Uniwersytet Przyrodniczy w Poznaniu

Wydział Rolnictwa, Ogrodnictwa i Biotechnologii

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Wpływ roślin fitosanitarnych na właściwości biochemiczne gleby i wzrost jabłoni (*Malus* Mill.) w szkółce po replantacji

Influence of phytosanitary plants on soil biochemical properties and apple growth (*Malus* Mill.) in a nursery after replantation

Rozprawa doktorska w dziedzinie nauk rolniczych

w dyscyplinie rolnictwo i ogrodnictwo

Doctoral dissertation in the field of agriculture

in the discipline of agriculture and horticulture

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Spis treści

1. Wykaz artykułów stanowiących rozprawę doktorską

1. **Wieczorek R**., Zydlik P. (2024): The use of biofumigation in orchards with apple replant disease - a review. Journal of Elementology, 29(1), 135-151, https://doi.org/10.5601/jelem.2023.28.3.3115.

Lista MEiN: 70 pkt. IF_{2024} 0,7

2. **Wieczorek R.,** Zydlik Z., Wolna-Maruwka A., Niewiadomska A., Kajzer D. (2023): The Effect of Biofumigation on the Microbiome Composition in Replanted Soil in a Fruit Tree Nursery. Agronomy 2023, 13, 2507. https://doi.org/ 10.3390/agronomy13102507

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3. **Wieczorek R.**, Zydlik Z., Wolna-Maruwka A., Kubiak A., Bocianowski J., Niewiadomska A. (2024): The Response of the Mycobiome to the Biofumigation of Replanted Soil in a Fruit Tree Nursery. Agronomy 2024, 14, 1961. https://doi.org/10.3390/ agronomy14091961

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4. **Wieczorek R.,** Zydlik Z., Zydlik P. (2024): Biofumigation Treatment Using *Tagetes patula*, *Sinapis alba* and *Raphanus sativus* Changes the Biological Properties of Replanted Soil in a Fruit Tree Nursery. Agriculture 2024, 14, 1023. https:// doi.org/10.3390/agriculture14071023

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2. Wykaz skrótów

R1 - gleba po płodozmianie (kombinacja kontrolna);

R2 - gleba replantowana;

R3 - gleba replantowana, z wykorzystaniem przedplonu z aksamitki rozpierzchłej *(Tagetes patula* L.);

R4 - gleba replantowana, z wykorzystaniem przedplonu z gorczycy białej (*Sinapis alba*);

R5 - gleba replantowana, z wykorzystaniem przedplonu z rzodkwi oleistej (*Raphanus sativus* var. *oleifer*a).

ARD - Apple Replant Disease – choroba replantacyjna jabłoni

OUT - Operational Taxonomic Units - liczba jednostek taksonomicznych

PCR - Polymeraze Chain Reaction – reakcja łańcuchowa polimerazy

PCA - Principal Component Analysis - analiza składowych głównych

TTC - chlorek trójfenylotetrazolu

ADh - aktywność dehydrogenaz

AP - aktywność proteaz

ASA - Absorpcyjna Spektrometria Atomowa

3. Streszczenie

Choroba replantacyjna jabłoni (ARD – Apple Replants Disease) jest często spotykanym, negatywnym zjawiskiem w sadach głównie jabłoniowych i szkółkach produkcyjnych. Czynnikiem sprawczym choroby jest przede wszystkim brak równowagi w strukturze mikrobioty i mykobioty w glebie i akumulacja szkodliwych mikroorganizmów. Jednym ze sposobów ograniczenia negatywnych skutków replantacji jest biofumigacja prowadząca do wytwarzana w glebie lotnych związków o właściwościach biobójczych.

Celem podjętych w latach 2019-2021 badań była ocena wpływu zastosowania wybranych roślin fitosanitarnych - aksamitki rozpierzchłej (*Tagetes patula* L.), gorczycy białej (*Sinapis alba*) i rzodkwi oleistej (*Raphanus sativus* var. oleifera) na: skład mikrobiomu i mykobiomu w glebie, liczebność nicieni oraz właściwości biochemicznych gleby po uprawie szkółkarskiej oraz wzrost drzewek i biometrię liści drzewek jabłoni.

Badania przeprowadzono w szkółce produkcyjnej. Drzewa jabłoni zostały posadzone w glebie z dwóch stanowisk. Gleba z pierwszego stanowiska nie była wcześniej wykorzystywana do produkcji materiału szkółkarskiego (gleba po płodozmianie). Glebę z drugiego stanowiska wykorzystano wcześniej do produkcji drzewek jabłoni (gleba replantowana). W glebie replantowanej jako przedplon zastosowano trzy gatunki roślin: aksamitkę rozpierzchłą (*Tagetes patula* L.), gorczycę białą (*Sinapis alba*) i rzodkiew oleistą (*Raphanus sativus* var. *oleifera*).

Na podstawie wyników analiz różnorodności taksonomicznej i funkcjonalnej zbiorowisk bakterii w glebie replantowanej (ARD), replantowanej z przedplonem z aksamitki rozpierzchłej (*Tagetes patula* L.), gorczycy białej (*Sinapis alba*) i rzodkwi oleistej (*Raphanus sativus var. oleifer*a) oraz w glebie po płodozmianie ustalono, iż zabieg biofumigacji zmieniał strukturę i liczebność mikrobiomu gleby replantowanej w szkółce drzewek owocowych. Wzrosła liczebność jednostek taksonomicznych OTU typów (*Proteobacteria, Bacteroidota, Patescibacteria, Chloroflexi, Fatescibacteria Verrucomicrobiota)* oraz rodzajów bakterii (*Flavobacterium, Massila, Sphingomonas, Arenimonas,* czy *Devosia).* Ustalono, iż biofumigacja poprawiła właściwości fizykochemiczne gleby (gęstość nasypowa i próchnica) oraz zwiększyła liczebności operacyjnych jednostek taksonomicznych (OTU) królestwa grzybów. Ponadto zabieg biofumigacji z aksamitką rozpierzchłą (R3) i rzodkwią oleistą (R5) zmniejszył

liczebności rodzaju *Fusarium,* do którego zalicza się kilka gatunków patogenicznych dla roślin.

Stwierdzono zmiany w liczebność nicieni fitopatogenicznych w glebie replantowanej po biofumigacji, gdzie ich liczba została znacznie obniżona, przy czym największa redukcja nastąpiła w glebie poddanej fumigacji aksamitką rozpierzchłą *Tagetes patula* L.) (R3). Szczególnie ważne jest zredukowanie osobników *Pratylenhus penetrans* z ok. 33 osobników w 100 cm^{-3} gleby (R2) do zera (R3).

Biofumiganty, zastosowane jako przedplon na glebie replantowanej, istotnie zwiększyły jej aktywność enzymatyczną i oddechową. Właściwości biologiczne gleby miały istotny wpływ na badane wybrane parametry wzrostu drzewek jabłoni w szkółce. Zastosowanie przedplonu z roślin fitosanitarnych poprawiło właściwości biologiczne gleby co przełożyło się na istotną poprawę siły wzrostu drzew. Na glebie replantowanej z wykorzystaniem roślin fitosanitarnych, w porównaniu do kombinacji bez tego zabiegu, wysokość drzew wzrosła o ponad 50%. Powierzchnia liści i ich masa oraz całkowita długość pędów bocznych były również istotnie wyższe.

Uzyskane wyniki dają podstawę do stwierdzenia, iż zastosowanie roślin fitosanitarnych w produkcji szkółkarskiej może stanowić skuteczną alternatywę dla chemicznej fumigacji gleby. Jednym z najbardziej obiecujących gatunków może być aksamitka rozpierzchła (*Tagetes patula* L.).

Słowa kluczowe: biofumigacja, mikrobiom, nicienie, aktywność enzymatyczna, wzrost drzewek

4. Abstract

Apple Replant Disease (ARD) commonly occurs in orchards, mainly with apple-trees, and in fruit nurseries. The disease is mostly caused by the lack of balance in the structure of the soil microbiota and mycobiota and due to the accumulation of harmful microorganisms. The negative effects of ARD can be reduced through biofumigation, which leads to the production of volatile biocidal compounds in the soil.

The aim of the study conducted between 2019 and 2021 was to assess the influence of selected phytosanitary plants, i.e. the French marigold (*Tagetes patula* L.), white mustard (*Sinapis alba*), and oil radish (*Raphanus sativus* var. *oleifer*) on: the composition of the soil microbiome and mycobiome, the number of nematodes, the physicochemical properties of the soil after nursery cultivation, the growth of apple trees and the biometry of their leaves.

The study was conducted in a production nursery. Apple trees were planted into the soil from two sites. The soil from the first site had not been used for the production of nursery material (crop rotation soil). The soil from the other site had been used for the production of apple trees (replanted soil). Three species of plants were used as forecrops: French marigold (*Tagetes patula* L.), white mustard (*Sinapis alba*), and oil radish (*Raphanus sativus* var. *oleifer*).

The analyses of taxonomic and functional diversity of bacterial communities showed that biofumigation changed the structure and abundance of the microbiome in the replanted soil in the fruit tree nursery. The abundance of operational taxonomic units (OTUs) (*Proteobacteria, Bacteroidota, Patescibacteria, Chloroflexi, Fatescibacteria, Verrucomicrobiota*) and bacterial genera (*Flavobacterium, Massila, Sphingomonas, Arenimonas,* and *Devosia*) increased. Biofumigation improved the physicochemical properties of the soil (bulk density and humus) and increased the abundance of operational taxonomic units (OTU) of the fungi kingdom. Apart from that, biofumigation with French marigold and oil radish reduced the abundance of the *Fusarium* genus, which includes several species of plant pathogens.

After biofumigation the number of phytopathogenic nematodes in the replanted soil decreased significantly. The greatest decrease was observed in the soil where French marigold (*Tagetes patula* L.) was used for biofumigation. It is particularly important that the population of *Pratylenhus penetrans* – a nematode species considered to be the main cause of ARD, was completely eliminated.

The biofumigants applied as forecrops to the replanted soil significantly increased its enzyme activity and respiration. In consequence, selected growth parameters of the apple trees in the nursery improved. In comparison with the soil where no phytosanitary plants were used, as a result of biofumigation, the height of the trees growing on the replanted soil increased by over 50%. The area of the leaves and their mass, as well as the total length of lateral shoots were also significantly greater.

The results of our study let us conclude that phytosanitary plants can be successfully used as an alternative to chemical soil fumigation in nursery production. The French marigold (*Tagetes patula* L.) seems to be one of the most promising species.

Keywords**:** biofumigation, microbiome, nematodes, enzyme activity, tree growth

5. Wprowadzenie

5.1. Choroba replantacji

Produkcja szkółkarska jest bardzo specyficzna i wymagająca, gdyż w stosunkowo krótkim czasie musimy zabudować mocny system korzeniowy i zapewnić warunki do prawidłowego rozwoju części nadziemnej. Sukces działań szkółkarza w dużej mierze uzależniony jest od gleby. Według licznych definicji gleba to kompleks bio-organo-koloidalno-mineralny. Składa się on z organizmów żywych, martwej substancji organicznej, minerałów, wody i powietrza. Dla rolnika zasadniczym elementem odróżniającym glebę od innych produktów geologicznych jest jej zdolność do stworzenia warunków do wzrostu i plonowania roślin. Podstawową cechą, która w sposób istotny wyróżnia glebę jest jej zdolność do samoreprodukcji, do spontanicznego odnawiania zasobów substancji koniecznych dla wzrostu i rozwoju roślin oraz innych organizmów zasiedlających glebę (Russel, 2005). Bardzo ważnym elementem jest wybór stanowiska glebowego. Chodzi tu nie tylko o rodzaj gleby, ale również stosowany przedplon. Już na początku ubiegłego stulecia zwracano uwagę, aby szkółki zakładać na tzw. glebie dziewiczej gdzie wcześniej nie prowadzono produkcji szkółkarskiej. W ostatnich latach nastąpiło nasilenie przeznaczania gruntów rolnych na cele inne niż rolnicze. Obecnie wysoki stopień specjalizacji gospodarstw oraz brak nowych terenów zmusza producentów do wchodzenia z produkcją szkółkarską na te same stanowiska, co może prowadzić do wystąpienia choroby replantacyjnej. Jest to problem coraz częściej występujący w sadach oraz szkółkach na całym świecie.

W literaturze występują takie terminy jak: zmęczenie gleby – soil fatigue (Wolińska i in., 2018), choroba gleby – soil sickness (Cesarano i in., 2017) lub choroba replantacyjna - replant disease (Nicola i in., 2018). Najczęściej stosowaną nazwą jest jednak choroba replantacyjna jabłoni (ARD – Apple Replant Disease). Zjawisko następuje na skutek sadzenia po sobie roślin tego samego lub spokrewnionego gatunku. Zdaniem Winkelmann i in. (2019), jest to zaburzona fizjologiczna i morfologiczna reakcja roślin na glebę, mikrobiom, który uległ zmianom pod wpływem wcześniejszych upraw tego samego lub spokrewnionego gatunku. W wyniku takich zaburzeń dochodzi do pogorszenia właściwości fizyczno-chemicznych i biologicznych gleby. ARD powoduje duże straty ekonomiczne i może znacząco ograniczać produkcję owoców, szczególnie w regionach o dużej koncentracji produkcji sadowniczej.

5.2. Czynniki sprawcze choroby replantacji

Badania w kierunku ustalenia przyczyn występowania ARD są prowadzone od lat 80 ubiegłego wieku. Pomimo licznych publikacji związanych z tą tematyką, wciąż brakuje jednoznacznego stanowiska na temat głównych sprawców choroby replantacyjnej. Przyjmuje się, iż mogą ją wywołać czynniki zarówno abiotyczne jak i biotyczne. Do tych pierwszych można zaliczyć np. niedostateczną wilgotność gleby, niską zawartość składników pokarmowych, niskie pH gleby oraz zaburzenia jej struktury. Zdaniem Spath i in. (2015), wpływ czynników abiotycznych na występowanie ARD jest stosunkowo niewielki, podczas gdy Sobiczewski i in. (2018) traktują czynniki pochodzenia abiotycznego jako głównych sprawców ARD.

Większość badań dotyczących sposobów przywracania żyzności glebie replantowanej dotyczy eliminacji czynników biologicznych. Najczęściej, zaliczają do nich określone gatunki nicieni, grzybów i bakterii. Jak uważa Winkelmann i in. (2019), w warunkach ARD następuje naruszenie równowagi pomiędzy gatunkami drobnoustrojów zasiedlających glebę. Dochodzi do ograniczenia rozwoju mikroorganizmów pożytecznych (Long i in., 2019) oraz obserwuje się wzrost aktywności mikrobiomu szkodliwego. Zdaniem Manici i in. (2013), to właśnie zmiana struktury gatunkowej drobnoustrojów glebowych jest główną przyczyną powstawania ARD.

Na świecie znanych jest około 4 100 gatunków nicieni (*nematod*) pasożytujących na roślinach. Migrują one przez glebę w poszukiwaniu rośliny żywicielskiej, atakują korzenie i żywią się cytoplazmą komórek. Tak uszkodzone korzenie są bardziej narażone na działanie innych patogenów glebowych. Fitopatogenne nicienie *Pratylenchus* spp. są często wymieniane jako główny biotyczny czynnik powstawania ARD (Mazzola i Manici, 2012; Singh i in., 2015; Kanfra i in., 2018). Inną grupą mikroorganizmów glebowych wymienianych wśród biologicznych sprawców ARD są grzyby rodzajów: *Alternaria* spp., *Rhizoctonia* spp., *Phythium* spp., *Cylindrocarpon* spp. i *Fusarium* spp. (van Schoor i in., 2009; Manici i in., 2013). W doświadczeniu Cavael i in. (2020) ilość w glebie replantowanej grzybów z rodzaju *Alternaria* stanowiła 2% całkowitej populacji grzybów glebowych. Jest to 10-krotnie większa ilość aniżeli w glebie rolniczej. Patogeny grzybowe atakują system korzeniowy roślin. Zdaniem Yin i in. (2014), w zainfekowanych korzeniach dominującym gatunkiem jest *Fusarium proliferatum*. Podobnego zdania są Zhao i in. (2022), którzy przypisują *F. proliferatum* istotną rolę w wywoływaniu ARD. Rola bakterii w wywoływaniu choroby replantacyjnej jest

przebadana w nieco mniejszym stopniu aniżeli grzybów. Jako sprawców ARD, badacze wymieniają bakterie należące do rodzajów *Bacillus i Pseudomonas* (Mazzola i Manici, 2012). Innego zdania są Franke-Whittle i in. (2015) według których, *Pseudomonas* nie odgrywa istotnej roli w zmniejszaniu żyzności gleby replantowanej.

Jak już wspominano wcześniej, w warunkach występowania ARD następuje pogorszenie właściwości fizyko-chemicznych i biologicznych gleby. Na glebie replantowanej spada aktywność enzymatyczna i oddechowa (Zydlik i in., 2019; Zydlik Z. i Zydlik P., 2020) co świadczy o spadku aktywności drobnoustrojów glebowych, odpowiedzialnych m.in. za mineralizację materii organicznej. To właśnie enzymy w glebie i jej aktywność oddechowa są miarodajnymi wskaźnikami aktywności mikroflory glebowej (Błońska i in., 2017; Meena i in., 2021). Zmniejszenie tępa mineralizacji materii organicznej w glebie replantowanej skutkuje mniejszą ilością dostępnych dla roślin składników pokarmowych. Udowodniono to m.in. w doświadczeniach Zydlik i in. (2020, 2021). Mniejsza ilość dostępnych składników odżywczych w glebie replantowanej powoduje osłabienie wzrostu wegetatywnego drzew owocowych rosnących w takich warunkach. Taki efekt występuje w przypadku podkładek jabłoni (Weiß i in., 2017; Weiß i in., 2017; Zydlik i in., 2023) oraz drzew tego gatunku (Manici, 2013; Liu i in., 2014; Zhao i in., 2022) (Publikacja 1 fot. 1). Objawami słabszego wzrostu drzew są mniejsze przyrosty oraz mniejsza powierzchnia asymilacyjna liści (Emmett i in., 2014; Yim i in., 2016 i 2017; Sobiczewski i in., 2018). Na słabszy wzrost wegetatywny drzew jabłoni rosnących w warunkach ARD może mieć wpływ nie tylko mniejsza ilość dostępnych składników pokarmowych, ale również ograniczone możliwości ich pobierania przez system korzeniowy roślin. Lukas i in. (2018) doszli do wniosku, iż podkładki jabloni w warunkach ARD, pobierają azot w formie azotanowej znacznie gorzej aniżeli podkładki rosnące w warunkach optymalnych.

5.3.Sposoby ograniczania choroby replantacji

Jednym ze sposobów ograniczenia negatywnych skutków replantacji jest stosowanie odpowiednich przedplonów, ze szczególnym uwzględnieniem roślin fitosanitarnych, które mogą przyczynić się do ograniczenia występowania szkodliwych nicieni lub grzybów patogenicznych występujących w glebie. Do roślin fitosanitarnych zaliczamy m.in. aksamitkę (*Tagetes* L.), gorczycę białą (*Sinapis alba*), rzodkiew oleistą (*Raphanus sativus var. oleifer*a), rzepak jary (*Brassica napus*), owies zwyczajny (*Avena*

sativa) oraz żyto (*Secale cereale* L.) i szparag lekarski (*Asparagus officinalis*). Działanie roślin fitosanitarnych związane jest ze specyficznymi związkami wytwarzanymi w roślinach i uwalnianymi do środowiska glebowego przez korzenie lub w wyniku rozkładu biomasy (Zhang i in., 2020; Sennett i in., 2021).

Produkcja materiału szkółkarskiego odbywa się w najważniejszym dla młodej rośliny okresie wzrostu. Rośliny w pierwszym stadium swojego rozwoju najwięcej energii zużywają na wytworzenie mocnego sytemu korzeniowego a dopiero po jego zbudowaniu wytwarzane są następne organy między innymi pędy boczne, dlatego szkółkarze powinni zadbać o stworzenie optymalnych warunków dla prawidłowego rozwoju systemu korzeniowego, gdyż zdrowy korzeń to zdrowe rośliny i wyrównana produkcja. Jednym ze sposobów jest wzbogacenie gleby w materię organiczną lub zastosowanie biowęgla (Zydlik i in., 2023).

W związku z tym, iż w dużych zagłębiach sadowniczych występuje problem z nabyciem gruntów pod nowe nasadzenia szkółkarskie oraz produkcję sadowniczą. Duża część gruntów dotąd użytkowana jest wyeksploatowana i wykazuje oznaki zmęczenia (choroba replantacji). Dlatego niemożliwe staje się prowadzenie produkcji sadowniczej i szkółkarskiej, dającej produkty na najwyższym poziomie oraz zapewniającej sukces ekonomiczny.

6. Cel pracy i hipoteza badawcza

Głównym celem podjętych badań była ocena wpływu zastosowania wybranych roślin fitosanitarnych - aksamitki rozpierzchłej (*Tagetes patula* L.), gorczycy białej (*Sinapis alba*) i rzodkwi oleistej (*Raphanus sativus* var. oleifera) na właściwości biologiczne i biochemiczne gleby oraz parametry biometrycne jabłoni. Cel ten osiągnięto przez realizację następujących celów szczegółowych:

- Ocenę mikrobiomu (liczebność i skład gatunkowy bakterii) i mykobiomu (liczebność i skład gatunkowy grzybów) w glebie po uprawie szkółkarskiej jabłoni oraz ocena liczebności w niej nicieni;
- Ocenę właściwości biochemicznych gleby po uprawie szkółkarskiej jabłoni (aktywność enzymatyczna i oddechowa gleby, zawartość próchnicy, odczyn, zawartość makro- i mikroelementów w glebie)
- Ocenę parametrów biometrycznych jabłoni (wzrost drzewek i biometria liści jabłoni) produkowanych w szkółce.

Hipoteza badawcza niniejszej dysertacji zakłada, że zastosowanie wybranych roślin fitosanitarnych wpłynie na zmniejszenie liczebności nicieni oraz mikroorganizmów szkodliwych w glebie i przyczyni się do poprawy wzrostu drzewek jabłoni w szkółce.

7. Materiały i metody badań

Eksperyment przeprowadzono w latach 2019-2021 w szkółce produkcyjnej w miejscowości Puszczykowo Zaborze, Zachodnia Polska (52°25′49,10″N 17°11′34,08″E) na glebie płowej właściwej. Wykorzystano w nim glebę pochodzącą z dwóch różnych stanowisk. W pierwszym była to gleba po uprawach rolniczych, optymalnie przygotowana pod uprawę jabłoni w szkółkę (dalej – gleba po płodozmianie). Stanowisko drugie to gleba, na której wcześniej produkowano przez trzy lata drzewka jabłoni – gleba z objawami ARD (gleba replantowana). W doświadczeniu wykorzystano trzy różne rośliny fitosanitarne: *Tagetes patula* L., *Sinapis alba, Raphanus sativus* var. *oleifer*a. Badania obejmowały pięć kombinacji: R1 – gleba po płodozmianie (kombinacja kontrolna); R2 - gleba replantowana; R3 - gleba replantowana, z wykorzystaniem przedplonu z aksamitki rozpierzchłej *(Tagetes patula* L.); R4 – gleba replantowana, z wykorzystaniem przedplonu z gorczycy białej (*Sinapis alba*); R5 - gleba replantowana, z wykorzystaniem przedplonu z rzodkwi oleistej (*Raphanus sativus* var. *oleifera*).

