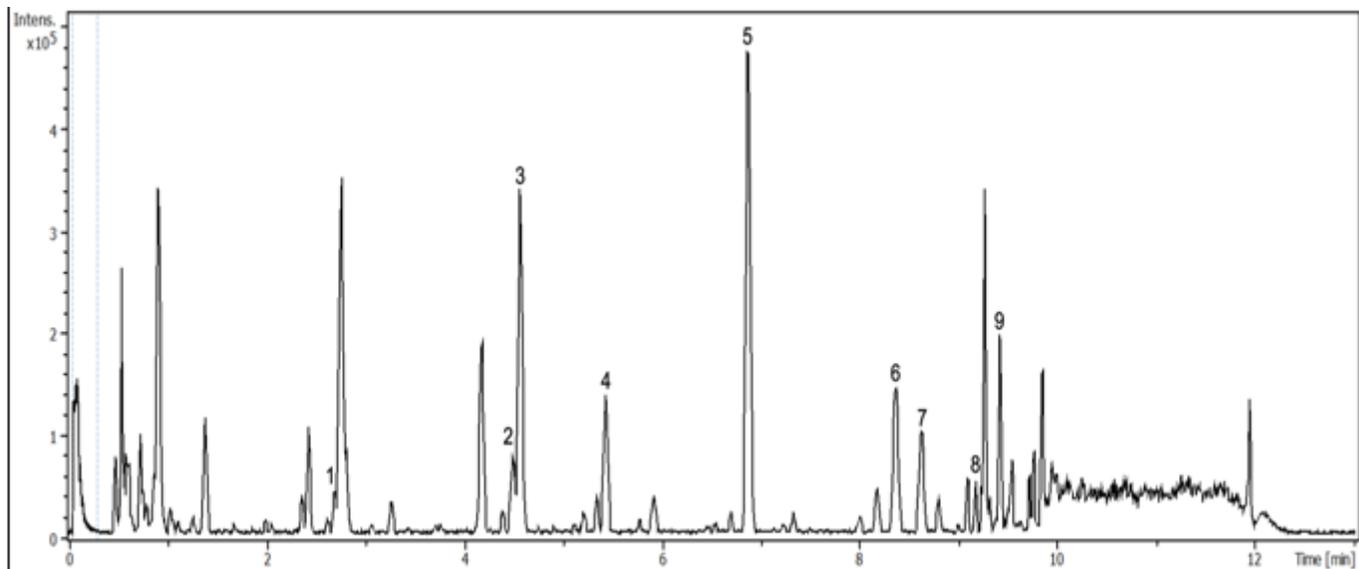
Figure 7

UHPLC chromatogram obtained from the injection of a concentrated EtOA extract of Czapek yeast medium after culturing of *P. aurantiogriseum* for four weeks. Annotated peaks: 1: maculosin (cyclo(L-Pro-L-Tyr); 2: penicillic acid; 3: cyclo(Leu-Pro); 4: cyclo(Phe-Pro); 5: aurantiamine; 6: patulin; 7: auranthin; 8: verrocosidin; 9. fuegin. Refer to Table S2 for compound identification.
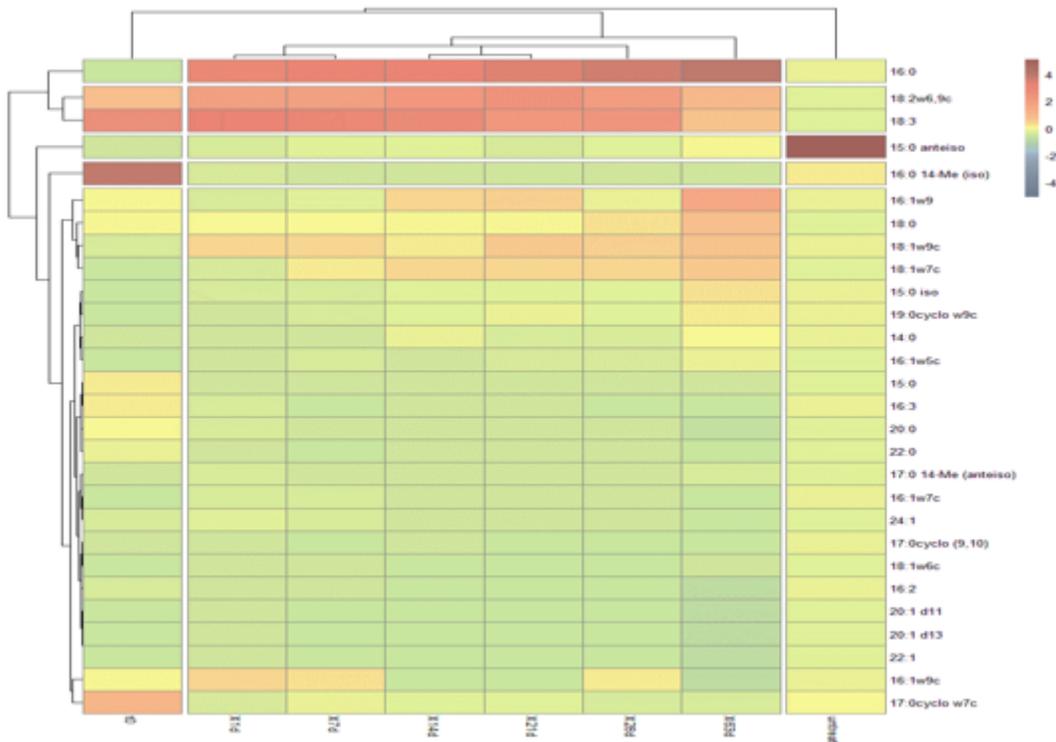
Figure 8

Heat map with cluster dendrogram for visualization of PLFA alterations over time in soil enriched with *Camelina*shoot material. The fatty acid profile in the untreated soil (untreat) indicates a high biodiversity of bacterial species which are predominately present in low abundance. Application of the glucosinolate-free *Camelina*shoot material reduces the biodiversity in favor of defined groups of microorganisms, including fungi. Samples were drawn directly after application (t0), then after 1 (X1d), 7 (X7d), 14 (X14d), 21 (X21d), 28 (X28d) and 63 (X63d) days; untreat: untreated soil. Phospholipid-derived fatty acids (PLFA)are on the right side.

Figure 9

The cluster plot analysis identified two groups of fatty acids. Cluster 1 contains all prokaryotic fatty acids, cluster 2 eucaryotic fatty acids.

Figure 10

Principal component analysis confirms the differentness of fatty acid compositions determined in untreated, d0 and d63 soil samples by corrlations of the variables.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.111022.pdf