Wszystkie rośliny fitosanitarne wysiano do gleby jesienią, po wykopaniu drzewek jabłoni. Wczesną wiosną (marzec) rozdrobiono je i wymieszano z glebą. Na początku maja, tak przygotowaną glebą wypełniono pojemniki o pojemności 7,5 l do których posadzono szczepy jabłoni. Wykorzystano drzewka jabłoni odmiany `Golden Delicious` na podkładce M.9 otrzymane z zimowego szczepienia w ręku. Każda kombinacja była reprezentowana przez 30 pojemników.

Do realizacji powyżej podanych celów wykonano szereg analiz mikrobiologicznych, nematologicznych, biochemicznych gleby.

7.1. Ocena składu gatunkowego mikroorganizmów w glebie

Wszystkie analizy zostały wykonane w próbkach pobieranych we wrześniu każdego roku okresu badawczego. Z każdego pojemnika w kombinacji, łyżką laboratoryjną pobierano próbkę gleby o wadze 30 g. Po ich wymieszaniu otrzymywano próbkę zbiorczą o łącznej masie 900 g. Analizy gleby obejmowały:

– identyfikację mikroorganizmów glebowych. Ekstrakcja DNA - Całkowite DNA ekstrahowano z 500 mg każdej próbki za pomocą zestawu Genomic Mini AX Soil (A&A Biotechnology) zgodnie z instrukcją producenta. Wyekstrahowane DNA oznaczono ilościowo za pomocą zestawu Quant-iT HS dsDNA assay kit

(Invitrogen) na fluorometrze Qubit2; 2 µl ekstraktów zbadano na 0,8% żelu agarozowym.

– analizę metagenomiczną bakterii opartą na hiperzmiennym regionie V3-V4 genu 16S rRNA. Do amplifikacji tego regionu i przygotowania bibliotek użyto specyficznych starterów 341F i 785R. PCR przeprowadzono przy użyciu zestawu Q5 Hot Start High-Fidelity DNA Polymerase (NEB Inc., Ipswich, MA, Stany Zjednoczone). Warunki reakcji były utrzymywane zgodnie ze specyfikacją producenta. Do sekwencjonowania wykorzystano sekwenator Illumina MiSeq PE300 (Genomed S.A., Warszawa, Polska) w technologii 2 × 250 bp paired-end (PE) z zestawem chemii Illumina v2. Reakcje przeprowadzono zgodnie z protokołem amplifikacji 16S RNA Illumina v3-v4 (Illumina, San Diego, CA, Stany Zjednoczone). Dane analizowano automatycznie za pomocą MiSeq i w środowisku chmury Illumina BaseSpace zgodnie z protokołem 16S Metagenomics (wersja 1.0.1).
– analizę metagenomiczną grzybów na podstawie regionu ITS1. Specyficzne sekwencje starterów ITS1FI2 i 5.8S zostały użyte do amplifikacji wybranego regionu i przygotowania biblioteki. Do reakcji łańcuchowej polimerazy (PCR) wykorzystano Q5 Hot Start High-Fidelity 2X Master Mix. PCR przeprowadzono zgodnie z zaleceniami podanymi przez producenta urządzenia. Do sekwencjonowania użyto sekwenatora MiSeq z technologią paired-end (PE), 2x300nt i zestawem Illumina v3. Szczegółowe informacje są dostępne na stronach internetowych producentów odczynników. Do automatycznej wstępnej analizy danych wykorzystano urządzenie MiSeq i oprogramowanie MiSeq Reporter (MSR) v2.6. Analiza składała się z dwóch etapów: 1. automatycznego demultipleksowania próbki; 2. generowania plików fastq zawierających surowe odczyty. Bazy danych sekwencji referencyjnych UNITE v8 i pakiet oprogramowania QIIME zapewniły klasyfikację odczytów w analizie bioinformatycznej do poziomu gatunku. Analiza składała się z sześciu etapów: 1. usuwanie sekwencji adapterowych - program Cutadapt; 2. analiza jakości odczytów i usuwanie sekwencji niskiej jakości (jakość < 20, minimalna długość 30) - program Cutadapt; 3. łączenie sparowanych sekwencji - algorytm fastq-join; 4. grupowanie na podstawie wybranej bazy sekwencji referencyjnych -

algorytm uclust; 5. usuwanie chimer sekwencyjnych - algorytm usearch61; 6. przypisanie taksonomii do wybranej bazy sekwencji referencyjnych.

- analiza nematologiczna gleby. Próby gleby pobrano z obrębu systemu korzeniowego drzewek. Wstępna identyfikacja nicieni do gatunku przeprowadzona była z wykorzystaniem istniejących kluczy (Brzeski 1998; Andrássy 2005). Uzyskane na podstawie morfologii oznaczenia potwierdzone zostały za pomocą sekwencjonowania markerów molekularnych (D2-D3 28S rDNA).

7.2. Ocena biochemicznych i chemicznych właściwości gleby.

Glebę pobierano w trzech terminach - wiosną tuż po rozpoczęciu wegetacji, latem po zakończeniu fazy intensywnego wzrostu wegetatywnego oraz jesienią przed opadnięciem liści. W każdej kombinacji próby pobierano z obrębu systemu korzeniowego. W świeżej glebie oznaczano:

- aktywność dehydrogenaz (ADh) – metodą kolorymetryczną wg. Thalmanna (1968), stosując jako substrat 1 % roztwór TTC (chlorek trójfenylotetrazolu), po 24–godzinnej inkubacji w temperaturze 30 ^{o}C przy długości fali 485 nm (test TTC), wyrażając jej aktywność w cm^3 H_2 $24h^{-1}$ kg^{-1}s.m. gleby.
- aktywność proteaz (AP) – metodą spektrofotometryczną wg. Ladda i Butlera (1972), używając jako substratu 1% roztwór kazeinianu sodu, po 1-godzinnym czasie inkubacji w temperaturze 50 ^{o}C przy długości fali 578 nm, aktywność enzymu wyrażono w mg tyrozyny $h^{-1}kg^{-1}$s.m. gleby.
- aktywność oddechową gleby oznaczono zgodnie z metodyką podaną przez Gołębiowską i Pędziwilk (1984). Oznaczenie wykonano na podstawie ilości wydzielanego CO_2 metodą absorpcyjną a wynik wyrażono w CO_2 w mg kg^{-1} $48h^{-1}$.
- analizy chemiczne gleby. Próbki gleby zostały pobrane do analiz chemicznych pod koniec lipca i zostały analizowane chemicznie metodą uniwersalną. Ekstrakcję makroskładników (N-NH_4, N-NO_3, P, K, Ca, Mg, S-SO_4, Cl i Na) przeprowadzono w 0,03 M CH_3COOH w stosunku ilościowym 1:10 substratu do roztworu ekstrakcyjnego. Po ekstrakcji wykonano następujące oznaczenia: N-NH_4, N-NO_3 - przez mikrodestylację według Bremera w modyfikacji Starcka; P - kalorymetrycznie z anadomolibdenianem amonu; K, Ca, Na - fotometrycznie;

Mg - metodą absorpcyjnej spektrometrii atomowej (ASA); $S\text{-}SO_4$ - nefelometrycznie z $BaCl_2$; Cl - nefelometrycznie z $AgNO_3$. Mikroelementy (Fe, Mn, Zn i Cu) ekstrahowano z gleby roztworem Lindsaya zawierającym w $1dm^3$: 5 g EDTA (kwas etylenodiaminotetraoctowy); 9 cm^3 25% roztworu NH_4, 4 g kwasu cytrynowego; 2 g Ca $(CH_3COO)_2\text{-}2H_2O$. Mikroelementy oznaczono metodą 150 ASA. Zasolenie określono konduktometrycznie jako przewodność elektrolityczną (EC w $mS\text{-}cm^{-1}$) (substrat:woda = 1:2), a pH - metodą potencjometryczną (substrat:woda = 1:2).

7.3. Ocena parametrów biometrycznych drzewek

W celu oceny parametrów biometrycznych drzewek wykonano następujące pomiary:

- średnicy pnia. Pomiar wykonano na wysokości 10 cm nad miejscem okulizacji, zgodnie z wytycznymi dotyczącymi oceny jakości materiału szkółkarskiego a wyniki wyrażono w mm. Ze średnicy pnia obliczono pole powierzchni przekroju poprzecznego pnia wyrażając wynik w mm^2. Dodatkowo obliczono przyrost średnicy, a wyniki wyrażono w mm;
- wysokości drzewek dokonując pomiaru od szyjki korzeniowej do wierzchołka pędu głównego z dokładnością do 0,5 cm;
- liczby pędów bocznych oraz ich długości mierząc wszystkie pędy długości powyżej 1 cm co było podstawą do obliczenia sumy przyrostów oraz ich średniej długość;
- masy i powierzchni liści. Liście zebrano dnia 24 lipca. W każdej kombinacji dokonano pomiarów w czterech powtórzeniach wybierając po 10 liści w powtórzeniu. Wszystkie liście zostały zaważone i zeskanowane. Następnie przy pomocy programu DigiShape 1.9, obliczono ich powierzchnię, a wynik wyrażono w cm^2.

8. Wyniki i ich omówienie

8.1. Skład gatunkowy mikroorganizmów w glebie

Bakterie (Publikacja 2)

Przeprowadzona analiza metapopulacyjna dokonana na podstawie analizy sekwencji 16S rRNA wykazała, że wcześniejszy sposób użytkowania gleby w szkółce, wykorzystane w doświadczeniu rośliny fitosanitarne oraz lata badań miały wpływ na liczbę jednostek taksonomicznych bakterii występujących w glebie. W zależności od stanowiska glebowego oraz roku badań otrzymano od 20 do 34 typów oraz od 378 do 554 rodzajów bakterii (Publikacja 2, tab. 2, ryc. 2-4). Na podstawie uzyskanych wyników badań wykazano, że niezależnie od roku badań, we wszystkich testowanych kombinacjach doświadczalnych zawartość OTU należących do typu *Proteobacteria* była niższa aniżeli w kombinacji kontrolnej (R1). Należy jednak podkreślić, że różnica w zawartości OTU należących do typu *Proteobacteria* dla gleb poddanych indukowanej biofumigacji, z zastosowaniem roślin fitosanitarnych - *Tagetes patula* L. (R3); *Sinapis alba* (R4); *Raphanus sativus var. oleifer*a (R5), w stosunku do gleby po płodozmianie (R1) była znacznie niższa, aniżeli różnica odnotowana dla gleby z ARD. Ponadto w roku 2020 zanotowano istotny wzrost zawartości OTU należących do typu *Proteobacteria* w przypadku aplikacji aksamitki rozpierzchłej (R3-R1). Na tej podstawie można twierdzić, że zastosowanie roślin fitosanitarnych na glebie z ARD powoduje wzrost zawartości OTU należących do typu *Proteobacteria*. Taki wniosek jest zbliżony do wcześniejszych doniesień autorów badań dotyczących rekultywacji gleby ARD przez dodanie kompostu (Fierrer i in., 2007; Swędrzyńska i Małecka-Jankowiak, 2017) lub w wyniku działania promieniowania gama (Rawat i Joshi, 2019).

Podobna tendencja wystąpiła również w przypadku typu *Firmicutes*. Analiza metagenomiczna gleby prowadzona w latach 2019-2021 wykazał obniżenie zawartości sekwencji siedmiu typów bakterii w testowanych próbkach glebowych w porównaniu do wariantu kontrolnego. Wspomniane różnice nie występowały jednakże we wszystkich wariantach doświadczalnych (Publikacja 2, ryc. 5-7). Uszeregowano je w zależności od częstotliwości występowania w glebie w sposób następujący: *Bacteroidota* > *Acidobacteriota* > *Actinobacteriota* = *Gemmatimonadota* > *Patescibacteria*.

Typy bakterii *Verrucomicrobiota, Chloroflexi,* a w szczególności *Planctomycetota* oraz *Cyanobacteria* izolowane były z mniejszą częstotliwością z gleby po płodozmianie (R1), szczególnie w stosunku do gleby replantowanej poddanej

biofumigacji. Typ *Chloroflexi* należy do bakterii nitryfikacyjnych rozwijających się w warunkach beztlenowych lub mikroaerofilnych i charakteryzuje się zdolnością do przetrwania w intensywnie zmieniających się, ekstremalnych warunkach. Zwiększona częstotliwość występowania typu *Chloroflexi* w glebach z ARD po biofumigacji w stosunku do gleby kontrolnej, może być tłumaczona tym, że rozwój społeczności tych bakterii opiera się na wykorzystaniu związków komórkowych pochodzących już z martwych mikroorganizmów i ich metabolitów co charakteryzuje gleby z ARD (Niewiadomska i in., 2020). Podobną dominację tej grupy mikroorganizmów zaobserwował Tang i in. (2018), który zastosował słomę ryżową i biowęgiel w celu skutecznej poprawy jakości gleby. Literatura przedmiotu wskazuje, iż istotnym typem wskaźnikowym, świadczącym o odbudowie gleb jest typ *Cyanobacteria*, któremu przypisuje się doniosłą rolę w wiązaniu azotu atmosferycznego oraz syntezie egzopolisacharów, co przekłada się na zwiększenie żyzności gleby i retencji wody oraz poprawie jej struktury i stabilności (Liang i in. 2018). Jak wynika z obecnego badania, na skutek indukowanej fumigacji przez zastosowanie m.in. aksamitki rozpierzchłej (R3), zawartość OTU wskazanego typu wzrosła o 83% w stosunku do gleby z ARD oraz gleby po uprawach rolniczych.

Przedstawione powyżej zmiany w składzie ilościowym i jakościowym mikrobiomu bakteryjnego w zależności od wcześniejszego sposobu użytkowania gleby i wpływu roślin fitosanitarnych, pozwoliły na wyznaczenie mikroorganizmów, które uznać można za biowskaźniki określające stan żyzności gleby. Uzyskane wyniki badań, zaprezentowane w postaci diagramów Venna (Publikacja 2, ryc. 13), potwierdzają wpływ wcześniejszego sposobu użytkowania gleby oraz okresu badawczego na strukturę mikrobiomu bakteryjnego. Biorąc pod uwagę obecność wszystkich taksonów w obrębie danej kategorii systematycznej oraz okresu badawczego wytypowano od 482 do 512 rodzajów wspólnych dla wszystkich obiektów. Przykładowo, w obrębie rodzaju we wszystkich wariantach doświadczalnych zidentyfikowano bakterie należące min. do rodzajów *Pseudomonas Bacillus, Arthrobacter, Streptomyces. Chujaibacter, Sphingomonas, Flavobacterium, Devosia.*

Grzyby (Publikacja 3)

W wyniku przeprowadzonej analizy ITS, stwierdzono, że w przypadku każdej z analizowanych próbek gleby *Fungi* i *Viridiplantae* stanowiły największy odsetek organizmów (Publikacja 3, ryc.1).

Liczba operacyjnych jednostek taksonomicznych (OTU) reprezentujących grzyby zidentyfikowane w badaniach, w glebie po płodozmianie wynosiła 54,19%, podczas gdy udział OTU w glebie z ARD wynosił tylko 25,65%. Biofumigacja zastosowana na glebie ARD, głównie z przedplonem aksamitki rozpierzchłej (*Tagetes patula* L.) (R3), najbardziej zwiększyła liczebność grzybów. Takich zależności nie zaobserwowano w glebie z przedplonami gorczycy białej (*Sinapis alba*) i rzodkwi oleistej (*Raphanus sativus* var. *oleifera*), gdzie udział OTU reprezentujących grzyby wynosił odpowiednio 31,38% i 18,94% (Publikacja 3, ryc. 1).

Znaczne obniżenie obfitości taksonów grzybów w środowisku glebowym nie zawsze należy do zjawisk korzystnych. Musimy mieć na uwadze fakt, że do tej grupy *Eucariota* obok patogenów należą symbionty (grzyby mykoryzowe) i rozkładacze. Jest to królestwo, które odgrywa kluczową rolę w cyklach biogeochemicznych (Frąc i in., 2022). Ponadto, grzybnia grzybów rozciąga się pod ziemią jak naczynia krwionośne w ludzkim ciele, przenosząc wodę i składniki odżywcze do i z różnych roślin. Grzyby wspierają wiele procesów ekosystemowych i pełnią funkcje, które są niezbędne dla zrównoważonego rozwoju przyszłego rolnictwa (Fernandes i in., 2022), w tym interakcji między roślinami a glebą, rozkładem materii organicznej (Vétrovský i in., 2019), promocją zdrowia roślin i odżywianie (Põlme i in., 2020).

Występujące w znacznych ilościach *Viridiplantae* stanowią uklad organizmów eukariotycznych, obejmujący kilkaset tysięcy gatunków, które według danych literaturowych odgrywają także ważną rolę zarówno w ekosystemach lądowych, jak i wodnych. (Leebens-Mack i in., 2019). Należą do nich m.in. rośliny lądowe (embriofity), które w drodze ewolucji wyłoniły się z zielenic (Cocquyt i in., 2009; Becker, 2007).

W badaniach własnych największą liczbę jednostek taksonomicznych OTU dla wskazanego Królestwa, zanotowano w glebie z ARD i wynosiła ona 18,69%, a mniejszą w glebie rolniczej oraz w glebach replantowanych z przedplonem aksamitki rozpierzchłej (*Tagetes patula* L.), gorczycy białej (*Sinapis alba*) i rzodkwi oleistej (*Raphanus sativus var. oleifer*a), gdzie kształtowała się ona odpowiednio dla tych gleb na poziomie 14,18%, 14,23%, 14,28%, 14,13% OTU. Podobne zależności redukcji *Viridiplante* zanotował

Qiao i in. (2017). Zaobserwował redukcję obfitości omawianej grupy organizmów, głównie z rodzaju *Chlamydomonas* po aplikacji nie roślin o zdolnościach biofumigacyjnych, ale korzystnych mikroorganizmów należących do grupy PGPR aplikowanych do gleby, w uprawie pomidora. Drobnoustroje te, wyizolowane z urodzajnych gleb, miały zdolność wydzielania kilku antagonistycznych związków, takich jak antybiotyki lipopeptydowe, do których należy m.in. surfaktyna, ituryna i fengycyna powodujących istotne ograniczenie *Viridiplante*.

Można przypuszczać, że w badaniach własnych rośliny o właściwościach biofumigacyjnych wydzielające metabolity wtórne również ograniczyły obecność omawianego królestwa grzybów, w stosunku do gleby z ARD. Dane literaturowe wskazują, że *Sinapis alba* oraz *Raphanus sativus* zastosowane w tym doświadczeniu jako rośliny biofumigacyjne, wytwarzają m.in. glukozynolany po hydrolizie których powstają biologicznie aktywne związki izotiocyjaniany – alifatyczny izotiocyjanian allilu, izotiocyjaniany aromatyczne, izotiocyjanian 2-fenyloetylu i benzylu (Gimsing i Kirkegaard, 2009). Z kolei *Tagetes* L. wytwarzają związki tiofenowe, takie jak α-tertienyl (Hamaguchi i in., 2019).

W przeprowadzonych obserwacjach w obrębie królestwa grzybów wykazano ponadto obecność mikroorganizmów eukariotycznych do tej pory niesklasyfikowanych, stanowiących od 30,05% i 30,28% odpowiednio dla gleby replantowanej, z wykorzystaniem przedplonu z aksamitki rozpierzchłej *(Tagetes patula* L.) (R3) i gleby po płodozmianie (R1-kontrola), do 58,05% dla gleby replantowanej, z wykorzystaniem przedplonu z rzodkwi oleistej (*Raphanus sativus* var. *oleifer*a) i 54,02% dla gleby z ARD (Publikacja 3, ryc.3).

Poza oceną i wskazaniem dominujących królestw w obrębie organizmów eukariotycznych występujących w badanych próbkach gleby, dokonano względnej analizy różnic w liczbie jednostek taksonomicznych (OTU), pomiędzy badanym wariantem gleby z ARD oraz gleb z ARD poddanych biofumigacji, a glebą kontrolną: R2*vs*R1, R3*vs*R1, R4*vs*R1, R5*vs*R1 (Publikacja 3, ryc. 2). Największe różnice zaobserwowano pomiędzy R5*vs*R1 dla *Fungi* i dla mikroorganizmów niesklasyfikowanych, z kolei najmniejsze różnice wykazano pomiędzy R3*vs*R1.

Przeprowadzona analiza metapopulacyjna dokonana na podstawie analizy hiperzmiennego regionu ITS1 wykazała, że wcześniejszy sposób użytkowania gleby w szkółce, oraz wykorzystane w doświadczeniu rośliny fitosanitarne miały wpływ na liczbę

jednostek taksonomicznych (OTU) w obrębie *Phylum* tylko dla *Fungi*, występujących w glebie. W zależności od badanego wariantu glebowego, w Królestwie *Fungi* otrzymano od 7 do 11 ich *Phylum* (Publikacja 3, ryc. 3).

Dla wszystkich wariantów doświadczenia zdecydowanie największą liczbę jednostek taksonomicznych OTU na poziomie *Phylum*, odnotowano dla *Ascomycota*, gdzie zwielokrotnioną ich liczbę odnotowano dla gleby kontrolnej oraz gleby replantowanej, z wykorzystaniem przedplonu z aksamitki rozpierzchłej *(Tagetes patula* L.) (R3) i wynosiła ona odpowiednio 42.78% i 39.82% (Publikacja 3, ryc. 3). Znacznie zredukowana ich liczba występowała w glebie z ARD, gdzie ich wartość bezwzględna wynosiła 14,48%.

Dane literaturowe wskazują, iż *Ascomycota* powszechnie występują w glebach rolniczych. Członkowie tej grupy mają wiele genów związanych z odpornością na stres związany z zabiegami agrotechnicznymi, a przede wszystkim niedotlenieniem środowiska. Badania własne wskazują, iż zastosowanie przedplonu z aksamitki rozpierzchłej *(Tagetes patula* L.) (R3) w glebie z ARD znacznie podniosła względną liczbę omawianej grupy grzybów podnosząc ją do wartości zbliżonej w glebie rolniczej.

Innym typem grzybów dominującym w badanych glebach był *Mortierellomycota*, którego najwyższy poziom odnotowano dla gleby replantowanej, z wykorzystaniem przedplonu z aksamitki rozpierzchłej *(Tagetes patula* L.) (R3) (7,73%), a najniższy dla gleby replantowanej, z wykorzystaniem przedplonu z rzodkwi oleistej (*Raphanus sativus var. oleifer*a) (R5) (2,37%) (Publikacja 3, ryc. 3). W glebie użytkowanej rolniczo (kontrolnej) OTU dla *Mortierellomycota* wynosiła 4,07%, a w glebie z ARD 4,68%. Według danych literaturowych członkowie *Mortierellomycota* należą do tzw. grzybów pożytecznych. Ich rola polega m.in. wspomaganiu produkcji fitohormonów (np. gibereliny, kwas indolooctowy), dostarczania symbiotycznym roślinom składników odżywczych, w tym głównie fosforu (Gaiero i in., 2021) m.in. przez uwalnianie różnych kwasów organicznych, które rozpuszczają oporne nieorganiczne formy fosforu (Zhang i in., 2020).

W oznaczonych typach najmniejsze różnice OTU występowały pomiędzy R3vsR1, a największe pomiędzy R5*vs*R1. Więcej niż 2% różnicy zaobserwowano dla *Ascomycota* pomiędzy wszystkimi analizowanymi wariantami. Ponadto różnica ponad 2% została zaobserwowana pomiędzy wariantami R2*vs*R1, R3*vs*R1 dla niesklasyfikowanych i *Mortierellomycota* pomiędzy R3*vs*R1 (Publikacja 3, ryc.4).

Kolejne analizy dotyczyły oceny poziomu dominujących klas w obrębie mykobiomu w glebie z ARD oraz w glebie z ARD replantowanej z przedplonem z aksamitki rozpierzchłej (*Tagetes patula* L.) (R3), gorczycy białej (*Sinapis alba*) (R4) i rzodkwi oleistej (*Raphanus sativus* var. *oleifer*a) (R5) oraz w glebie po płodozmianie (R1). Przeprowadzone obserwacje metapopulacji na poziomie Class wykazały dominację *Eurotiomycetes* dla wszystkich wariantów (od 6,10% dla R5 do 37,78% dla R1) oprócz R4, gdzie największą OTU stanowiły *Sordariomycetes* (9,29%). Generalnie we wszystkich obiektach doświadczalnych odnotowano najmniejszą zawartość jednostek taksonomicznych, należących do klasy *Tremellomycetes* (Publikacja 3, ryc.5).

Największe różnice (ujemne) dla OTU na poziomie Class pomiędzy glebą z ARD (R1) i glebami z ARD poddanymi biofumigacji, a glebą kontrolną (R1) zaobserwowano dla *Eurotiomycetes* (-31,68% - -9,21%). Największą dodatnią różnicę (6,53% OTU) zaobserwowano dla klasy *Sordariomycetes* pomiędzy R4*vs*R1. Ponadto w R3*vs*R1 w 4 przypadkach różnica OTU była większa co do wartości bezwzględnej 2%: dla niesklasyfikowanych (-2,9%), *Mortierellomycetes* (3,66%), *Sordariomycetes* (2,35%), *Ascomycota* (3,05%) (Publikacja 3, ryc.6).

Nicienie (Publikacja 4)

Nicienie zaliczane są do biologicznych czynników sprawczych odpowiedzialnych za powstawanie ARD (Yin i in., 2016; Kanfra i in., 2018). W doświadczeniu, stwierdzono obecność w glebie 11 gatunków tych mikroorganizmów (Publikacja 4, tab.7). W wariancie kontrolnym (R1) wykryto 4 gatunki nicieni, z czego w najwięcej było przedstawicieli gatunku *Ecumenicus monohystera*. (ok. 34 osobników w 100 cm^{-3} gleby). Znacznie mniej było *Mesorhabditis spiculigera.* W glebie replantowanej (R2) stwierdzono najwięcej nicieni należących do gatunku *Mesorhabditis spiculigera,* i *Tylenchorhynchus dubius* (odpowiednio 79 i 60 osobników w 100 cm^{-3} gleby).

Zdaniem Dutta i in. (2019) rośliny fitosanitarne są dobrze zbadane pod kątem ich właściwości nicieniobójczych. W doświadczeniu, wszystkie trzy gatunki roślin fitosanitarnych skutecznie ograniczały liczebność nicieni, szczególnie w porównaniu z glebą replantowaną (R2). Wysoką skutecznością pod tym względem cechowała się aksamitka rozpierzchła (*Tagetes patula* L.) (R3). W kombinacji z jej stosowaniem zredukowano liczebności nicieni gatunku *Pratylenhus penetrans* z ok. 33 osobników w 100 cm^{-3} gleby do zera. Jest to gatunek nicieni z rodziny *Pratylenchidae* zaliczany do

jednego z najważniejszych szkodników w uprawach sadowniczych, warzywnych i ozdobnych. Żerując na korzeniach, powoduje on powstawanie nekrotycznych plam co znacząco zmniejsza aktywną powierzchnię korzeni. W doświadczeniach Weerakoon i in. (2012), Mazolla i in. (2015), Wang i in. (2019), potwierdzono redukuję liczebności fitopatogennych nicieni z gatunku *Pratylenhus penetrans* w glebie replantowanej po wykorzystaniu roślin z rodziny kapustowatych (*Brassica juncea, Sinapis alba*) lub rzodkwi zwyczajnej (*Raphanus sativus*) (Yim i in., 2016). W kombinacjach z przedplonem z aksamitki rozpierzchłej (R3), w porównaniu do kombinacji bez przedplonów (R2), stwierdzono kilkudziesięciokrotną redukcję *Tylenchorhynchus dubius*, kilkunastokrotną - *Prismatolaimus* sp. i *Geocenamus nothus*, oraz kilkukrotną – *Mononhoides* sp.. Należy zwrócić uwagę na znaczne zmniejszenie liczebności w glebie replantowanej *Tylenchorhynchus dubius* – kolejnego po *Pratylenhus penetrans* wewnętrznego pasożyta roślin żerującego na korzeniach. Jego zdolność do przeżywania i rozwoju w najróżniejszych warunkach środowiskowych powoduje, iż *Tylenchorhynchus dubius* występuje w strefie korzeni ponad 100 gatunków roślin. W kombinacji z przedplonem z gorczycy białej *(Sinapis alba*) (R4), całkowicie zredukowano ilość w glebie takich gatunków jak *Ecumenicus monohystera* i *Mononhoides* sp. Kilkunastokrotnie zmniejszyła się również ilość *Cephalobus persegnis* i *Geocenamus nothus*. W przypadku takich gatunków jak *Cuticularia oxycerca* nie stwierdzono istotnego zróżnicowania ich liczebności w zależności od kombinacji. Relatywnie najmniejszą skutecznością w redukowaniu liczny nicieni w glebie replantowanej wykazała się rzodkiew oleista (*Raphanus sativus* var. oleiformis) (R5). W kombinacji z przedplonem z tej rośliny zredukowano do zera ilość w glebie nicieni należących do gatunków *Geocenamus nothus* i *Terrtocephalus terrestris*. Nie zmieniła się natomiast liczebność *Ecumenicus monohystera* i *Mononhoides* sp.

Z analizy liczebności nicieni w glebie w poszczególnych latach badań wynika, iż efekt nicieniobójczy roślin fitosanitarnych był widoczny już po roku od ich zastosowania. Z jedenastu wykrytych w 2020 roku gatunków nicieni, w kolejnym roku badań istotnie zmniejszyła się liczebność ośmiu z nich (Publikacja 4, ryc. 3). W szczególności dotyczy to gatunków *Geocenamus nothus, Mesorhabditis spiculigera, Mononhoides* sp., *Pratylenhus penetrans, Prismatolaimus* sp.

8.2. Biochemiczne i chemiczne właściwości gleby (Publikacja 4)

Jakość gleby oceniano na podstawie zawartości mikro- i makroelementów, wilgotności, pH, jej aktywności enzymatycznej i oddechowej. Przeprowadzone doświadczenie wykazało istotne różnice w parametrach fizykochemicznych gleby, które zależały od jej wcześniejszego użytkowania. Gleba po replantacji (R2) była bardziej kwaśna niż gleba optymalnie przygotowana po szkółkę (R1) (pH odpowiednio 4,8 i 5,0) i zawierała prawie trzykrotnie mniej materii organicznej (0,8% i 2,17%) (Publikacja 4, tab. 2). Jakość gleby replantowanej była istotnie lepsza w wariantach z biofumigacją. Odczyn gleby wzrósł z 4,8 do 5,0 (aksamitka rozpierzchła - *Tagetes patula* L.) (R3) i 5,5 (rzodkiew oleista - *Raphanus sativus* var. *oleifera* (R5). Zawartość próchnicy również znacząco wzrosła i była najwyższa w wariancie z gorczycą białą. Była ona o ponad 50% wyższa niż w wariancie z glebą optymalnie przygotowaną pod szkółkę (R1), tj. 0,8% i 1,09% (Publikacja 4, tab. 2).

Próchnica jest podstawowym źródłem składników odżywczych dostępnych dla roślin. Szybkość mineralizacji materii organicznej zależy od efektywności działania mikroorganizmów glebowych, którą można zmierzyć za pomocą aktywności enzymów glebowych. Uważa się, że znajomość aktywności enzymatycznej w połączeniu z innymi właściwościami gleby, stanowi podstawę oceny jej jakości (Furtak i Gajda, 2018). Podstawowym źródłem enzymów są drobnoustroje glebowe (głównie bakterie) oraz resztki korzeni roślin w glebie. W glebie najważniejszą rolę odgrywają enzymy należące do oksydoreduktaz (dehydrogenazy) oraz hydrolaz (proteaza, ureaza). W doświadczeniu stwierdzono istotne zróżnicowanie aktywności enzymatycznej gleby w zależności od jej wcześniejszego sposobu użytkowania. Największe różnice wystąpiły w przypadku aktywności dehydrogenaz (0,56 i 1,22 cm^{-3} H_2 $24h^{-1}$ kg^{-1} s.m odpowiednio) (Publikacja 4, tab. 3). Te enzymy są uznawane za bardzo czułe wskaźniki zmian właściwości gleby (Gałązka i in., 2017). Innym parametrem określającym aktywność mikroorganizmów glebowych jest aktywność oddechowa gleby, mierzona ilością wydzielanego CO_2 (Meene i Rao, 2021). Również i w tym przypadku wcześniejszy sposób użytkowania gleby miał istotny wpływ na ten parametr. Aktywność oddechowa gleby replantowanej (R2) – 19,25 CO_2 mg kg^{-1} 48 h^{-1} była istotnie niższa, aniżeli gleby optymalnie przygotowanej pod szkółkę (R1) – 27,70 CO_2 mg kg^{-1} 48 h^{-1}. W kombinacjach z przedplonem z roślin fitosanitarnych stwierdzono istotny wzrost aktywności zarówno enzymatycznej jak i oddechowej gleby replantowanej (Publikacja 4, tab. 3). Średnio za dwa lata badań, w

kombinacji z przedplonem z aksamitki rozpierzchłej (*Tagetes patula* L.) (R3) i rzodkwi oleistej (*Raphanus sativus* var. *oleifera*) (R5), aktywność dehydrogenaz w glebie replantowanej była ponad dwukrotnie wyższa aniżeli w glebie bez przedplonów (1,3 oraz 0,56 cm^{-3} H_2 $24h^{-1}$ kg^{-1} s.m. odpowiednio). Podobny wniosek można wyciągnąć przy analizie aktywności oddechowej gleby (32,6 i 19,25 CO_2 mg kg^{-1} 48 h^{-1}). Aktywność proteaz w glebie replantowanej była najwyższa w wariancie z aksamitką rozpierzchłą. Warto podkreślić, iż zarówno aktywność enzymatyczna jak i oddechowa gleby replantowanej w kombinacjach z roślinami fitosanitarnymi była istotnie wyższa aniżeli w wariancie kontrolnym z glebą optymalnie przygotowaną pod szkółkę (R1).

Aktywność enzymów w glebie zależy od takich czynników jak: właściwości fizykochemiczne gleby (odczyn, zawartość materii organicznej, zanieczyszczenia metalami ciężkimi), warunki klimatyczne i systemy uprawy (Błońska, 2012; Zhang and Sun, 2014). Jak stwierdzają Weaver i in. (2012) niewystarczająca ilość wody w glebie może być czynnikiem istotnie ograniczającym jej aktywność enzymatyczną. W doświadczeniu, analizy aktywności enzymatycznej i oddechowej gleby wykonywano w okresie wiosny, lata oraz jesieni. Wykazano zróżnicowanie uzyskanych wyników analiz w zależności od okresu wegetacji. Zarówno aktywność dehydrogenaz jak i proteaz glebowych była najwyższa w okresie jesiennym, a najniższa – wiosną (Publikacja 4, tab. 4). O wysokiej aktywności dehydrogenaz w glebie w okresie jesiennym informują również (Yuan i Yue, 2012; Styla, 2014; Zydlik i in., 2021). Odmienna zależność wystąpiła w przypadku aktywności oddechowej gleby. Średnio w latach 2020 – 2021 była ona najniższa pod koniec wegetacji. Jesienią wilgotność podłoża jest na ogół wysoka, co w połączeniu z optymalną temperaturą, stwarza dogodne warunki do rozwoju mikroorganizmów glebowych. Dostępność wody ma duży wpływ na aktywność enzymów glebowych, ponieważ zwiększona wilgotność umożliwia rozpuszczenie w glebie materii organicznej (Geisseler i in., 2011).

Wykazany w doświadczeniu wzrost aktywności mikroorganizmów, mierzony aktywnością enzymatyczną i oddechową gleby, w kombinacjach z przedplonem trzech gatunków roślin fitosanitarnych, przełożył się na wzrost zawartości w glebie makro i mikroelementów. W kombinacjach z roślinami fitosanitarnymi, w szczególności aksamitką rozpierzchłą (*Tagetes patula* L.) (R3), stwierdzono istotny wzrost zawartości w glebie replantowanej takich pierwiastków jak N, P, K, Zn, Cu, Fe. Skuteczna pod tym względem była również rzodkiew oleista (*Raphanus sativus* var. *oleifera*). W kombinacji

z jej stosowaniem, w porównaniu do gleby replantowanej bez przedplonów (R2), stwierdzono wzrost ilości składników mineralnych od kilkunastu procent (Fe) do około 90% (Zn) (Publikacja 4, tab. 5).

8.3. Parametry biometrycznye drzewek (Puplikacja 4)

Prowadzone w latach 2019-2021 badania wykazały, iż wcześniejszy sposób użytkowania gleby oraz zastosowanie roślin fitosanitarnych w przygotowaniu gleby pod szkółkę drzew owocowych miały wpływ na parametry biometrycne jabłoni takie jak: wzrost drzewek wyrażonych ich wysokością, liczbę pędów bocznych, czy długości pędów bocznych (Publikacja 4, tab. 6). Wzrost drzewek w pierwszym roku produkcji w szkółce skupia się na wzroście wydłużeniowym pędu głównego (przewodnika). Najwyższe drzewka wyrosły na glebie po płodozmianie (R1). Zastosowanie replantacji (R2) osłabiło istotnie wzrost pędu głównego. Zastosowanie roślin fitosanitarnych na glebie po szkółce poprawiło wzrost drzewek jabłoni odmiany `Golden Delicious`. Korzystny wpływ zastosowania roślin fitosanitarnych stwierdzono nie tylko w odniesieniu do wysokości drzewek, ale także w liczbie pędów bocznych, ich średniej długości oraz sumie przyrostów (Publikacja 4, tab. 6). Szczególnie oceniając długość pędów bocznych należy podkreślić korzystny wpływ zastosowania jako przedplonu *Tagetes patula* L.(R3) i *Raphanus sativus var. oleiferus* L.(R5).

Dla optymalnego wzrostu rośliny potrzebują nie tylko zdrowego i dobrze rozwiniętego systemu korzeniowego, ale również liści. Przeprowadzone w szkółce badania wykazały, że zastosowanie replantacji w szkółce istotnie zmniejszyło masę liści oraz ich powierzchnię. Zastosowane roślin fitosanitarnych istotnie zwiększyło masę liści oraz ich powierzchnię. Z pośród wszystkich zastosowanych roślin przedplonowych najbardziej korzystny wpływ na poprawę parametrów określających wzrost liści miała wcześniejsza uprawa i przyoranie *Synapis alba* L. (R4) (Publikacja 4, ryc. 2). Weiß i in. (2017) również zaobserwowali w swoim doświadczeniu słaby wzrost wegetatywny podkładek jabłoni rosnących w warunkach ARD. Sobiczewski i in. (2018) stwierdzili, że powierzchnia liści jabłoni rosnących w warunkach ARD była mniejsza. Jedną z przyczyn słabego wzrostu roślin rosnących w warunkach ARD jest ograniczony wzrost ich systemu korzeniowego. Według Grunewaldt-Stöcker i in. (2018), ARD powoduje nekrozę komórek korzeniowych i hamuje wzrost korzeni włośnikowych co w konsekwencji ogranicza pobieranie wody i składników odżywczych przez rośliny

9. Podsumowanie i wnioski

Wyniki badań prowadzonych w latach 2019-2021 potwierdziły fakt, że gleba replantowana (wcześniej użytkowana pod produkcje szkółkarską) charakteryzowała się niższą wartością produkcyjną. Zawierała ona mniej składników mineralnych niż gleba po płodozmianie i miała gorsze parametry biologiczne - mniejszą różnorodność mikrobiomu, mykobiomu oraz niższą aktywność enzymatyczną i respiracyjną. W takich warunkach jabłonie rosły gorzej. Poprawę właściwości biologicznych gleby replantowanej uzyskano poprzez zastosowanie trzech gatunków roślin fitosanitarnych jako przedplonów - aksamitki rozpierzchłej (*Tagetes patula* L. – R3), gorczycy białej (*Sinapis alba* L.– R4) i rzodkwi oleistej (*Raphanus sativus var. oleiferus* L. – R5). W kombinacjach z ich stosowaniem w glebie replantowanej odnotowano ponad dwukrotny wzrost zawartości próchnicy, a także istotnie wyższą aktywność enzymatyczną i oddechową. Po zabiegu biofumigacji odnotowano również znaczny wzrost liczby bakterii w glebie, zwłaszcza w wariancie z wykorzystaniem aksamitki rozpierzchłej (*Tagetes patula* L. – R3). Potwierdziły to badania wykorzystujące analizę funkcjonalną materiału genetycznego wyizolowanego z gleby (metagenomika) jako narzędzia do oceny bioróżnorodności gleby w szkółce po replantacji. Analizy składu mikrobiomu wykazały, że biofumigacja roślinami fitosanitarnymi – aksamitką rozpierzchłą (*Tagetes patula* L.), gorczycą białą (*Sinapis alba* L.) i rzodkwią oleistą (*Raphanus sativus var. oleifera* L.), zmieniła strukturę i liczbę bakterii w glebie replantowanej, w szkółce drzew owocowych. Rośliny fitosanitarne zwiększyły liczebność operacyjnych jednostek taksonomicznych (OTU) z rodzajów *Proteobacteria, Bacteroidota, Patescibacteria, Chloroflexi, Fatescibacteria* i *Verrucomicrobiota*, ale zmniejszyły liczebność *Firmicutes, Cidobacteriota* i *Actinobacteriota*. Biofumigacja zwiększyła również zawartość niektórych dominujących rodzajów bakterii w glebie replantowanej, takich jak *Flavobacterium, Massila, Sphingomonas, Arenimonas* i *Devosia*. Rodzaje te są uważane za kluczowe w promowaniu wzrostu roślin i indukowaniu odporności ogólnoustrojowej roślin, co może wskazywać na regenerację gleby zdegradowanej. Jak wykazała analiza ITS, zastosowanie jako przedplonu aksamitki rozpierzchłej (*Tagetes patula* L. – R3) przyczyniło się do wzrostu jednostek taksonomicznych dla królestwa grzybów. Co więcej, liczba OTU dla gromady *Ascomycota* w tym wariancie gleby była kilkakrotnie większa i zbliżona do wartości w glebie po płodozmianie (R1). Populacja niesklasyfikowanych grzybów w glebie replantowanej z przedplonem aksamitki

rozpierzchłej (*Tagetes patula* L.) była zredukowana. Grzyby te mogły obejmować niektóre gatunki, które negatywnie wpływały na kondycję drzew w szkółce. Kolejną dominującą grupą w tym wariancie eksperymentalnym była *Mortierellomycota*. Analizy klas i rzędów wykazały, że biofumigacja, głównie z przedplonem nagietka, spowodowała dominację klasy *Eurotiomycetes* i rzędu E*urotiales*. Ta klasa grzybów odgrywa ważną rolę w zwalczaniu chorób grzybowych roślin, a także w glebach ARD. Zastosowanie gorczycy białej (*Sinapis alba* L.) i rzodkwi oleistej (*Raphanus sativus var. oleifera* L. – R5) jako przedplonów nie miało tak silnych analogicznych efektów. Biofumigacja aksamitka rozpierzchłą (*Tagetes patula* L. – R3) i rzodkwią oleistą (*Raphanus sativus var. oleifera* L. – R5) przyczyniła się do zmniejszenia liczebności grzybów z rodzaju *Fusarium,* który obejmuje kilka ważnych gatunków patogenicznych dla roślin. Tłumaczyć to może znaczącą poprawę siły wzrostu drzewek jabłoni rosnących na glebie replantowanej z przedplonem roślin fitosanitarnych. Liście drzew w wariantach z biofumigacją miały większą powierzchnię i masę (wzrost o 50%), niż liście drzew rosnących na glebie replantowanej bez przedplonów. Ponadto drzewa były wyższe i miały większy całkowity wzrost pędów bocznych. Doświadczenie potwierdziło również działanie nicieniobójcze trzech gatunków biofumigantów, zwłaszcza aksamitki rozpierzchłej *Tagetes patula* L. – R3). Zabieg biofumigacji z jego użyciem umożliwił redukcję populacji nicieni z gatunku *Pratylenhus penetrans* z 33 do 0 osobników w 100 cm^{-3} gleby.

Wykorzystanie roślin fitosanitarnych na glebie replantowanej należy uznać za bezpieczniejszą i przyszłościową alternatywę dla odkażania termicznego i fumigacji chemicznej, pozwalającej na poprawę właściwości biologicznych gleby replantowanej, w tym redukowanie liczebności nicieni pasożytujących na roślinach. Pozwoli to na przywrócenie równowagi mikroorganizmów glebowych oraz poprawę siły wzrostu roślin w szkółkach drzew owocowych.

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10. Bibliografia

Andrássy, I. (2005): Free-living Nematodes of Hungary: Nematoda Errantia, Tom 2, Hungarian Natural History Museum, pp. 496, ISSN 1785-1025

Becker, B. (2007): Function and evolution of the vacuolar compartment in green algae and land plants (Viridiplantae). Int. Rev. Cytol., 264, 1–24.

Błońska, E., Lasota, J., Januszek, K. (2012): Aktywność enzymatyczna leśnych gleb gruntowoglejowych. Rocz. Glebozn., 63, 3–8.

Błońska, E., Lasota, J., Zwydak, M. (2017): The relationship between soil properties, enzyme activity and land use, Forest Research Papers, 78, 39-44, available: DOI: 10.1515/frp-2017-0004

Brzeski, M. W. (1998): Nematodes of *Tylenchida* in Poland and temperate Europe. Muzeum i Instytut Zoologii, Polska Akademia Nauk. Warszawa, 396 pp.

Cavael, U., Diehl, K., Lentzsch, P. (2020): Assessment of growth suppression in apple production with replant soils. Ecological Indicators, 109, 105846, available: https://doi.org/ 10.1016/j.ecolind.2019.105846

Cesarano, G., Zotti, M., Antignani, V., Marra, R., Scala, F., Bonanomi, G. (2017): Soil sickness and negative plant-soil feedback: a reappraisal of Hypotheses. J.Plant Pathol., 99(3), 545-570, available: https://doi.org/10.4454/jpp.v99i3.3960

Cocquyt, E., Verbruggen, H., Leliaert, F., Zechman, F.W., Sabbe, K., De Clerck, O. (2009): Gain and loss of elongation factor genes in green algae. BMC Evol. Biol., 9, 39.

Dutta, T.K., Khan, M.R., Phani, V. (2019): Plant-parasitic nematode management via biofumigation using brassica and non-brassica plants: Current status and future prospects. Curr. Plant Biol., 17, 17–32. https://doi.org/10.1016/j.cpb.2019.02.001

Emmett, B., Nelson, E.B., Kessler, A., Bauerle, T.L. (2014): Fine-root system development and susceptibility to pathogen colonization. Planta, 239, 325-340, available: DOI: 10.1007/ s00425-013-1989-7

Fernandes, M.L.P., Bastida, F., Jehmlich, N., Martinovi´c, T., V˘etrovský, T., Baldrian, P., Delgado-Baquerizo, M., Starke, R. (2022): Functional soil mycobiome across ecosystems. J. Proteom., 252, 104428

Fierer, N., Bradford, M.A., Jackson, R.B. (2007): Toward an ecological classification of soil bacteria. Ecology, 88, 1354–1364. https://doi.org/10.1890/05-1839.

Frąc, M., Hannula, E.S., Bełka, M., Salles, J.F., Jedryczka, M. (2022): Soil mycobiome in sustainable agriculture. Front. Microbiol., 13, 1033824.

Franke-Whittle, I. H., Manici, L. M., Insam,, H., Stres, B. (2015): Rhizosphere bacteria and fungi associated with plant growth in soils of three replanted apple orchards. Plan Soil, 395, 317-333, available: https://doi.org/10.1007/s11104-015-2562-x

Furtak, K., Gajda, A.M. (2018): Biochemiczne metody oceny różnorodności funkcjonalnej i strukturalnej mikroorganizmów glebowych. Post. Mikrobiol., 57, 2, 194–202 http://www.pm.microbiology.pl

Gaiero, J.R., Tosi, M., Bent, E., Boitt, G., Khosla, K., Turner, B.L., Richardson, A.E., Condron, L.M., Dunfield, K.E. (2021): Soil microbial communities influencing organic phosphorus mineralization in a coastal dune chronosequence in New Zealand. FEMS Microbiol. Ecol., 97, fiab034.

Gałązka, A., Gawryjołek, K., Perzyński, A., Gałązka, R., Księżak, J. (2017): Changes in Enzymatic Activities and Microbial Communities in Soil under Long-Term Maize Monoculture and Crop Rotation. Pol. J. Environ. Stud., 26, 39–46. https://doi.org/10.15244/pjoes/64745.

Geisseler, D., Horwath, W., Scow, K. (2011): Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. Pedobiologia, 54, 71–78. https://doi.org/10.1016/j.pedob i.2010.10.001.

Gimsing, A. L., Kirkegaard, J.A. (2009): Glucosinolates and biofumigation: Fate of glucosinolates and their hydrolysis products in soil. Phytochem Rev (2009) 8:299–310, DOI: 10.1007/s11101-008-9105-5.

Gołębiowska, J., Pędziwilk, Z. **(**1984): CO_2 release as on index of biological activity of cultivated soils. Acta Microbiol. Polon. 33: 249-256.

Grunewaldt-Stöcker, G., Mahnkopp, F., Popp, C., Maiss, E., Winkelmann, T. (2018): Diagnosis of apple replant disease (ARD): Microscopic evidence of early symptoms in fine roots of different apple rootstock genotypes. Sci. Hortic., 243, 583–594 https://doi.org/10.1016/j.scienta.2018.09.014.

Hamaguchi, T., Sato, K., Vicente, C.S.L., Hasegawa, K. (2019): Nematocidal actions of the marigold exudate -terthienyl: Oxidative stress-inducing compound penetrates nematode hypodermis. Biol. Open, 8, bio038646

Kanfra, X., Liu, B., Beerhues, L., Sørensen, S.J., Heuer, H. (2018): Free-living nematodes together with associated microbes play an essential role in apple replant

disease. Frontiers of Plant Science, 9, 1666, available: DOI: 10.3389/fpls.2018.01666

Ladd, N., Butler, J.H.A. (1972): Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol. Biochem., 4, 19–30.

Leebens-Mack, J.H., Barker, M.S., Carpenter, E.J., Deyholos, M.K., Gitzendanner, M.A., Graham, S.W., Grosse, I., Li Z., Melkonian, M., Mirarab, S. (2019): One Thousand Plant Transcriptomes and the Phylogenomics of Green Plants. Nature, 574, 679–685.

Liang, B., Ma, C., Fan, L., Wang, Y., Yuan, Y. (2018): Soil amendment alters soil physicochemical properties and bacterial community structure of a replanted apple orchard. Microbiol. Res., 216, 1–11. https://doi.org/10.1016/j.micres.2018.07.010.

Liu, E., Wang, G., Li, Y., Shen, X., Chen, X., Song, F., Wu, S., Chen, Q., Mao, Z. (2014): Replanting affects the tree growth and fruit quality of Gala apple, Journal of Integrative Agriculture, 13, 8, available: www.sciencedirect.com

Long, F., Lin, Y.M., Hong, T., Wu, C.Z., Li, J. (2019): Soil sickness in horticulture and forestry: A review. Allelopathy Journal, 47, 57-72, available: DOI: 10.26651/allelo.j/2019-47-1-1219

Lucas, M., Balbín-Suárez, A., Smalla, K., Vetterlein, D. (2018): Root growth, function and rhizosphere microbiome analyses show local rather than systemic effects in apple plant response to replant disease soil. PLoS ONE, 1-21, available: DOI: 10.1371/journal. pone.0204922

Manici, L.M., Kelderer, M., Franke-Whittle, I.H., Rühmer, T., Baab, G., Nicoletti, F., Caputo F., Topp A., Insam H., Naef A. (2013): Relationship between root-endophytic microbial communities and replant disease in specialized apple growing areas in Europe. Applied Soil Ecology 72, 207-214.

Mazzola, M., Manici, L.M. (2012): Apple replant disease: Role of microbial ecology in cause and control. Annual Review of Phytopathology, 50, 45-65, available: DOI: 10.1146/annurevphyto-081211-173005

Mazzola, M., Hewavitharana, S.S., Strauss, S.L. (2015): Brassica seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen reinfestation. Phytopathology, 105, 460–469.

Meena, A., Rao, K.S. (2021): Assessment of soil microbial and enzyme activity in the rhizosphere zone under different land use/cover of a semiarid region India. Ecological Processes, 10, 1-12, available: https://doi.org/10.1186/s13717-021-00288-3

Nicola, L., Insam, H., Pertot, I., Stres, B. (2018): Reanalysis of microbiomes in soils affected by apple replant disease (ARD): Old foes and novel suspects lead to the proposal of extended model of disease development. Applied Soil Ecology, 129, 24-33, available: https://doi.org/ 10.1016/j.apsoil.2018.04.010

Niewiadomska, A., Majchrzak, L., Borowiak, K., Wolna-Maruwka, A., Waraczewska, Z., Budka, A., Gaj, R. (2020): The Influence of Tillage and Cover Cropping on Soil Microbial Parameters and Spring Wheat Physiology. Agronomy, 10, 200. https://doi.org/10.3390/agronomy10020200.

Polme, S., Abarenkov, K., Nilsson, R.H., Lindahl, B.D., Clemmensen, K.E., Kauserud, H., Nguyen, N., Kjøller, R.,, Bates, S.T., Baldrian, P. (2020): Fungal Traits: A user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Divers., 105, 1–16.

Qiao, J., Yu, X., Liang, X., Liu, Y., Borriss, R., Liu, Y. (2017): Addition of plant-growth-promoting Bacillus subtilis PTS-394 on tomato rhizosphere has no durable impact on composition of root microbiome. BMC Microbiol., 17, 131.

Rawat, N., Joshi, G.K. (2019): Bacterial community structure analysis of a hot spring soil by next generation sequencing of ribosomal RNA. Genomics, 111, 1053–1058. https://doi.org/10.1016/j.ygeno.2018.06.008.

Russel, S. (2005): Znaczenie badań enzymów w środowisku glebowym. 5-9. W: Acta Agrophysica. Metody oznaczania aktywności enzymów w glebie pod redakcją Russel S., Wyczółkowski A.I. Instytut Agrofizyki im. Bohdana Dobrzańskiego PAN w Lublinie. ss.74, ISSN 2300-6501.

Sennett, L., Burton, D.L., Goyer, C. Zebarth, B.J. (2021): Influence of chemical fumigation and biofumigation on soil nitrogen cycling processes and nitrifier and denitrifier abundance. Soil Biol. Biochem., 162, 108421.

Singh, S., Singh, B., Singh ,A.P. (2015) ‘Nematodes: A threat to sustainability of agriculture’, Procedia Environmental Science, 29, 215-216, available: https://doi.org/10.1016/j. proenv.2015.07.270

Sobiczewski, P., Treder, W., Bryk, H., Klamkowski, K., Krzewińska, D., Mikiciński, A., Berczyński, S., Tryngiel-Gać, A. (2018): The impact of phytosanitary treatments in the soil with signs of fatigue on the growth of apple seedlings and populations of bacteria and fungi. Polish Journal of Agronomy, 34, 11-22, available: DOI: 10.26114/pja.iung.361.2018. 34.02

Spath, M., Insam, H., Peintner, U., Kelderer, M., Kuhnert-Finkernagel, R., Franke-Whittle, I.H. (2015): Linking soil biotic and abiotic factors to apple replant disease: a greenhouse approach. Journal of Phytophatology., 163, 287-299, available: DOI: 10.1111/jph.12318

Styła, K. (2014): Effect of long-term apple-tree cultivation and cultural practices in the orchard after replantation on the contents of indole-3-acetic acid and chemical compounds, EJPAU 17(3), #05, http://www.ejpau.media.pl/volume17/issue3/art-05.html

Swędrzyńska, D., Małecka-Jankowiak, I. (2017): The Impact of Tillaging Spring Barley on Selected Chemical, Microbiological, and Enzymatic Soil Properties. Pol. J. Environ. Stud., 26, 303–313. https://doi.org/10.15244/pjoes/64911

Thalmann, A. (1968): Zur methodik der bestimmung der dehydrogenase aktivität in boden mittels triphenyltetrazoliumchlorid (TTC). Landwirtsch. Forsch. 21: 249-258.

Tang, Z.; Zhang, L.; He, N.; Gong, D.; Gao, H.; Ma, Z.; Fu, L.; Zhao, M.; Wang, H.; Wang, C.; et al. Soil bacterial community as impacted by addition of rice straw and biochar. Sci. Rep. 2021, 11, 22185. https://doi.org/10.1038/s41598-021-99001-9.

van Schoor, L., Denman, S., Cook, N.C. (2009): Characterisation of apple replant disease under South African conditions and potential biological management strategies. Scientia Horticulturae, 119, 153-162, available: DOI: 10.1016/j.scienta.2008.07.032

Vétrovský, T., Kohout, P., Kopecký, M., Machac, A., Man, M., Bahnmann, B.D., Brabcová V., Choi J., Meszárošová L., Human Z.R. (2019): A meta-analysis of global fungal distribution reveals climate-driven patterns. Nat. Commun., 10, 5142.

Wang, Y., Wei, K., Han, X., Zhao, D., Zheng, Y., Chao, J., Gou, J., Kong, F., Zhang, C.S. (2019): The antifungal effect of garlic essential oil on Phytophthora nicotianae and the inhibitory component involved. Biomolecules, 9, 632.

Weiß, S., Liu,B., Reckwell, D., Beerhues, L., Winkelmann, T. (2017): Impaired defense reactions in apple replant disease-affected roots of Malus domestica ‘M26’. Tree Physiol., 37, 1672–168.. https://doi.org/10.1093/treephys/tpx108.

Weiß, S., Bartsch, M., Winkelmann, T. (2017): Transcriptomic analysis of molecular responses in Malus domestica ‘M26’ roots affected by apple replant disease. Plant Molecular Biology, 94, 303-318, available: https://doi.org/10.1007/s11103-017-0608-6

Weaver, M., Zablotowicz, R., Krutz, L., Bryson, C., Locke, M. (2012): Microbial and Vegetative Changes Associated With Development of a Constructed Wetland. Ecol. Indic., 13, 37–45

Weerakoon, D.M.N., Reardon, C.L., Paulitz, T.C., Izzo, A.D., Mazzola, M. (2012): Long-term suppression of Pythium abappressorium induced by Brassica juncea seed meal amendment is biologically mediated. Soil Biol. Biochem., 51, 44–52.

Winkelmann, T., Smalla, K., Amelung, W., Baab, G., Grunewaldt-Stöcker, G., Kanfra, X., Meyhöfer, R., Reim, S., Schmitz, M., Vetterlein, D. (2019): Apple replant disease: Causes and mitigation strategies. Current Issues Molecular Biology, 30, 89-106, available: https:// dx.doi.org/10.21775/cimb.030.089

Wolińska, A., Kuźniara, A., Zielenkiewicz, U., Banach, A., Błaszczyk, M. (2018): Indicators of arable soils fatigue – bacterial families and genera: a metagenomic approach. Ecological Indicators, 93, 490-500, available: https://doi.org/10.1016/j.ecolind.2018.05.033

Yim, B., Nitt, H., Wrede, A., Jacquiod, S., Sørensen, S.J., Winkelman, T., Smalla, K. (2017): Effects of soil pre-treatment with Basamid®granules, *Brassica juncea*, *Raphanus sativus*, and *Tagetes patula* on bacterial and fungal communities at two apple replant disease sites. Front. Microbiol., 8, 1604, available: DOI: 10.3389/fmicb.2017.01604

Yim, B., Hanschen, F.S., Wrede, A., Nitt, H., Schreiner, M., Smalla, K., Winkelmann, T. (2016): Effects of biofumigation using Brassica juncea and Raphanus sativus in comparison to disinfection using Basamid on apple plant growth and soil microbial communities at three field sites with replant disease. Plant Soil, 406, 389–408. https://doi.org/10.1007/s11104-016-2876-3.

Yin, C., Xiang, L., Wang, G., Wang, Y., Shen, X., Chen, X. (2016): How to Plant Apple Trees to Reduce Replant Disease in Apple Orchard: A Study on the Phenolic Acid

of the Replanted Apple Orchard. PLoS ONE, , 11, e01673470 https://doi.org/10.1371/journal.pone.0167347.

Yin, C.M., Wang, G.S., Li, Y.Y., Chen, X.S., Wu, S.J., Mao, Z.Q. (2014): Assessment of fungal diversity in apple replanted orchard soils by T-RFLP analysis. Acta Ecologica Sinica, 34, 837-846, available: DOI: 10.5846/stxb201306241765

Yuan, B., Yue, D. (2012): Soil Microbial and Enzymatic Activities Across a Chronosequence of Chinese Pine Plantation Development On The Loess Plateau of China. Pedosphere, 22, 1–12.

Zhan, J., Sun, Q. (2014): Development of microbial properties and enzyme activities in copper mine wasteland during natural restoration. Catena, 116, 86–94.

Zhang, D., Yan, D., Cheng, H., Fang, W., Huang, B., Wang, X., Wang, X., Yan, Y. Ouyang C., Li Y. (2020): Effects of multi-year biofumigation on soil bacterial and fungal communities and strawberry yield. Environ. Pollut., 256, 113415.

Zhang, J., Feng, L., Ouyang, Y., Hu, R., Xu, H., Wang, J. (2020): Phosphate-solubilizing bacteria and fungi in relation to phosphorus availability under different land uses for some latosols from Guangdong, China. Catena, 195, 104686

Zhao, L., Wang, G., Liu, X., Chen, X., Shen, X., Yin, C., Mao, Z. (2022): Control of apple replant disease using mixed cropping with Brassica juncea or Allium fistulosum. Agriculture, 12, 68, available: https://doi.org/10.3390/agriculture12010068

Zydlik, Z., Zydlik, P., Wieczorek, R. (2021): The Effects of Bioinoculants Based on Mycorrhizal and *Trichoderma* spp. Fungi in an Apple Tree Nursery under Replantation Conditions. Agronomy, 11, 2355, available: DOI: 10.3390/agronomy11112355

Zydlik, Z., Zydlik, P. (2020): Effect of a preparation containing humic acids on selected physicochemical and biological properties of replanted soil. Journal of Elementology, 25(3), 993-1004, available: DOI: 10.5601/jelem.2020.25.2.2002

Zydlik, Z., Zydlik, P., Jarosz, Z., Wieczorek, R. (2023): The use of Organic Additives For Replanted Soil in Apple Tree Production in a Fruit Tree Nursery. Agriculture, 13, 973, available: https://doi.org/10.3390/agriculture13050973

Zydlik, Z., Zydlik, P., Kleiber, T. (2019): The effect of the mycorrhization on the content of macroelements in the soil and leaves of blueberry cultivated after replantation. Zemdirbyste-Agriculture, 106(4), 345-350, available: DOI: 10.13080/z-a.2019.106.044

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Oświadczam, że mój udział w przygotowaniu pracy polegał na:

Wieczorek R., Zydlik Z., **Wolna-Maruwka A.,** Niewiadomska A., Kajzer D. (2023): The Effect of Biofumigation on the Microbiome Composition in Replanted Soil in a Fruit Tree Nursery. Agronomy 2023, 13, 2507. https://doi.org/ 10.3390/agronomy13102507 (Publikacja 2) współuczestniczeniu w opracowaniu koncepcji i metodyki badań oraz opracowaniu wyników i redagowaniu manuskryptu

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REVIEW PAPER

The use of biofumigation in orchards with apple replant disease – a review

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Abstract

Apple replant disease (ARD) is the consequence of replantation of the same or related species of crops. As a result of the occurrence of ARD, the physicochemical and biological properties of the soil deteriorate. It causes considerable economic losses and may significantly reduce fruit production, especially in regions with high density of orchards. It is difficult to indicate an effective method of preventing the consequences of ARD due to the diversity of causative factors. The development of ARD can be effectively limited by chemical fumigation. However, there are numerous limitations to this method because it is a nuisance to the environment. Not only soil pathogens but also the beneficial microflora may be destroyed, especially by broad-spectrum fumigants. Biofumigation, which involves the use of plants with phytosanitary properties, is an environment-friendly alternative. These plants produce potentially bioactive compounds which, apart from their fungicidal effect, also have nematicidal, insecticidal, antiviral, and cytotoxic properties. This article is a review of the results of research on the effects of biofumigation in orchards with ARD. It shows how the plants used for biofumigation, mainly plants of the Brassicaceae, Asteraceae family, reduced the occurrence of biological causes of ARD (fungi and especially nematodes), and promoted the development of beneficial microorganisms in replanted soil. As a result of biofumigation, both physical and biological properties of the soil are improved, and that leads to improvements of vegetative growth of fruit trees and apple trees in a fruit tree nursery.

Keywords: Apple Replant Disease, biofumigation, *Brassicaceae*, *Tagetes*, soil microbial balance

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APPLE REPLANT DISEASE (ARD) SYNDROME

Apple Replant Disease (ARD) is a disturbed physiological and morphological response of plants to the soil whose microbiome has been changed by previous crops of the same or related species (Winkelmann et al. 2019). In consequence of such disturbances, the physicochemical and biological properties of the soil deteriorate. For a long time, ARD has been the subject of numerous scientific studies, using various nomenclature, often interchangeably, e.g. soil fatigue (Wolińska et al. 2018), soil sickness (Cesarano et al. 2017) or replant disease (Nicola et al. 2018). The most common name is Apple Replant Disease (ARD).

ARD, which is the result of planting orchards in place of grubbed ones, has been mentioned for over 200 years. Symptoms of ARD have been found in plantations of ornamental plants (roses), vegetables (asparagus) – Elmer (2018), and medicinal plants (Wu et al. 2015). However, it is the biggest problem in orchards, especially with apple trees. ARD occurs in all major areas with apple orchards around the world (Mazolla, Manici 2012), especially in intensive orchards, where replanting is frequent due to the aging of trees, market requirements concerning species and cultivars, as well as changes in fruit consumers' preferences.

This article is a review of research on the use of biological methods to improve the physicochemical and biological properties of replanted soil in orchards, with a special focus on biofumigation.

CAUSATIVE FACTORS OF ARD

The causes of ARD have been investigated since the 1980s. Despite numerous publications on this subject, researchers have not assumed a clear position on the main causes of ARD. It is assumed that the disease can be caused by both abiotic and biotic factors. The former include insufficient soil moisture, low content of nutrients, low soil pH, and disturbances in soil structure. According to Spath et al. (2015), the influence of abiotic factors on the occurrence of ARD is relatively small, whereas Sobiczewski et al. (2018) treat abiotic factors as the main causes of ARD.

Most authors of studies on methods for restoring fertility to replanted soil discuss the elimination of biological factors, usually specific species of nematodes, fungi, and bacteria. According to Winkelmann et al. (2019), ARD disturbs the soil microbial balance, limits the development of beneficial microorganisms (Long et al. 2019) and increases the activity of harmful microflora. According to Manici et al. (2013), the change in the species structure of soil microorganisms is the main cause of ARD.

There are about 4,100 species of nematodes parasitising plants around the world. They migrate through the soil in searching of a host plant, invade the roots, and feed on the cytoplasm. Roots damaged in this way are more vulnerable to other soil pathogens. The phytopathogenic nematodes *Pratylenchus* spp. are often mentioned as the main biotic factor of ARD (Mazzola, Manici 2012, Singh et al. 2015, Kanfra et al. 2018). Another group of soil microorganisms mentioned as the biological causes of ARD are fungi of the following genera: *Alternaria* spp., *Rhizoctonia* spp., *Phythium* spp., *Cylindrocarpon* spp., and *Fusarium* spp. (van Schoor et al. 2009, Manici et al. 2013). In the experiment conducted by Cavael et al. (2020), the share of fungi of the *Alternaria* genus in replanted soil amounted to 2% of the total population of soil fungi, which was 10 times more than in agricultural soil. Fungal pathogens attack the root system. According to Yin et al. (2014), *Fusarium proliferatum* is the dominant species in infected roots. Zhao et al. (2022) also found *F. proliferatum* to be an important causative factor of ARD. The role of bacteria in causing ARD has been investigated to a lesser extent than the role of fungi. Researchers usually listed bacteria of the *Bacillus* and *Pseudomonas* genera as the causes of ARD (Mazzola, Manici 2012). However, according to Franke-Whittle et al. (2015), *Pseudomonas* bacteria do not significantly reduce the fertility of replanted soil.

Other causative factors of ARD are phenolic compounds contained in replanted soil. Apple roots contain large amounts of specific polyphenols, such as phlorizin, phloretin, benzoic acid and rutin (Hofmann et al. 2009, Emmet et al. 2014, Yin et al. 2016, Leisso et al. 2017). Phenolic compounds accumulated in the soil in old orchards may strongly inhibit the growth of apple trees.

THE CONSEQUENCES OF ARD IN ORCHARDS

Apple replant disease poses a major challenge for fruit producers. It causes noticeable economic losses and seriously limits the development of fruit production. The profitability of an apple orchard on replanted soils is 50% lower because the yield of fruit is lower and it is harvested later (van Schoor et al. 2009). Fruiting may be delayed by 2-3 years (Mazolla, Manici 2012). The productivity of apple trees, measured with the pppp index, is lower on replanted soils (Cavael 2020). The cross-sectional area of the trunk is commonly used as an indicator of the yield of apple trees in fields.

As mentioned above, ARD deteriorates the physicochemical and biological properties of soil. The enzyme and respiratory activity on replanted soil is reduced (Zydlik et al. 2019, 2020), which indicates lower activity of soil microorganisms responsible for the mineralisation of organic matter. Soil enzymes and soil respiratory activity are reliable indicators of the acti-

vity of soil microflora (Błońska et al. 2017, Meena, Rao 2021). The reduced rate of mineralisation of organic matter in replanted soil results in a smaller amount of nutrients available to plants. This fact was proved in the experiments conducted by Zydlik et al. (2020, 2021*a*). A smaller amount of available nutrients in replanted soil causes weaker vegetative growth of fruit trees. This effect can be observed in apple rootstocks (Weiß et al. 2017, Zydlik et al. 2023) and apple trees (Manici 2013, Liu et al. 2014, Zhao et al. 2022) – Photo 1.

trees on replanted soil (third replantation)

trees on non-replanted soil

Photo 1. An eight-year-old apple trees of the Topaz cultivar (photo by Z. Zydlik)

The weaker growth of trees is manifested by smaller growth and a smaller assimilation area of leaves (Emmett et al. 2014, Yim et al. 2016, Sobiczewski et al. 2018). The weaker vegetative growth of apple trees growing on soil with ARD may be affected not only by a lower amount of available nutrients, but also by limited possibilities of their uptake by the root system. Lukas et al. (2018) concluded that apple rootstocks affected by ARD absorb nitrogen in the form of nitrate much worse than rootstocks growing in optimal conditions.

The yield of apples in an orchard affected by ARD is significantly reduced and their quality is worse (Zydlik et al. 2021*b*). For example, apples may be about 10% smaller (Nikola et al. 2018). The appearance and taste of apples are also worse (Lucas et al. 2018).

The roots are an important organ responsible for the uptake, storage,

and transport of minerals and water to the aerial parts of plants. ARD inhibits these processes because it damages the roots and weakens their function (Photo 2).

agricultural soil | replanted soil | replanted soil with *Trihoderma*

Photo 2. The growth of the root system of apple trees on soil with ARD compared to the other variants (photo by Z. Zydlik)

The roots become discoloured and form necrotic tips. The number of root hairs decreases and their growth is limited. These effects can be observed in both fruit trees and nursery material. As early as two weeks after being in the soil with ARD, necrosis appeared on M26 rootstocks and the growth of root hairs weakened (Grunewaldt-Stöcker et al. 2019). The root system usually becomes damaged by the microbiome causing ARD, e.g. nematodes or products of their metabolism in the immediate vicinity of the roots (Lucas et al. 2018).

METHODS OF ALLEVIATING THE CONSEQUENCES OF ARD

It is difficult to indicate an effective method of preventing or alleviating the consequences of ARD due to the diversity of causative factors and the interrelations between them. According to Berg et al. (2017), the effect of ARD causative factors largely depends on the cultivation history, environmental conditions (type of soil, weather), and the physiological state of plants. Moreover, the quality and quantity of causative factors may change during one growing season. The most effective solution is to avoid replanting the same species of crops in the same place. However, in practice, this is difficult

or impossible due to the limited number of suitable places for new plantations in orchards and nurseries. All actions aimed at the eradication of ARD symptoms should be targeted at the improvement of the physicochemical properties of the replanted soil and restoration of the species composition of its microflora. According to Deldago-Baqueriro et al. (2016), it is the high diversity of soil microorganisms that determines its multifunctionality and provides protection against pathogens. The physicochemical and biological properties of replanted soil can be improved with agricultural, chemical or biological methods.

Agricultural methods

Proper agricultural practice includes crop rotation and organic fertilisation with manure or compost (Forge et al. 2016, Franke-Whittle et al. 2018). Crop rotation is not often used in perennial orchard plantations. Organic additives, such as biochar, can also be added to the replanted soil. When organic carbon was added to the replanted soil in an apple tree nursery, the enzyme activity of the soil more than doubled. The rate of photosynthesis in the leaves also increased significantly, which improved the vegetative growth of the apple trees (Zydlik et al. 2023) – Photo 3.

agricultural soil | replanted soil | replanted soil with organic carbon

Photo 3. The influence of the soil types on the growth of apple trees (photo by Z. Zydlik)

The productivity of replanted soil can also be improved by adding humic acids, which are components of the soil humus. The spraying with humic acids decreases the salinity of replanted soil, increases the activity of enzymes

(dehydrogenases and proteases) and respiratory activity (Zydlik et al. 2020). Humic acids used in an apple orchard with ARD increase the yield of trees and improve the quality of fruit (Zydlik et al. 2021*b*).

Agricultural treatments may improve the physicochemical properties of replanted soil, stimulate the development of microflora, as a result of which the soil reaches a state of microbial balance. However, such procedures may be time-consuming or not always sufficient to alleviate the consequences of ARD.

Chosen chemical substances

Chemicals may also limit the development of pathogenic microbiome in replanted soil very effectively, especially before planting crops. They can be applied into replanted soil by fumigation or sterilisation of the soil. Until recently, methyl bromide was the most common chemical fumigant used to combat soil pathogens (Zhang et al. 2019). However, it was withdrawn because it destroyed stratospheric ozone. Current soil fumigation chemicals mainly include dazomet or metam sodium (both releasing methyl isothiocyanate) and 1,3-dichloropropene/chloropicrin (Nicola et al. 2017, Nyoni et al. 2019). Such broad-spectrum fumigants are used in many countries to combat soil-borne diseases (Li et al. 2017, 2021).

The results of numerous studies have confirmed the effectiveness of chemicals in reducing the effects of ARD. According to Spatch et al. (2015) and Yim et al. (2016), this may indicate that ARD is primarily caused by biotic factors, especially by nematodes. Chemical fumigation of replanted soil limits the development of pathogenic soil microorganisms, e.g. *Fusarium* spp. (Jiang et al. 2022), and plants are affected by the consequences of ARD to a lesser extent (Cheng et al. 2020). When chloropicrin was applied into soil, the populations of *Fusarium* spp. (Li et al. 2017*b*) and *Phytophtora* spp. (Li et al. 2021) were significantly reduced, which significantly increased the strawberry yield.

The disadvantage of using chemical soil fumigants is their high toxicity. Not only soil pathogens but also the beneficial microflora may be destroyed, especially by broad-spectrum fumigants (Li et al. 2017, Fang et al. 2018). Li et al. (2021) observed that chemical fumigation reduced the population of proteobacteria (responsible for the proper binding and accumulation of nitrogen) and *Acidobacteria*, which are considered a reliable indicator of the degree of plant nutrition. As chemical fumigation is a nuisance to the environment, researchers are searching for non-chemical methods for combating phytopathogenic soil microflora.

Biological methods

In horticultural practice, the effects of ARD can be alleviated biologically by applying biopreparations containing various groups of beneficial micro-

organisms into soil, which are antagonistic to pathogens. These may be arbuscular mycorrhizal fungi competing with phytopathogenic bacteria or *Trichoderma* spp. Zydlik et al. (2021*a*) observed that the *Trichoderma harzianum* species increased the enzyme and respiratory activities of the soil. Noteworhty is the fact that it is difficult to control soil fungi due to their extensive mycelia and numerous spores.

Plants used for biofumigation

The term *biofumigation* introduced in 1993 is defined as a process of decomposition of plant or animal tissues, leading to the production of volatile biocidal compounds. Biofumigation consists in using phytosanitary plants, which are antagonistic to pathogenic microorganisms in soils with ARD and support the development of beneficial soil microflora. For example, these are plants of the *Brassicaceae* family, with about 350 genera and about 4,000 species. Species of the *Brassica*, *Raphanus*, and *Sinapis* genera are the most common plants of the *Brassicaceae* family used for biofumigation (Hanschen, Winkelmann 2020, Morris et al. 2020). In general, these are usually plants eaten by humans and animals and those used for the production of edible and industrial oils. The most common phytosanitary plants used for biofumigation are: red mustard (*Brassica juncea*), white mustard (*Sinapis alba*), field mustard (*Brassica rapa*), rape (*Brassica napus*), arugula (*Eruca sativa*), and radish (*Raphanus sativus*) – Neubauer et al. (2014), Rios et al. (2016), Ntali and Caboni (2017).

Plants of the *Brassicaceae* family produce secondary metabolites, glucosinolates, which after hydrolysis produce bioactive isothiocyanates: aliphatic allyl isothiocyanate, aromatic isothiocyanates, 2-phenylethyl isothiocyanate, and benzyl isothiocyanate (Gimsing and Kirkegaard 2009). These plants produce over 200 types of glucosinolates, which are present in all parts of the plant. The amounts of these compounds vary considerably depending on the cultivation phase, species, cultivars, and growing season. The highest concentration of glucosinolates was found in the tissues of plants of the *Brassicaceae* family in the summer (Ngala et al. 2015). According to Doheny-Adams et al. (2018), the highest concentration of glucosinolates in the tissues of mustard can be observed at the intensive flowering phase, but later their amount gradually decreases as the plant develops. Other compounds formed during the decomposition of *Brassicaceae* biomass can also be used for biofumigation. These are volatile compounds containing sulphur, e.g. carbon disulphide, dimethyl sulphide, and dimethyl disulphide (Wang et al. 2009).

Plants from the *Asteraceae* family (*Asteraceae* Dum) contain various fungicidal components which kill fungi with short reproductive cycles, producing large numbers of spores and easily developing resistance (Perera et al. 2019). These plants produce potentially bioactive compounds which, apart from their fungicidal effect, also have nematicidal, insecticidal, antiviral, and

cytotoxic properties (Karakas et al. 2019). The *Asteraceae* kill nematodes with metabolites released from the roots of mature plants, e.g. thiophene compounds such as α-terthienyl (Hamaguchi et al. 2019). The greatest amount of such compounds is produced by the roots of intensively growing marigolds (*Tagetes* L). The amount of thiophene compounds depends on the species. The highest content was found in *T. tenuifolia*, followed by *T. patula* and *T. erecta* (Marotti et al. 2010).

Allium L. is another genus of bioactive plants which can limit the development of soil diseases and improve plant growth (Arnault et al. 2013) thanks to the substances secreted by the roots, including propyl disulphide and methyl disulphide (Ngala et al. 2015).

The radish (*Raphanus sativus*), also classified as a cruciferous plant, is less frequently used for biofumigation because it has high requirements concerning soil fertility. However, *Raphanus sativus* may combat soil nematodes more effectively than white mustard (*Sinapis alba*).

The methods of using plants of the *Brassicaceae* family may vary depending on the species, the organism being controlled and the soil cultivation method. The most common methods are growing as preceding crop, ploughing green manure, adding fresh or dried plant residues (e.g. meal) to the soil. They are recommended in fruit production before fruit trees or berry bushes are planted. Fresh biomass is particularly recommended because it has high content of glucosinolates. The plant material should be thoroughly crushed and then applied into the soil at a depth of 15-20 cm (Kumar et al. 2018).

As results from reference publications, there have been various studies on using plants from the *Brassicaceae* family to reduce the occurrence of pathogens causing ARD. Experiments have been conducted on tomatoes, cucumbers, peppers, lettuce, onions, napa cabbage, potatoes, sugar beets, wheat, maize, gourds, grasses, pine, and ginger. As regards fruit cultivation, such experiments are usually conducted in apple orchards, fruit tree nurseries, and less often on strawberry plantations.

Effects of biofumigation

Mustard is often used in experiments with fruit plants to limit the occurrence of phytopathogens (fungi and nematodes) in soil. The nematicidal effect is one of the best-investigated effects of biofumigation (Dutta et al. 2019). Hollister et al. (2012) observed that mustard meal effectively inhibited the development of such fungal pathogens as *Bacillus*, *Pseudomonas*, and *Streptomyces*. Mustard seed powder limited the infection of the roots of apple trees growing in replanted soil by *Pythium* spp. (Weerakoon et al. 2012). Barrau et al. (2009) used Ethiopian mustard (*Brassica carinata*) for biofumigation and observed that it reduced the occurrence of the *Phytophthora cactorum* pathogen in a strawberry plantation, which resulted in a higher yield of fruits. Mazzola et al. (2009) and Weerakoon et al. (2012) found that *Brassicaceae* seed meal reduced damage to apple rootstocks by *Pythium*.

It also reduced the population of the phytopathogenic nematode *Pratylenchus penetrans* in the soil. The researchers observed that *B. juncea* was more effective than *B. napus* or *Sinapis alba*.

The marigold (*Tagetes* L.) is an annual herbaceous plant of the *Asteraceae* genus, which has antifungal, bacteriostatic, and insecticidal properties (Padalia, Chanda 2015). Thus far, about 40 species of marigolds have been identified, but nematodes in soil are most effectively destroyed by the French marigold (*Tagetes patula* L.). *Tagetes* plants are the most effective when they are applied before planting fruit trees. A marigold preceding crop significantly increased the vegetative growth of apple trees in an orchard on soil with ARD (Yim et al. 2016, 2017). Du et al. (2017) conducted an experiment with *T. erecta* and observed its fungicidal effect on *F. oxysporum*, which is one of the main causes of ARD. Wang et al. (2022) applied *Tagetes erecta* to replanted soil and observed a decrease in the population of *Fusarium oxysporum* – the fungus which is one of main causative factors of ARD. The number of parasitic nematodes damaging plant roots also decreased significantly. However, Kanfra et al. (2021) observed that *T. patula* was more effective than *T. tenuifolia*.

Phytosanitary plants used for biofumigation not only reduce the populations of pathogenic microorganisms responsible for the development of ARD, but they also introduce significant amounts of organic material into soil through the production of large amounts of biomass. This improves the soil structure, increases the amount of nutrients and stimulates the development of beneficial microflora. These may be bacteria from the *Pseudomonas* genus, which are antagonistic to pathogenic fungi in soil (Behera et al. 2014), *Actinobacteria*, which stimulate plant growth, or *Bacillus* (Sobiczewski et al. 2018), which are considered the main biological factor protecting plants from soil-borne diseases. When mustard seed meal was applied to replanted soil and when white mustard and winter wheat were grown as preceding crops before the orchard was established, the content of *Trichoderma* fungi in the soil increased significantly (Sobiczewski et al. 2018). These fungi play a significant role in the decomposition of organic matter and in the reduction of many soil pathogens.

Biofumigation reduces the population of pathogenic microorganisms in replanted soil and thus improves the vegetative growth of fruit trees. This fact was observed by various scientists, including Yim et al. (2017) on marigold and Krzewińska et al. (2008) on mustard. Kanfra et al. (2021) used *Tagetes* for the biofumigation of replanted soil in a fruit tree nursery. As a result, the diameters of the trunks of apple trees grafted on M26 rootstocks were several dozen per cent larger than those in the control variant. The biofumigation of replanted soil with the dry powder of *Tagetes erecta* increased the intensity of photosynthesis in the leaves of apple trees and the respiration rate of their roots, which improved the vegetative growth of the trees (Wang et al. 2022). The activity of root antioxidative enzymes,

which protect plant cells from free radicals, also increased. In consequence, the plants' resistance to pathogens increased.

Good results can also be achieved by combining different methods of using plants for biofumigation. When mustard seed meal was applied to the soil and white mustard and winter wheat were grown as preceding crops before establishing an orchard, the height of apple trees, the area of their leaves and the intensity of photosynthesis increased (Sobiczewski et al. 2018).

Mixtures of plants used for biofumigation also very effectively limit the development of pathogenic microbiome. The mixed cultivation of *Allium fistulosum* and *Brassica juncea* limited the development of *Fusarium proliferatum* for a long time due to the continuous release of bioactive compounds and improved the growth of apple trees growing on soil with ARD (Zhao et al. 2022). The combined use of *B. juncea* and *S. alba* effectively reduced the population of *Pratylenchus penetrans* in the replanted soil, which improved the growth and yield of apple trees (Mazzola et al. 2015, Wang et al. 2019). Yim et al. (2016) also observed better growth of apple trees after using *B. juncea* with *R. sativus* on replanted soil.

SUMMARY

The biofumigation of replanted soil is a promising method of mitigating the consequences of ARD in fruit plantations. This fact has been proved by the results of numerous studies, which showed that this method effectively limited the development and reduced the populations of biological causative factors of replantation disease – mainly fungi and nematodes. Biofumigation enables the restoration of microbiological balance in replanted soil, which improves the health, growth, and yield of fruit trees and berry plants growing on soil with ARD.

Further research is necessary due to the variety of causative factors of ARD, the large number of plant species with phytosanitary properties, and the influence of external factors on the effectiveness of biofumigation. New plant species with the potential to combat pathogenic microorganisms in orchards should be sought. It is also necessary to pay greater attention to the timing of biofumigation, selection of the right amounts of phytosanitary plants and optimal methods of their application in soil, and find the most effective mixtures of these plants. New experiments should be conducted not only on apple trees but also on other species of fruit plants that are sensitive to ARD.

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R.W. – writing – original draft preparation, funding acquisition, data curation; P.Z. – writing – original draft preparation, supervision, writing – review and editing.

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REFERENCES

Arnault, I., Fleurance, C., Vey, F., Fretay, G.D., Auger J. (2013) 'Use of Alliaceae residues to control soil-borne pathogens,' *Industrial Crops and Production*, 49, 265-272, available: https://doi.org/10.1016/j.indcrop.2013.05.007

Barrau, C., Porras, M., Romero, E., Zurera, K., Ramos, N., Soares, C., Neto, E., Marreiros, A., Entrudo, J., Romero, F. (2009) '*Brassica carinata* for control of *Phytophthora* spp. in strawberry field crops', *Revista de Ciências Agrárias*, 32(2), 135-138.

Behera, B.C., Patra, M., Dutta, S.K., Thatoi, H.N. (2014) 'Isolation and characterisation of sulphur oxidising bacteria from mangrove soil of Mahanadi river delta and their sulphur oxidising ability', *Journal of Applied and Environmental Microbiology*, 2,1-5, available: DOI: 10.12691/jaem-2-1-1

Berg, G., Köberl, M., Rybakova, D., Müller, H., Grosch, R., Smalla, K. (2017) 'Plant microbial diversity is suggested as the key to future biocontrol and health trends', *Fems Microbiology Ecology*, 93, available: https://doi.org/10.1093/femsec/fix050

Błońska, E., Lasota, J., Zwydak, M. (2017) 'The relationship between soil properties, enzyme activity and land use', *Forest Research Papers*, 78, 39-44, available: DOI: 10.1515/frp-2017-0004

Cavael, U., Diehl, K., Lentzsch, P. (2020) 'Assessment of growth suppression in apple production with replant soils', *Ecological Indicators*, 109, 105846, available: https://doi.org/10.1016/j.ecolind.2019.105846

Cesarano, G., Zotti, M., Antignani, V., Marra, R., Scala, F., Bonanomi, G. (2017) 'Soil sickness and negative plant-soil feedback: a reappraisal of Hypotheses', *Journal of Plant Pathology*, 99(3), 545-570, available: https://doi.org/10.4454/jpp.v99i3.3960

Cheng, H., Zhang, D., Huang, B., Song, Z., Ren, L., Hao, B., Liu, J., Zhu, J., Fang, W., Yan, D., Li, Y., Wang, Q., Cao, A. (2020) 'Organic fertilizer improves soil fertility and restores the bacterial community after 1,3-dichloropropene fumigation', *Science of the Total Environment*, 738, 140345, available: DOI: 10.1016/j.scitotenv.2020.140345

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B. (2016) 'Microbial diversity drives multifunctionality in terrestrial ecosystems', *Nature Communications*, 7, 1-8, available: https://doi.org/10.1038/ncomms10541

Doheny-Adams, T., Lilley, C.J., Barker, A. (2018) 'Constant isothiocyanaterelease potentials across biofumigant seeding rates', *Journal of Food and Agricultural Food Chemistry*, 66, 5108-16, available: DOI: 10.1021/acs.jafc.7b04610

Du, R., Liu, J., Sun, P., Li, H., Wang, J. (2017) 'Inhibitory effect and mechanism of *Tagetes erecta* L. fungicide on *Fusarium oxysporum* f. sp. *niveum*', *Scientific Reports*, 7, 14442, available: https://doi.org/10.1038/s41598-017-14937-1

Dutta, T.K., Khan, M.R., Phani, V. (2019) 'Plant-parasitic nematode management via biofumigation using brassica and non-brassica plants: Current status and future prospects', *Current Plant Biology*, 17, 17-32, available: https://doi.org/10.1016/j.cpb.2019.02.001

Elmer, W. (2018) 'Asparagus decline and replant problem: A look back and a look forward at strategies for mitigating losses', *Acta Horticulturae*, 1223, 195-204, available: https://doi.org/10.17660/ActaHortic.2018.1223.27

Emmett, B., Nelson, E.B., Kessler, A., Bauerle, T.L. (2014) 'Fine-root system development and susceptibility to pathogen colonization', *Planta*, 239, 325-340, available: DOI: 10.1007/s00425-013-1989-7

Fang, W., Yan, D., Wang, X. (2018) 'Responses of nitrogen-cycling microorganisms to dazomet fumigation', *Front Microbiology*, 9, 1-13, available: https://doi.org/10.3389/fmicb.2018.02529

Forge, T., Neilsen, G., Neilsen, D. (2016) 'Organically acceptable practices to improve replant success of temperate tree-fruit crops', *Scientia Horticulturae*, 200, 205-214, available: https://doi.org/10.1016/j.scienta.2016.01.002

Franke-Whittle, I. H., Manici, L. M., Insam, H., Stres, B. (2015) 'Rhizosphere bacteria and fungi associated with plant growth in soils of three replanted apple orchards', *Plan Soil*, 395, 317-333, available: https://doi.org/10.1007/s11104-015-2562-x

Franke-Whittle, I.H., Juárez, M.F.-D., Insam, H., Schweizer, S., Naef, A., Topp, A.-R., Kelderer, M., Rühmer, T., Baab, G., Henfrey, J. (2018) 'Performance evaluation of locally available composts to reduce replant disease in apple orchards of central Europe', *Renewable Agriculture and Food Systems*, 34, 543-557, available: https://doi.org/10.1017/S1742170518000091

Gimsing, A.L., Kirkegaard, J.A. (2009) 'Glucosinolates and biofumigation: fate of glucosinolates and their hydrolysis products in soil', *Phytochemistry Reviews*, 8, 299-310, available: https://doi.org/10.1007/s11101-008-9105-5

Grunewaldt-Stöcker, G., Mahnkopp, F., Popp, C., Maiss, E., Winkelmann, T. (2019) 'Diagnosis of apple replant disease (ARD): Microscopic evidence of early symptoms in fine roots of different apple rootstock genotypes', *Scientia Horticulturae*, 243, 583-594, available: https://doi.org/10.1016/j.scienta.2018.09.014

Hamaguchi, T., Sato, K., Vicente, C.S.L., Hasegawa, K. (2019) 'Nematicidal actions of the marigold exudate α-terthienyl: Oxidative stress-inducing compound penetrates nematode hypodermis', Biology Open, 8,bio038646, available: DOI: 10.1242/bio.038646

Hanschen, F.S., Winkelmann, T. (2020) 'Biofumigation for fighting replant disease – a review', *Agronomy*,10, 3, available: https://doi.org/10.3390/agronomy10030425

Hofmann, A., Wittenmayer, L., Arnold, G., Schieber, A., Merbach, W. (2009) 'Root exudation of phloridzin by apple seedlings *(Malus X domestica* Borkh.*)* with symptoms of apple replant disease', *Journal of Appled Botany and Food Quality*, 82.

Hollister, E.B., Hu, P., Wang, A.S., Hons, F.M., Gentry, T.J. (2012) 'Differential impacts of brassicaceous and nonbrassicaceous oilseed meals on soil bacterial and fungal communities', *Fems Microbiology Ecology*, 83, 632-641, available: DOI: 10.1111/1574-6941.12020

Jiang, W., Chen, R., Zhao, L., Qin, L., Mao, Z. (2022) 'Chemical fumigants control apple replant disease: Microbial community structure-mediated inhibition of *Fusarium* and degradation of phenolic acids', *Journal of Hazardous Materials*, 440, available: https://doi.org/10.1016/j.jhazmat.2022.129786

Kanfra, X., Liu, B., Beerhues, L., Sørensen, S.J., Heuer, H. (2018) 'Free-living nematodes together with associated microbes play an essential role in apple replant disease', *Frontiers of Plant Science*, 9, 1666, available: DOI: 10.3389/fpls.2018.01666

Kanfra, X., Obawolu, T., Wrede, A., Strolka, B., Winkelmann, T., Hardeweg, B., Heuer, H. (2021) 'Alleviation of nematode-mediated apple replant disease by pre- cultivation of tagetes', *Horticulturae*, 7, 433, available: https://doi.org/10.3390/horticulturae7110433

Karakas, M. and Bolukbasi, E. (2019) 'A review: Using marigolds (*Tagetes* spp.) as an alternative to chemical nematicides for nematode management', *International Journal of Advanced Engineering, Management and Science*, 5, 556-560, available:DOI: 10.22161/ijaems.59.3

Krzewińska, D., Treder, W., Klamkowski, K., Tryngiel-Cać, A., Sobiczewski, P. (2008) 'Ocena

wzrostu siewek jabłoni 'Antonówka' uprawianych w glebie „zmęczonej" i po zabiegach poprawiających jej właściwości', *Zeszyty Naukowe Instytytutu Sadownictwa i Kwiaciarstwa*, 16, 51-60 (in Polish)

Kumar, G.N.K., Jayasudha, S.M., Kirankumar, K.C. (2018). 'Disease management by biofumigation in organic farming system', *Journal of Pharmacognosy and Phytochemistry*, 7(4), 676-679, available: www.phytojournal.comLeisso

Li, J., Huang, B., Wang, Q., Li Y., Fang, W., Han, D., Yan, D., Guo, M., Cao, A. (2017*a*) 'Effects of fumigation with metam-sodium on soil microbial biomass, respiration, nitrogen transformation, bacterial community diversity and genes encoding key enzymes involved in nitrogen cycling', *Science of the Total Environment*, 598(15), 1027-1036, available: DOI: 10.1016/j.scitotenv.2017.02.058

Li, Q., Zhang, D., Cheng, H., Song, Z., Ren, L., Hao, B., Zhu, J., Fang, W., Yan, D., Li Y., Wang, Q., Cao, A. (2021) 'Chloropicrin alternated with dazomet improved the soil's physicochemical properties, changed microbial communities and increased strawberry yield', *Ecotoxicology And Environmental Safety*, 220, 112362, available: https://doi.org/10.1016/j.ecoenv.2021.112362

Li, J., Huang, B., Wang, Q., Li, Y., Fang, W., Yan, D., Guo, M., Cao, A. (2017*b*) 'Effect of fumigation with chloropicrin on soil bacterial communities and genes encoding key enzymes involved in nitrogen cycling', *Environmental Pollution*, 227, 534-542, available: DOI: 10.1016/j.envpol.2017.03.076

Liu, E., Wang, G., Li, Y., Shen, X., Chen, X., Song, F., Wu, S., Chen, Q., Mao, Z. (2014) 'Replanting affects the tree growth and fruit quality of Gala apple', *Journal of Integrative Agriculture*, 13, 8, available: www.sciencedirect.com

Long, F., Lin, Y.M., Hong, T., Wu, C.Z., Li, J. (2019) 'Soil sickness in horticulture and forestry: A review', *Allelopathy Journal*, 47, 57-72, available: DOI: 10.26651/allelo.j/2019-47-1-1219

Lucas, M., Balbín-Suárez, A., Smalla, K., Vetterlein, D. (2018) 'Root growth, function and rhizosphere microbiome analyses show local rather than systemic effects in apple plant response to replant disease soil', *PLoS ONE*, 1-21, available: DOI: 10.1371/journal.pone.0204922

Manici, L.M., Kelderer, M., Franke-Whittle, I.H., Rühmer, T., Baab, G., Nicoletti, F., Caputo, F., Topp, A., Insam, H., Naef, A. (2013) 'Relationship between root-endophytic microbial communities and replant disease in specialized apple growing areas in Europe', Applied Soil Ecology 72, 207-214.

Marotti, I., Marotti, M., Piccaglia, R., Nastri, A., Grandi, S., Dinelli, G. (2010) 'Thiophene occurrence in different tagetes species: Agricultural biomasses as sources of biocidal substances', *Journal of Food Science and Agriculture*, 90, 1210-1217, available: DOI: 10.1002/jsfa.3950

Mazzola, M., Brown, J., Zhao, X., Izzo, A.D., Fazio, G. (2009) 'Interaction of brassicaceous seed meal and apple rootstock on recovery of *Pythium* spp. and *Pratylenchus penetrans* from roots grown in replant soils', *Plant Disease*, 93, 51-57, available: https://doi.org/10.1094/PDIS-93-1-0051

Mazzola, M., Hewavitharana, S.S., Strauss, S.L. (2015) 'Brassica seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen reinfestation', *Phytopathology*, 105, 460-469, available: DOI: 10.1094/PHYTO-09-14-0247-R

Mazzola, M., Manici L.M. (2012) 'Apple replant disease: Role of microbial ecology in cause and control', *Annual Review of Phytopathology*, 50, 45-65, available: DOI: 10.1146/annurev-phyto-081211-173005

Meena, A. and Rao, K.S. (2021) 'Assessment of soil microbial and enzyme activity in the rhizosphere zone under different land use/cover of a semiarid region India', *Ecological Processes*, 10, 1-12, available: https://doi.org/10.1186/s13717-021-00288-3

Morris, E.K., Fletcher, R., Veresoglou, S.D. (2020) 'Effective methods of biofumigation: a meta-analysis', *Plant and Soil*, 446, 379-392, available: https://doi.org/10.1007/s11104-019-04352-y

Neubauer, C., Heitmann, B., Müller, C. (2014) 'Biofumigation potential of Brassicaceae cultivars to *Verticillium dahlia*', *European Journal of Plant Pathology*, 140, 341-352, available: https://doi.org/10.1007/s10658-014-0467-9

Ngala, B.M., Wood, S.R., Back, M.A. (2015) 'Sinigrin degradation and G. *pallida* suppression in soil cultivated with brassicas under controlled environmental conditions', Applied Soil Ecology, 95, 9-14, available: https://doi.org/10.1016/j.apsoil.2015.05.009

Nicola, L., Insam, H., Pertot, I., Stres, B. (2018) 'Reanalysis of microbiomes in soils affected by apple replant disease (ARD): Old foes and novel suspects lead to the proposal of extended model of disease development', *Applied Soil Ecology*, 129, 24-33, available: https://doi.org/10.1016/j.apsoil.2018.04.010

Nicola, L., Turco, E., Albanese, D., Donati, C., Thalheimer, M., Pindo, M., Insam, H., Cavalieri, D., Pertot, I. (2017) 'Fumigation with dazomet modifies soil microbiota in apple orchards afected by replant disease', Applied Soil Ecology, 113, 71-79, available: https://doi.org/10.1016/j.apsoil.2017.02.002

Ntalli, N., Caboni, P. (2017) 'A review of isothiocyanates biofumigation activity on plant parasitic nematodes', *Phytochemistry Reviews*, 16, 827-834, available: https://doi.org/10.1007/s11101-017-9491-7

Nyoni, M., Mazzola, M., Wessels, J.P.B., McLeod, A. (2019) 'The efficacy of semiselective chemicals and chloropicrin/1,3-dichloropropene – containing fumigants in managing apple replant disease in South Africa', *Plant Disease*, 103, 1363-1373, available: DOI: 10.1094/PDIS-10-18-1844-RE

Padalia, H., Chanda, S. (2015). 'Antimicrobial efficacy of different solvent extracts of *Tagetes erecta* L. flower, alone and in combination with antibiotics', *Applied Microbiology*, 1, 106, available: DOI: 10.4172/2471-9315.1000106

Perera, W.H., Meepa, K.M., Fronczek, F.R., Cook, D.D., Wedge, D.E., Duke, S.O. (2019) 'Bioassay-Guided isolation and structure elucidation of fungicidal and herbicidal compounds from *Ambrosia salsola* (Asteraceae)', *Molecules*, 24, 835, available: DOI: 10.3390/molecules 24050835

Ríos, P., Obregón, S., González, M., de Haro, A., Sánche, M.E. (2016) 'Screening brassicaceous plants as biofumigants for management of *Phytophthora cinnamomi* oak disease', *Forest Pathology*, 46, 652-659, available: DOI: 10.1111/efp.12287

Singh, S., Singh, B., Singh, A.P. (2015) 'Nematodes: A threat to sustainability of agriculture', *Procedia Environmental Science*, 29, 215-216, available: https://doi.org/10.1016/j.proenv.2015.07.270

Sobiczewski, P., Treder, W., Bryk, H., Klamkowski, K.,Krzewińska, D., Mikiciński, A., Berczyński, S., Tryngiel-Gać, A. (2018) 'The impact of phytosanitary treatments in the soil with signs of fatigue on the growth of apple seedlings and populations of bacteria and fungi', *Polish Journal of Agronomy*, 34, 11-22, available: DOI: 10.26114/pja.iung.361.2018.34.02

Spath, M., Insam, H., Peintner, U., Kelderer, M., Kuhnert-Finkernagel, R., Franke-Whittle, I.H. (2015) 'Linking soil biotic and abiotic factors to apple replant disease: a greenhouse approach', *Journal of Phytophatology*., 163, 287-299, available: DOI: 10.1111/jph.12318

van Schoor, L., Denman, S., Cook, N.C. (2009) 'Characterisation of apple replant disease under South African conditions and potential biological management strategies', *Scientia Horticulturae*, 119, 153-162, available: DOI: 10.1016/j.scienta.2008.07.032

Wang, D., Rosen, C., Kinkel, L., Cao, A., Tharayil, N., Gerik, J. (2009) 'Production of methyl sulfide and dimethyl disulfide from soil-incorporated plant materials and implications for controlling soilborne pathogens', *Plant and Soil*, 324, 185-197, available: https://doi.org/10.1007/s11104-009-9943-y

Wang, X., Li, K., Xu, S., Duan, Y., Wang, H., Yin, C., Chen, X., Mao, Z., Xiang, K. (2022) 'Effects of different forms of *tagetes erecta* biofumigation on the growth of apple seedlings and replanted soil microbial environment', *Horticulturae*, 8, 633, available: https://doi.org/10.3390/horticulturae8070633

Wang, Y., Wei, K., Han, X., Zhao, D., Zheng, Y., Chao, J., Gou, J., Kong, F., Zhang, C.S. (2019) 'The antifungal effect of garlic essential oil on *Phytophthora nicotianae* and the inhibitory component involved', *Biomolecules*, 9, 632, available: DOI: 10.3390/biom9100632

Weerakoon, D.M.N., Reardon, C.L., Paulitz, T.C., Izzo, A.D., Mazzola, M. (2012) 'Long-term suppression of *Pythium abappressorium* induced by *Brassica juncea* seed meal amendment is biologically mediated', *Soil Biology Biochemistry*, 51, 44-52, available: DOI: 10.1016/j.soilbio.2012.03.027

Weiß, S., Bartsch, M., Winkelmann, T. (2017) 'Transcriptomic analysis of molecular responses in *Malus domestica* 'M26' roots affected by apple replant disease', *Plant Molecular Biology*, 94, 303-318, available: https://doi.org/10.1007/s11103-017-0608-6

Winkelmann, T., Smalla, K., Amelung, W., Baab, G., Grunewaldt-Stöcker, G., Kanfra, X., Meyhöfer, R., Reim, S., Schmitz, M., Vetterlein, D. (2019) 'Apple replant disease: Causes and mitigation strategies', *Current Issues Molecular Biology*, 30, 89-106, available: https://dx.doi.org/10.21775/cimb.030.089

Wolińska, A., Kuźniara, A., Zielenkiewicz, U., Banach, A., Błaszczyk, M. (2018) 'Indicators of arable soils fatigue – bacterial families and genera: a metagenomic approach', *Ecological Indicators*, 93, 490-500, available: https://doi.org/10.1016/j.ecolind.2018.05.033

Wu, L., Wang, J., Huang, W., Wu, H., Chen, J., Yang, Y., Zhang, Z., Lin, W. (2015) 'Plant-microbe rhizosphere interactions mediated by *Rehmannia glutinosa* root exudates under consecutive monoculture', *Scientific Reports*, 5, 15871, available: https://doi.org/10.1038/srep15871

Yim, B, Smalla, K, Winkelmann, T. (2013) 'Evaluation of apple replant problems based on different soil disinfection treatments links to soil microbial community structure', *Plant and Soil*, 366, 617-631, available: https://doi.org/10.1007/s11104-012-1454-6

Yim, B., Hanschen, F.S., Wrede, A., Nitt, H., Schreiner, M., Smalla, K., Winkelmann, T. (2016) 'Effects of biofumigation using *Brassica juncea* and *Raphanus sativus* in comparison to disinfection using Basamid on apple plant growth and soil microbial communities at three field sites with replant disease', *Plant and Soil*, 406, 389-408, available: https://doi.org/10.1007/s11104-016-2876-3

Yim, B., Nitt, H., Wrede, A., Jacquiod, S., Sørensen, S.J., Winkelmann, T., Smalla, K. (2017) 'Effects of soil pre-treatment with Basamid®granules, *Brassica juncea*, *Raphanus sativus*, and *Tagetes patula* on bacterial and fungal communities at two apple replant disease sites', *Frontiers in Microbiology*, 8, 1604, available: DOI: 10.3389/fmicb.2017.01604

Yin, C., Xiang, L., Wang, G., Wang, Y., Shen, X., Chen, X. (2016) 'How to plant apple trees to reduce replant disease in apple orchard: A study on the phenolic acid of the replanted apple orchard', *PLoS ONE*, 11, e0167347, available: DOI: 10.1371/journal.pone.0167347

Yin, C.M., Wang, G.S., Li Y.Y., Chen, X.S., Wu, S.J., Mao, Z.Q. (2014) 'Assessment of fungal diversity in apple replanted orchard soils by T-RFLP analysis', *Acta Ecologica Sinica*, 34, 837-846, available: DOI: 10.5846/stxb201306241765

Zhang, D., Yan, D., Fang, W., Huang, B., Wang, X., Wang, X., Zhu, J., Liu, J., Ouyang, C., Li Y., Wang, Q., Cao A., (2019) 'Chloropicrin alternated with biofumigation increases crop yield and modifies soil bacterial and fungal communities in strawberry production', *The Science of the Total Environment*, 675, 615-622, available: DOI: 10.1016/j.scitotenv.2019.04.222

Zhao, L., Wang, G., Liu, X., Chen, X., Shen, X., Yin, C., Mao, Z. (2022) 'Control of apple replant disease using mixed cropping with *Brassica juncea* or *Allium fistulosum*', *Agriculture*, 12, 68, available: https://doi.org/10.3390/agriculture12010068

Zydlik, Z., Zydlik, P. (2020) 'Effect of a preparation containing humic acids on selected physico-chemical and biological properties of replanted soil', *Journal of Elementology*, 25(3), 993-1004, available: DOI: 10.5601/jelem.2020.25.2.2002

Zydlik, Z., Zydlik, P., Kayzer, D. (2021*b*) 'The influence of a soil activator containing humic acids on the yield and quality of apples in conditions of replantation` *Journal of Elementoly*, 26(2), 333-347, available: DOI: 10.5601/jelem.2020.25.4.2091

Zydlik, Z., Zydlik, P., Kleiber, T. (2019) 'The effect of the mycorrhization on the content of macroelements in the soil and leaves of blueberry cultivated after replantation', *Zemdirbyste-Agriculture*, 106(4), 345-350, available: DOI: 10.13080/z-a.2019.106.044

Zydlik, Z., Zydlik, P., Jarosz, Z., Wieczorek, R. (2023) 'The use of organic additives for replanted soil in apple tree production in a fruit tree nursery', *Agriculture*, 13, 973, available: https://doi.org/10.3390/agriculture13050973

Zydlik Z., Zydlik, P., Wieczorek, R. (2021*a*) 'The effects of bioinoculants based on mycorrhizal and trichoderma spp. fungi in an apple tree nursery under replantation conditions', *Agronomy*, 11, 2355, available: DOI: 10.3390/agronomy11112355

Publikacja 2

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Article

The Effect of Biofumigation on the Microbiome Composition in Replanted Soil in a Fruit Tree Nursery

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Abstract: The imbalance of the soil microbiome is a primary indicator of ARD (apple replant disease). Biofumigation is a treatment that enables the restoration of microbiome balance. This study involved an analysis of the taxonomic and functional diversity of bacterial communities in replanted soil (ARD), in replanted soils with forecrops of French marigold (*Tagetes patula* L.), white mustard (*Sinapis alba*), and oilseed radish (*Raphanus sativus* var. *oleifera*), and in agricultural soil. The biofumigation treatment with phytosanitary plants changed the structure and abundance of the replanted soil microbiome in a fruit tree nursery. The count of operational taxonomic units (OTU) of the *Proteobacteria*, *Bacteroidota*, *Patescibacteria*, *Chloroflexi*, and *Verrucomicrobiota* phyla increased, whereas the count of the *Firmicutes*, *Acidobacteriota*, and *Actinobacteriota* phyla decreased. Biofumigation caused an increase in the content of some dominant bacterial genera, such as *Flavobacterium*, *Massilia*, *Sphingomonas*, *Arenimonas*, and *Devosia*, in the replanted soil. Their presence in the soil may improve the growth of plants, induce their systemic resistance, and thus improve the production properties of soil with ARD. The research results led to the conclusion that the use of phytosanitary plants in nursery production can be an effective alternative to the chemical fumigation of soil.

Keywords: phytosanitary plants; ARD; bacterial genera

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1. Introduction

The cultivation of fruit trees is a very specific and demanding procedure because it is a long-term monoculture. This problem also concerns nurseries producing fruit trees. As early as the beginning of the 20th century, researchers found it important to establish nurseries on the soil where such crops had not been grown before. Currently, due to the high specialisation of farms and the lack of new areas, nursery production needs to be done in the same places. This may lead to apple replant disease (ARD). This problem usually occurs in orchards with apple trees [1,2], especially those grown on dwarf rootstocks [3], peach trees [4,5], and cherry trees [6,7]. ARD is increasingly common in plantations with roses [8,9], vines [10,11], asparagus [12], medicinal plants such as Rehmannia glutinosa [13], and some forest tree species [14]. The high incidence of ARD in apple orchards results from the fact that the apple tree is one of the most common orchard species in the world. Due to the intensive fruit production and the emergence of new, more attractive varieties fruit growers increasingly often have to replace their plantings with new ones. Apple replant disease (ARD) has been investigated by many scientists from all over the world [15–23]. Research results have shown that when a new orchard is established in place of an old one, trees usually grow worse and the development of small hair roots is

impaired, which may result in the death of the roots. In consequence, the growth of the aerial part is strongly reduced, whereas the fruits from these orchards are characterised by low quality [24,25].

ARD is often described as a detrimentally disturbed physiological and morphological response of apple plants to soils that have experienced microbiome changes due to previous apple crops [1] or as a soil microbiome dysbiosis [26–28]. Biotic factors are considered to be the main causative agents of this disease. These are fungi (*Fusarium*, *Rhizoctonia*, *Phytium*, *Phytophthora* spp., and others), bacteria (the *Pseudomonas* and *Bacillus* genera and the *Actinobacteria* phylum) [2], as well as nematodes. According to Manici et al. [29], ARD may primarily be caused by an imbalance in the structure of the soil microbiota and the accumulation of harmful microorganisms. According to Zhao et al. [30], the intensity of ARD in apple orchards is associated with increased soil acidification and the resulting lack of available minerals. Due to the large diversity of causative factors and the complexity of their interactions, it is difficult to effectively reduce the negative effects of ARD [31–33].

ARD can be prevented by thermal decontamination within a temperature range of 50–100 °C, which may strongly reduce the total soil microbiota [34], or by gamma radiation [35]. Another option is chemical fumigation, i.e., disinfecting the soil with chemicals. It is considered an effective method, but it is expensive and harmful to the environment. The chemicals used for this type of soil fumigation are toxic. Currently, these are mainly dazomet or sodium methane (both release methyl isothiocyanate) as well as 1,3-dichloropropene/chloropicrin [36–39].

Due to the non-selective action of chemicals and the deposition of their residues in the soil environment, the abundance of microbiota is reduced, and the time of soil regeneration is usually extended. Therefore, researchers increasingly often talk about the need to reduce the amount of chemical crop protection products used in horticultural production, including nursery production. Anaerobic soil disinfection (ASD) is an alternative to the chemical decontamination of soils with ARD. The method consists of applying a rapidly biodegradable material (organic carbon) into the soil and covering the soil tightly with a transparent film. As a result, soil microorganisms that decompose organic matter consume oxygen completely. Such anaerobic conditions are not lethal for some organisms. However, it is important to note that as a result of the decomposition of organic material, free volatile fatty acids are released, which are toxic to many species of soil organisms, including facultative anaerobes. The ASD method proved to be effective in nurseries with apple trees and cherry trees [40,41]. Another strategy for fighting ARD is to change the biodiversity of the soil environment by introducing composts [42,43].

Biofumigation is a promising method of reducing the negative effects of replantation. It consists of using appropriate forecrops, especially phytosanitary plants, which may reduce the populations of harmful nematodes, bacteria, and pathogenic fungi in the soil. Phytosanitary plants include marigold (*Tagetes patula* L.), white mustard (*Sinapis alba*), oil radish (*Raphanus sativus* var. *oleifera*), spring rape (*Brassica napus*), oats (*Avena sativa*), rye (*Secale cereale* L.), and asparagus (*Asparagus officinalis*). Biofumigation is a process that leads to the production of volatile biocidal compounds. Plants of the Brassicaceae family (*Sinapis alba*, *Raphanus sativus*) produce secondary metabolites—glucosinolates—after hydrolysis, of which biologically active compounds are formed: isothiocyanates—aliphatic allyl isothiocyanate, aromatic isothiocyanates, 2-phenylethyl isothiocyanate, and benzyl isothiocyanate [44]. The use of fresh biomass is recommended, as this form is particularly rich in glucosinolates. Plants in the *Asteraceae* Dum. family (primarily *Tagetes* L.) produce compounds that exhibit, among other things, nematicidal and insecticidal effects, which are the result of metabolites released from the roots of mature plants. These include thiophene compounds such as α- tertienyl [45]. When using phytosanitary plants, it is important to remember that the plant material should be thoroughly crushed and then applied into the soil at a depth of 15–20 cm [46]. Phytosanitary plants produce specific

compounds that are released into the soil environment through the roots or through biomass decomposition and thus may cause changes in the soil microbiome [47–49].

It was assumed that the biofumigation process based on the use of selected phytosanitary plants would contribute to reducing the abundance of *Firmicutes* bacteria, which include, among others, bacteria of the genus *Bacillus* and *Clostridium*, producing persistent forms in unfavourable environmental conditions for growth, the abundance of the *Actinobacteriota* type indicating soil dryness, and the *Acidobacteriota* type indicating soil acidification.

The aim of the study was to understand the structure of bacterial communities in soil with ARD and to assess the direction of changes in the microbiome in replanted soil under the influence of phytosanitary plants—marigold (*Tagetes patula* L.), white mustard (*Sinapis alba*), and oil radish (*Raphanus sativus* var. *Oleifera*)—in a fruit tree nursery (apple tree).

2. Materials and Methods

2.1. Experiment Design

The experiment was conducted between 2019 and 2021 on stagnic luvisol (according to WRB) in a production nursery in Puszczykowo Zaborze, Poland (52°25′49.10″ N 17°11′34.08″ E). Soil from two different sites was used in the experiment. The soil from the first site had been used in agricultural production. It was optimally prepared for the cultivation of apple trees in a nursery (hereinafter referred to as agricultural soil). The soil from the other site had been used for growing apple trees for three years. It had ARD symptoms (hereinafter referred to as replanted soil). Three different phytosanitary plants were used in the experiment: *Tagetes patula* L., *Sinapis alba*, and *Raphanus sativus* var. *oleifer*. There were five variants of the experiment: R1—agricultural soil (control variant); R2—replanted soil; R3—replanted soil, with a French marigold forecrop (*Tagetes patula* L.); R4—replanted soil, with a white mustard forecrop (*Sinapis alba*); R5—replanted soil, with an oil radish forecrop (*Raphanus sativus* var. *oleifera*).

All phytosanitary plants were sown into the soil in the autumn after the apple trees had been dug out. In early spring (March), they were crushed and mixed with the soil. In early May, the soil with the crushed phytosanitary plants was put into containers with a capacity of 7.5 l, and the apple tree strains were planted there. Golden Delicious apple trees on M.9 rootstock obtained from winter grafting were used in the experiment. There were 30 containers in each variant of the experiment.

Before starting the experiment, the physicochemical properties of the soil from both sites were analysed. The analysis showed significant differences in the content of mineral components, humus, and soil pH. The replanted soil had a higher specific weight and significantly lower humus content (Table 1). The content of minerals P, K, Ca, and Mg in the replanted soil was lower than in the agricultural soil. The analysis showed that the replanted soil was characterised by low fertility, and the results indicated the possible occurrence of ARD.

Table 1. The chemical analysis of the soil before starting the experiment (R1—agricultural soil; R2—replanted soil).

Properties of the Soil	R1	R2
pH (H_2O)	7.6	5.8
Bulk density (g dm^{-3})	1600	1830
Salinity (g Na Cl dm^{-3})	0.23	0.23
Humus content (%)	4.88	1.70
Mineral content (mg dm^{-3}): $N\text{-}NO_3$	<3.9	<3.9
P	127	30
K	229	89
Ca	1333	240
Mg	188	38
Cl	<21.3	<21.3

The climatic conditions were characterised on the basis of data from a weather station located 6 km away from the research site. Between 2018 and 2021, the average annual temperature was much higher than the average temperature spanning a long-term period. The amount of rainfall was also much lower (Figure 1).

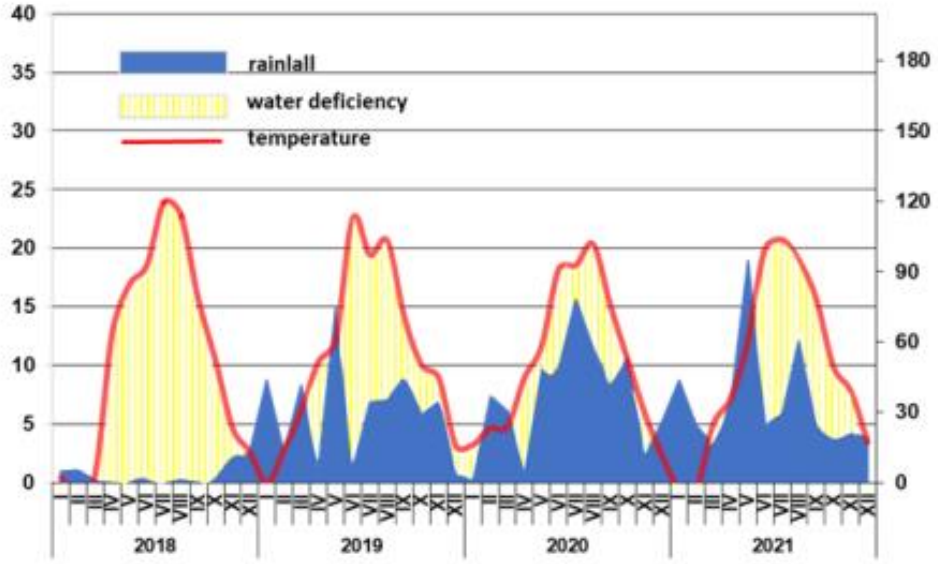


Figure 1. The course of temperature and rainfall between 2018 and 2021.

The analysis of the total rainfall and the average air temperature in individual months showed that in each growing season, there were dry periods, so irrigation was necessary to ensure optimal plant growth. The greatest water shortage occurred in the growing seasons of 2018 and 2019.

2.2. Soil Analyses

The composition of the soil microbiome was analysed in samples collected in September of each year of the research period. A soil sample weighing 30 g was collected with a laboratory spatula from each container in the variant. They were mixed, and an aggregate sample with a total weight of 900 g was obtained.

2.2.1. Identification of Soil Microorganisms—DNA Extraction

Total DNA was extracted from 500 mg of each sample with a Genomic Mini AX Soil kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions. The extracted DNA was quantified with a Quant-iT HS dsDNA assay kit (Invitrogen Carlsbad, CA, USA) on a Qubit2 fluorometer, and 2 µL of extracts were examined on a 0.8% agarose gel.

2.2.2. PCR Amplification

The metagenomic analysis was based on the hypervariable region V3–V4 of the 16S rRNA gene. Specific primers (341F and 785R) were used for the amplification of this region and to prepare libraries. A PCR was conducted with a Q5 Hot Start High-Fidelity DNA Polymerase kit (NEB Inc., Ipswich, MA, USA). The reaction conditions were maintained according to the manufacturer's specifications. An Illumina MiSeq PE300 sequencer (Genomed S.A., Warsaw, Poland) in 2 × 250 bp paired-end (PE) technology with a v2 Illumina chemistry kit was used for sequencing. The reactions were conducted according to the

Illumina V3–V4 16S RNA amplification protocol (Illumina, San Diego, CA, USA). The data were analysed automatically with the MiSeq and in the Illumina BaseSpace cloud environment according to the 16S Metagenomics protocol (ver. 1.0.1). The libraries were prepared in an analogous way to the attached Illumina protocol.

2.3. Statistical and Bioinformatics Analyses

The data were subjected to a conventional analysis of variance with the STATISTICA® 10 software (StatSoft, Krakow, Poland). Venn diagrams were used to present the similarities and differences in the genus composition of experimental variants, representing the relative abundance of bacteria according to the type of forecrops used. Differences in the mean abundance of bacteria between the soils, in which, before establishing the apple tree nursery, the forecrops of French marigold (*Tagetes patula* L.), white mustard (*Sinapis alba*), and oil radish (*Raphanus sativus* var. *oleifera*) had been used, and the soil after agricultural crops were calculated and visualised. The datasets were also subjected to principal component analysis (PCA), which showed the relationships between the experimental variants and the relative abundance of the phylum composition of bacteria to the type of forecrops used [50].

3. Results and Discussion

The obtained results of research on the bacterial microbiome of replanted soils subjected to biofumigation with selected phytosanitary plants confirmed the thesis of reducing the population of bacteria indicating poor soil condition (*Firmicutes*, *Actinobacteriota*, and *Acidobacteriota*) in favour of increasing the population indicating its revitalisation (*Proteobacteriota*, *Bacteroidota*, *Patescibacteria*, and *Chloroflexi*).

3.1. Bacterial Phyla

The metapopulation analysis based on the analysis of the 16S rRNA sequence showed that the previous use of the soil in the nursery, the phytosanitary plants used in the experiment, and the years of research influenced the number of operational taxonomic units of bacteria in the soil (Table 2). Next-generation sequencing is an increasingly popular and extremely sensitive method of determining similarities and differences within the soil microbiome. This fact was confirmed by the results of our research (Table 2, Figures 2–4) and the data provided in reference publications [51–53]. Depending on the soil site and the year of the research, there were 20–34 bacterial phyla and 378–554 genera identified (Table 2). Due to the large number of operational taxonomic units (OTUs), only those with an average share of more than 1% were shown in Figures 2–4.

Table 2. Number of bacterial taxonomic units according to experimental combinations (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

Taxonomic Units	R1	R2	R3	R4	R5
2019 year					
Phylum	20	-	22	23	23
Class	44	-	48	50	47
Order	87	-	96	93	96
Family	180	-	201	197	205
Genus	378	-	441	407	470
Species	456	-	591	532	704
2020 year					
Phylum	30	32	31	32	31
Class	84	89	89	90	85
Order	190	201	216	224	207

Family	280	294	320	338	317
Genus	457	499	554	510	513
Species	832	906	1007	935	940
			2021 year		
Phylum	32	30	34	33	30
Class	80	76	79	85	79
Order	180	170	195	200	189
Family	272	255	302	301	280
Genus	480	458	552	553	505
Species	838	774	953	942	988

Throughout the study period, the following bacterial phyla were dominant in the soil: Proteobacteria (the relative abundance ranged from 33.23% to 60.08%), *Firmicutes*, *Actinobacteriota*, *Acidobacteriota*, *Chloroflexi*, and *Verrucomicrobiota* (Figures 2–4). Depending on the year of the soil metagenomic analyses, the following phyla were also dominant: *Bacteroidetes*, *Planctomycetes*, *Tenericutes*, *Spirochaetes*, *Chlamydiae*, *Cyanobacteria*, *Gemmatimonadota*, *Bacteroidota*, and *Fatescibacteria* (Figures 2–4). Mahnkopp-Dirks et al. [54] found that *Proteobacteria* were the dominant phylum in both the ARD soil and unaffected soil (up to 83.7% of the OTU content).

Fierer et al. [55] proposed the concept of bacterial classification, in which *Proteobacteria* were described as fast-growing copiotrophs, i.e., microorganisms developing in environments with high carbon availability, whose abundance is closely correlated with the degree of carbon mineralisation in the soil. Our experiment showed that, regardless of the year of the study, the most intensive proliferation of Proteobacteria was observed in the soil in variant R3, slightly weaker—in the replanted soil with the white mustard forecrop (*Sinapis alba*) (R4), and then in variant R5 (Figures 2–4). The lowest percentage of the OTU content was found in the replanted soil (R2) in 2020. A year earlier, despite several attempts to isolate the bacterial DNA, it was impossible to obtain research material due to the degree of soil sterilisation.

In 2019, regardless of the experimental variant, *Firmicutes* bacteria were the most dominant phylum (Figure 2). In the following years of the research, the count of *Actinobacteriota* increased and was greater than the counts of other bacterial phyla (Figures 3 and 4). The intensive growth and development of *Actinobacteriota* were particularly noticeable in variant R2. According to Swędrzyńska and Małecka-Jankowiak [56] as well as Niewiadomska et al. [57], *Actinobacteriota* are a saprophytic group of actinobacteria that quickly adapt to unfavourable environmental conditions, such as desiccation. Therefore, they actively decompose organic matter when the soil moisture is low.

Figures 2–4 show relative differences between the dominant types of bacteria in the control variant (R1) and the other experimental variants, expressed as a percentage of sequence. The analysis of the research results showed that regardless of the year of the study, the OTU content of the Proteobacteria phylum in all experimental variants was lower than in the control variant (R1). However, it is necessary to stress the fact that the difference in the content of OTUs of the *Proteobacteria* phylum in the soils subjected to induced biofumigation with the phytosanitary plants—*Tagetes patula* L. (R3), *Sinapis alba* (R4), and Raphanus sativus var. *oleifera* (R5)—in relation to the agricultural soil was significantly lower than the difference observed in the soil with ARD (Figures 5–7). Apart from that, in 2020, the application of French marigold (R3-R1) caused a significant increase in the content in OTUs of the *Proteobacteria* phylum (Figure 6). Thus, it can be concluded that the application of phytosanitary plants to the soil with ARD causes an increase in the content of OTUs belonging to the *Proteobacteria* phylum. This conclusion is similar to the findings of the authors of studies on ARD soil recultivation by adding compost [58,59] or by exposure to gamma radiation [53].

A similar trend was also observed for the *Firmicutes* phylum. The metagenomic analysis of the soil conducted between 2019 and 2021 showed that the sequence content of seven bacterial phyla in the soil samples was lower than in the control variant. However, these differences were not observed in all experimental variants (Figures 5–7). They were ranked as follows according to the frequency of their occurrence in the soil: *Bacteroidota* > *Acidobacteriota* > *Actinobacteriota* = *Gemmatimonadota* > *Patescibacteria*.

The *Verrucomicrobiota* and *Chloroflexi* bacterial phyla, and especially *Planctomycetota* and *Cyanobacteria*, were isolated less often from the agricultural soil (R1), especially when compared with the replanted soil subjected to fumigation. The *Chloroflexi* phylum encompasses nitrifying bacteria developing in anaerobic or microaerophilic conditions. They can survive in intensively changing, extreme conditions. The incidence of the *Chloroflexi* phylum in soils with ARD after biofumigation was higher than in the control soil. This phenomenon can be explained by the fact that the development of the community of these bacteria is based on the use of cellular compounds from dead microorganisms and their metabolites, which is typical of soils with ARD [60]. A similar dominance of this group of microorganisms was observed by Tang et al. [61], who used rice straw and biochar to effectively improve soil quality. According to the information provided in reference publications, *Cyanobacteria* is an important phylum indicating the reconstruction of soils. They are credited with an important role in fixing atmospheric nitrogen and the synthesis of exopolysaccharides, which increase soil fertility and water retention and improve its structure and stability [62]. In our study, fumigation induced by the use of French marigold (R3) increased the content of OTUs in the *Cyanobacteria* phylum by 83% as compared with the soil with ARD and the agricultural soil.

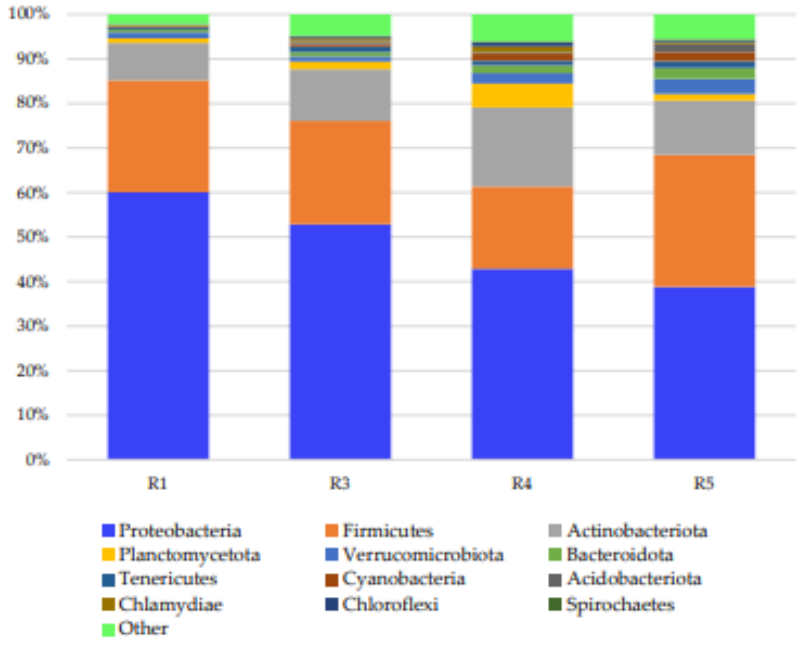


Figure 2. Relative abundance of dominant phyla of bacteria in 2019. The classifications with less than 1% abundance are gathered into the category "Other" (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

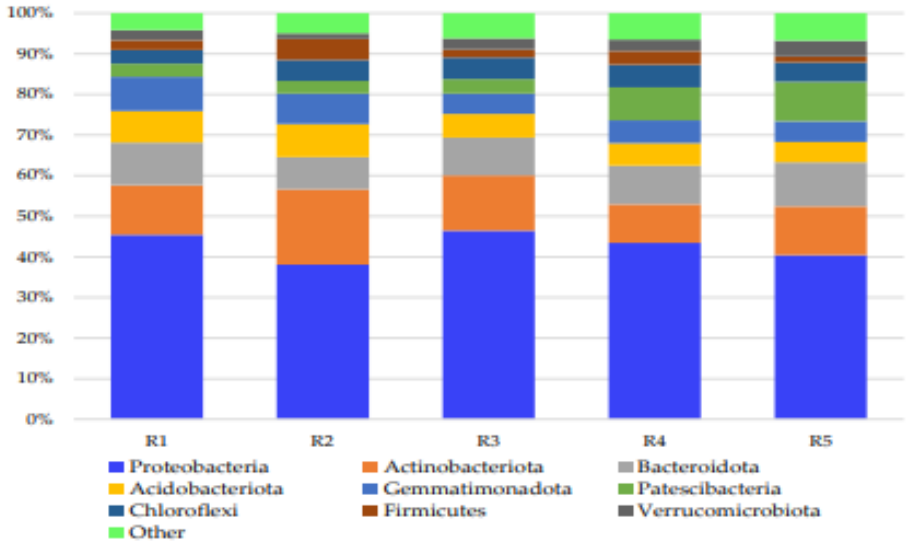


Figure 3. Relative abundance of dominant phyla of bacteria in 2020. The classifications with less than 1% abundance are gathered into the category "other" (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

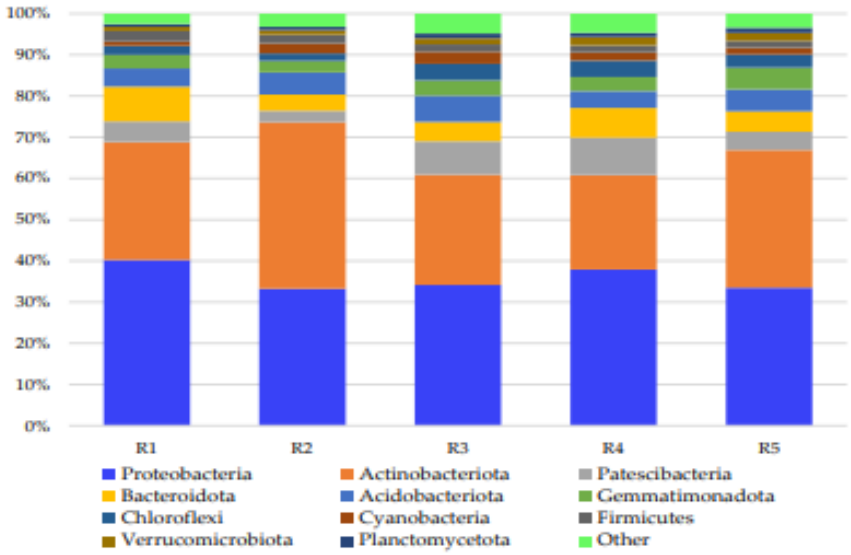


Figure 4. Relative abundance of dominant phyla of bacteria in 2021. The classifications with less than 1% abundance are gathered into the category "other" (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

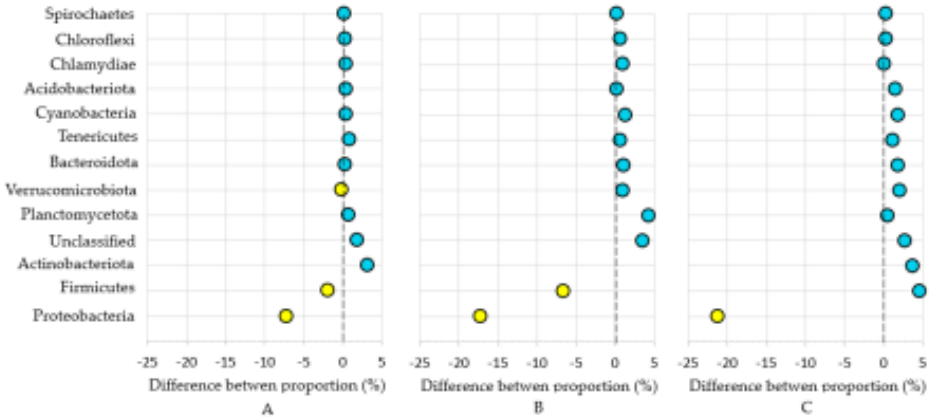


Figure 5. Relative abundance of dominant classes of bacteria phyla in 2019 (**A**—R3 via R1; **B**—R4 via R1; **C**—R5 via R1). The classifications with less than 1% abundance are gathered into the category "other" (R1—agricultural soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut; "yellow" means negative difference; "blue" means positive difference)

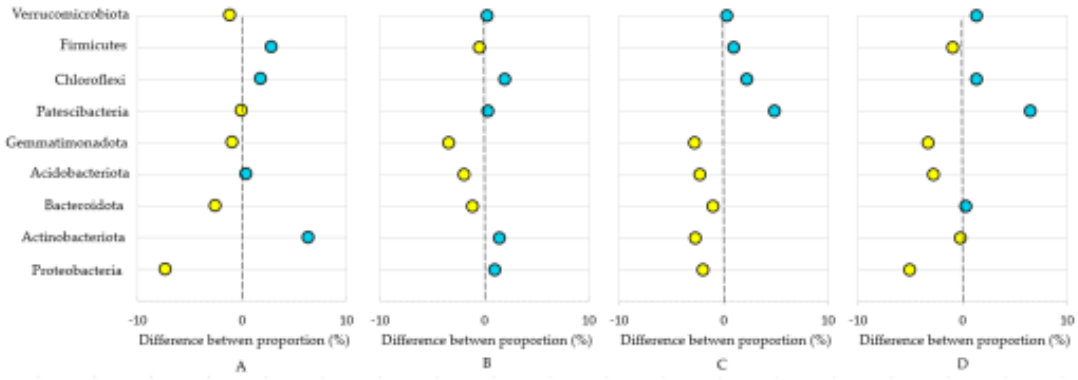


Figure 6. Relative abundance of dominant classes of bacteria phyla in 2020 (**A**—R2 via R1; **B**—R3 via R1; **C**—R4 via R1; **D**—R5 via R1). The classifications with less than 1% abundance are gathered into the category "other" (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut; "yellow" means negative difference; "blue" means positive difference).

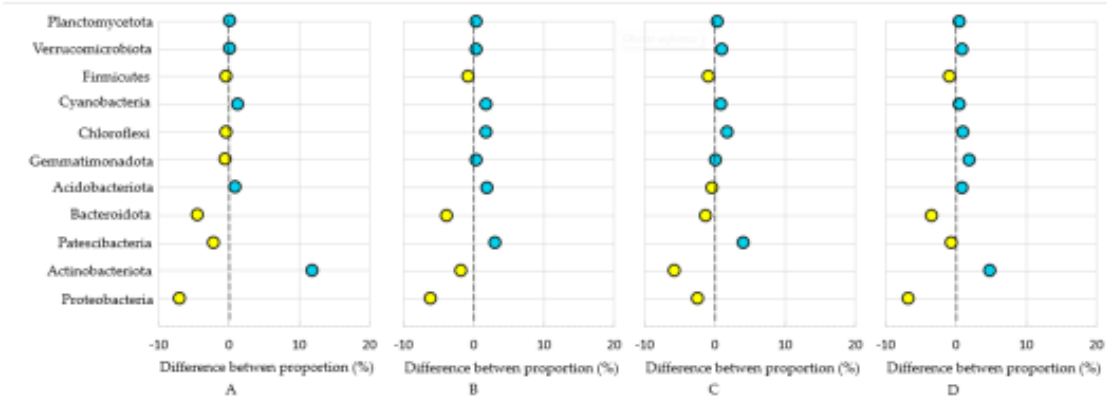
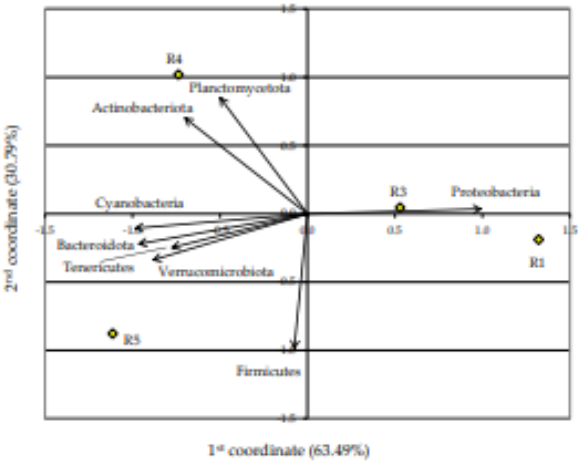


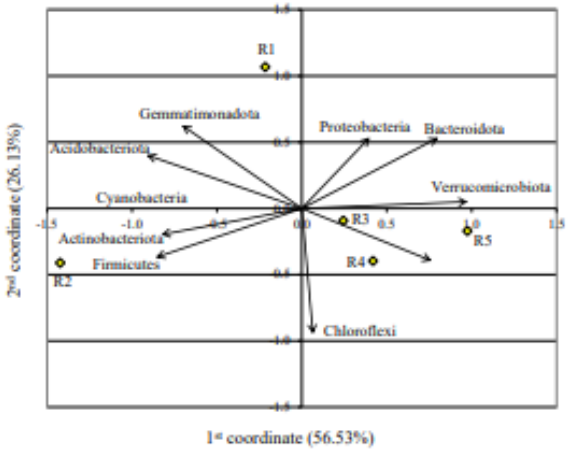
Figure 7. Relative abundance of dominant classes of bacteria phyla in 2020 (**A**—R2 via R1; **B**—R3 via R1; **C**—R4 via R1; **D**—R5 via R1). The classifications with less than 1% abundance are gathered

into the category "other". (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut; "yellow" means negative difference; "blue" means positive difference.

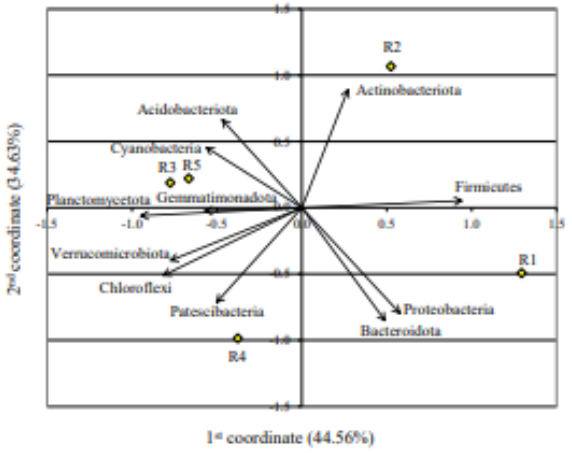
The aforementioned changes in the qualitative and quantitative composition of the bacterial microbiome caused by the previous use of soil and the effect of phytosanitary plants enabled the identification of microorganisms, which can be regarded as bioindicators of soil fertility. According to Fierer et al. [55], in order to better understand the soil regeneration process, it is important to know both the communities of microorganisms inhabiting the soil and their interrelationships. The principal component analysis (PCA) revealed the relationships between the different types of soil bacteria in the experimental variants during the three years of the research (Figure 8). It showed that soil biodiversity ranged from 71.19% to 94.28%. It also showed that the relationships between the different types of bacteria were related to the year of the study. Regardless of the experimental variant, in the first year of the study, the analysis revealed a clear correlation between the percentage of taxonomic sequences of the *Verrucomicrobiota*, *Bacteroidota*, *Cyanobacteria*, and *Tenericutes* phyla and between *Actinobacteriota* and *Planctomycetota*. A similar relationship between *Verrucomicrobiota* and *Bacteroidota* was also observed in the second year of the study (Figure 8). However, in 2020 and 2021, there was a correlation between the percentage of OTUs in the *Proteobacteria* phylum and *Bacteroidota*, which was in line with the results of the study by Fazi et al. [63].



2019



2020



2021

Figure 8. Principal component analysis of the relative abundance of dominant phyla of bacteria in the different soils (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

3.2. Bacterial Genera

Due to the fact that next-generation sequencing resulted in a relatively large number of sequences of bacterial genera, only the most numerous of them (>1%) were shown in Figures 9–11. In 2019, the dominant genera were: *Pseudomonas* (0.86–25.24%), *Bacillus* (3.76–27.76%), *Clostridium* (2.59–18.12%), and *Cohnella* (0.42–8.42%)—a highly cellulolytic bacterial genus belonging to the *Paenibacillaceae* family (Figure 9). The metagenomic analysis of the soil showed that in the next two years of the research, the most common bacterial genera in all experimental combinations were *Rhodanobacter* (1.63–8.66%) and *Sphingomonas* (2.80–5.01%), as well as *Gaillales* uncul. (2.64–7.12%) in 2020 and *Cellulosimicrobium* (8.69–25.34%) in 2021 (Figures 10 and 11).

The percentage of operational taxonomic units (OTUs) of individual bacterial genera depended on the experimental variant. In the first year of the study, the highest counts of

bacteria of the *Pseudomonas* and *Clostridium* genera were found in the agricultural soil (R1), whereas the lowest were found in the soil in variant R5 (Figure 9). In the next two years of research (Figures 10 and 11), it was mainly the *Rhodanobacter* genus of the *Gammaproteobacteria* class, *Xanthomonadales* order, and *Xanthomonadaceae* family that occurred more often in the variant with replanted soil (R2). This genus of bacteria is considered an indicator of soils degraded by agriculture. Wolińska et al. [64] selected the *Rhodanobacter* genus as a metagenomic analysis indicator characteristic of soils degraded by agriculture. According to the researchers, these bacteria are resistant to agricultural practices. They can be classified as oligotrophs with low nutritional requirements. This fact may account for the high content of their OTUs found in variants R1 and R2 in our study. The forecrop of phytosanitary plants (variants R3, R4, and R5) resulted in a lower count of bacteria of the *Rhodanobacter* genus (Figure 10). In 2020 and 2021, there was a smaller count of bacteria of the *Gaiellales* genus in the replanted soil (Figures 10 and 11). According to the data provided in reference publications, this genus plays a key role in the soil because it inhibits root rot caused by fungi of the *Fusarium* genus [65]. Moreover, according to Wu et al. [66], if the soil conditions are unfavourable for plant growth, this genus can adjust its metabolism so as to promote plant growth by increasing the availability of nutrients.

In 2020 and 2021, the presence of other resistant types of bacteria inhabiting the replanted soils with ARD was observed, i.e., *Peanibacillus* and *Chitinophagaceae* (Figures 10 and 11). According to the data provided in the reference publications, the former genus has all possible characteristics of plant growth-promoting rhizobacteria (PGPR), which can improve plant growth by induction of immunity, production of growth hormones, sharing of phosphorus, etc.). On the other hand, some species of PGPR cause diseases in honeybees. This has a negative influence on nurseries, which cannot produce high-quality trees with high yields [67].

Another genus found in the replanted soil (R2) was *Chitinophagaceae_uncul*. These bacteria are credited with an important role in the decomposition of organic carbon. They increase the intensity of its mineralisation, which is a phenomenon characteristic of agriculturally degraded soils [68]. Bacteria of the *Chitinophagaceae_uncul* genus were also identified in the soils after induced biofumigation, but their count was lower than in the replanted soil without the forecrop of phytosanitary plants (Figures 9 and 10).

In our study, apart from the bacterial genera resistant to soil degradation, there were also genera that did not react to soil dysfunction caused by ARD. The number of their OTUs in the soil in all variants of the experiment was similar. In 2020 and 2021, it was the *Sphingomonas* genus whose content in all variants of the experiment was similar (Figures 10 and 11). According to scientific publications, species belonging to this genus have multifaceted functions, ranging from the remediation of environmental pollution to the production of phytohormones, gibberellins, and indole acetic acid, which have indirect mutualistic effects on plants. Some species of this genus improve plant growth under soil stress, such as drought, salinity, or the content of heavy metals [69].

Other genera of microorganisms that occurred in identical counts in various combinations were *Devosia* and *Pseudolabrys* in 2020 (Figure 10) and *Chujaibacter* and uncultivated bacteria of the *Gemmatimonadaceae* family in 2021 (Figure 11). The presence of these bacterial genera in agriculturally degraded soils and soils with ARD was also confirmed in the available reference publications. The *Devosia* genus is well known for its dominance in soil habitats contaminated with various toxins, and it is best characterised by its bioremediation potential. In addition, the authors of studies on this genus of microorganisms stress their genomic plasticity to ensure adaptation, bioremediation, and the potential to use a wide range of substrates in degraded soils [70].

In 2020, the genus found in the soil collected from all experimental variants was *Pseudolabrys* bacteria (Figure 10), whereas in 2021 it was *Chujaibacter* (Figure 11). These are bacteria of the *Nitrobacteriaceae* family that are involved in the nitrification process [71]. They are also characterised by very high resistance to negative environmental factors and

by increased succession with decreasing soil pH, which is characteristic of soils with ARD [72].

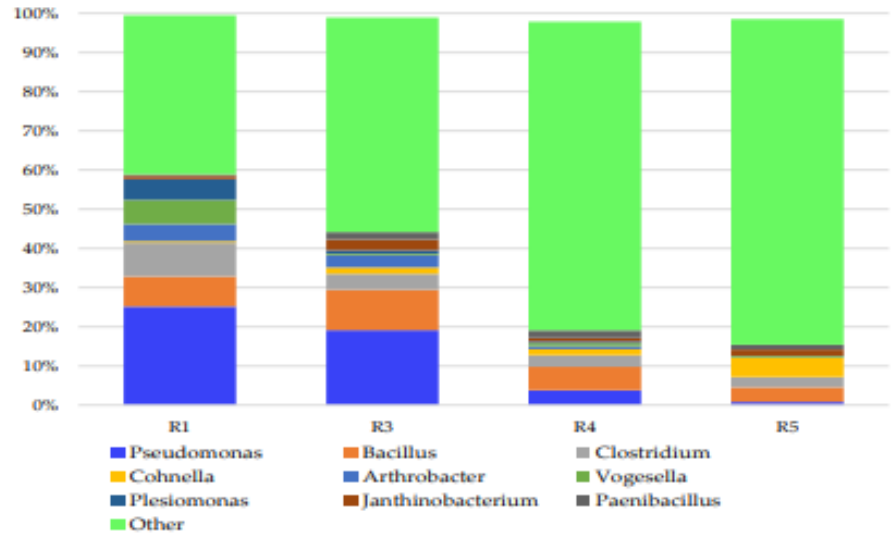


Figure 9. Relative abundance of the dominant (rodzaj) genus of bacteria in 2019. The classifications with less than 1% abundance are gathered into the category "other" (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

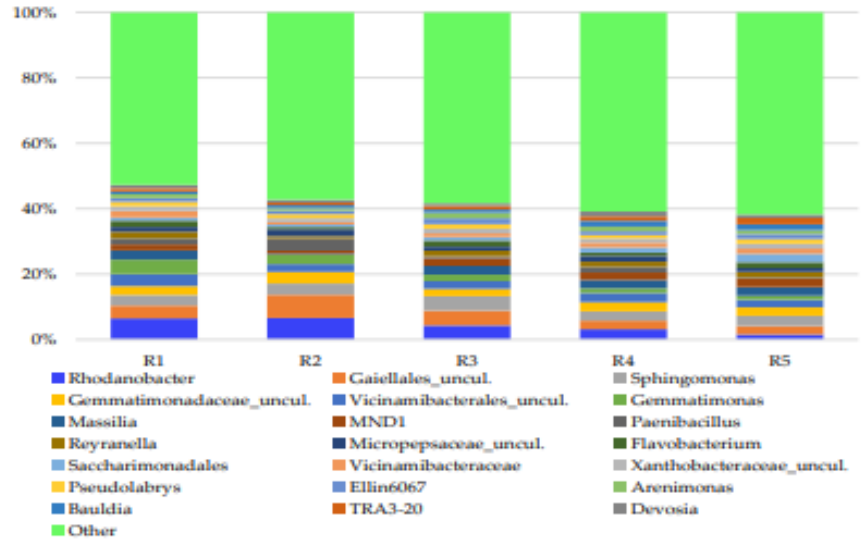


Figure 10. Relative abundance of the dominant genus of bacteria in 2020. The classifications with less than 1% abundance are gathered into the category "other" (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

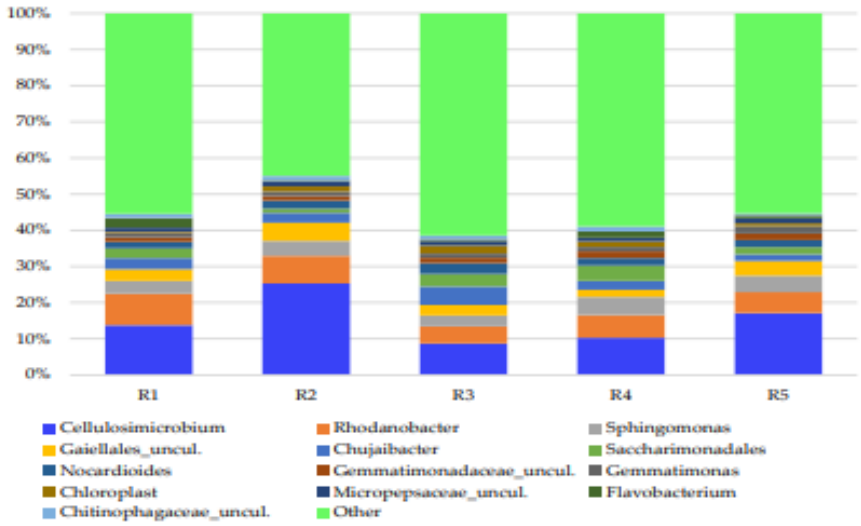
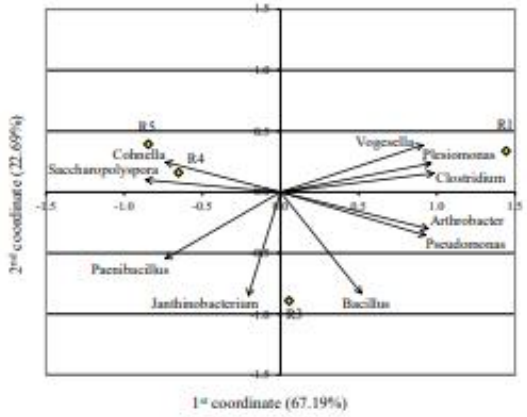


Figure 11. Relative abundance of the dominant genus of bacteria in 2021. The classifications with less than 1% abundance are gathered into the category "other" (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

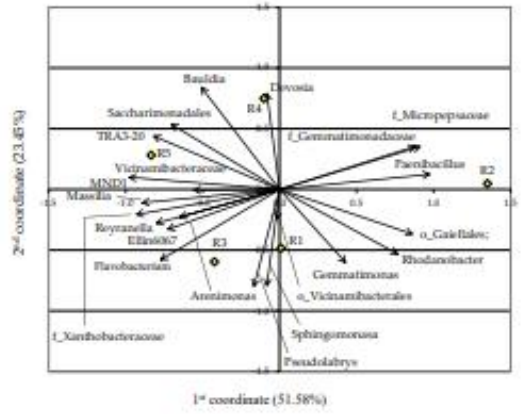
In 2020 and 2021, *Flavobacterium*, a sensitive bacterial genus, was detected in the replanted soil. Its smallest count was found in the replanted soil (R2), where it amounted to 0.82% in 2020 and 0.31% in 2021. After the application of the phytosanitary plants (variants R3, R4, and R5), the abundance of these bacteria in 2020 and 2021, respectively, increased to 1.08% and 0.46%, 1.61% and 1.68%, and 2.55% and 0.80% OTU (Figure 12). *Flavobacterium* bacteria are potential inhibitors of pathogens in root ecosystems. Apart from that, selected representatives of this genus are treated as plant growth-promoting rhizobacteria (PGPR) [73]. If ARD occurs, these microorganisms interact antagonistically with nematodes, thus alleviating the symptoms of soil disease [74]. The results of our experiment concerning the influence of French marigold, white mustard, and oilseed radish on the *Flavobacterium* genus were in line with the findings of other scientific publications. Hanschen and Winkelmann [75] observed that induced fumigation, e.g., by using *Brassica juncea* and *Sinapis alba*, increased the count of plant growth-promoting bacteria while inhibiting ARD.

In 2020, *Massilia*, which has similar characteristics and properties to *Flavobacterium*, was identified as an additional genus of sensitive bacteria in the replanted soil [76] (Figure 12). In 2021, apart from *Flavobacterium*, *Saccharimonadales* was another genus of ARD-sensitive bacteria. Like the *Massilia* genus, these microorganisms stimulate the growth of plants and play an important role in providing them with phosphorus and other nutrients [49].

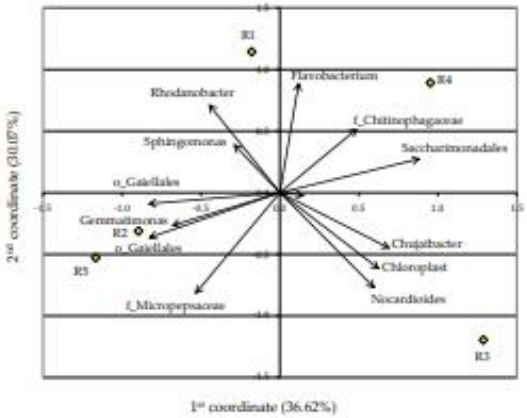
It is likely that the increase in the content of beneficial bacteria in the soils with ARD after induced fumigation (variants R3–R5) was caused by the phytosanitary plants, which produced bioactive compounds (including alpha-terthienyl). In consequence, they limited the development of pathogens such as *Rhizoctonia solani* and *Fusarium solani* and thus contributed to the succession of the abovementioned genera of beneficial bacteria [54].



2019



2020



2021

Figure 12. Principal component analysis of the relative abundance of dominant phyla of bacteria in the different soils (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula*

L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

The results of our experiment presented in the form of Venn diagrams confirmed the influence of the previous method of soil use and the research period on the structure of the bacterial microbiome (Figure 13). The presence of all taxa within a particular taxonomic category and the research period were taken into account. As a result, 482–512 genera common to all variants were selected. For example, the following bacterial genera were identified in all experimental variants: *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Streptomyces*, *Chujaibacter*, *Sphingomonas*, *Flavobacterium*, and *Devosia*.

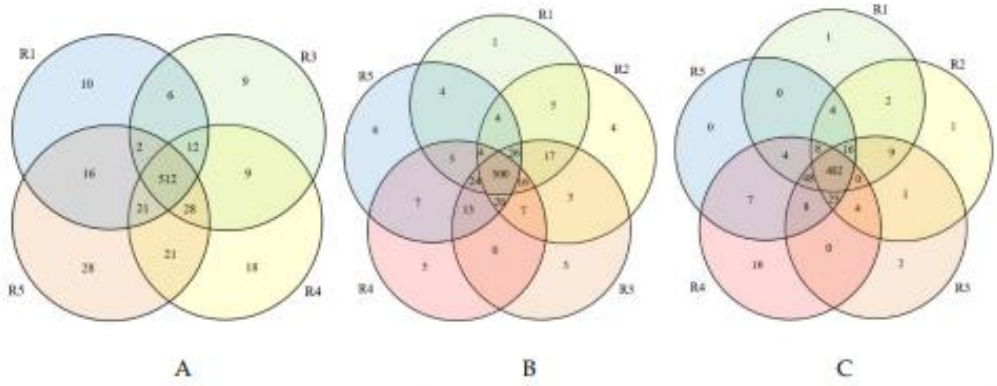


Figure 13. Venn diagram of overlapping bacterial communities (phyla) (**A**—2019; **B**—2020; **C**—2021). (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut.); the numbers indicate the number of unique bacterial sequences

The number of bacterial sequences identified in our study provides grounds for the conclusion that the cultivation of the phytosanitary plants contributed to the change in the qualitative composition of the soil microbiome. The highest number of unique taxa in the experimental variants was observed in 2019 in variant R5 (Table 3). These were 28 bacterial genera, which included saprophytic and plant growth-promoting species, as well as plant pathogens. In the consecutive years of the analyses, the number of unique genera in variants R5, R3, and R4 decreased significantly. During the analyses, the lowest number of unique bacterial taxa was found in the control variant, especially in the second and third years of the study, when the *Amycolatopsis* genus of the *Pseudonocardiacea* family was identified (Tables 4 and 5). This genus includes species recognised as biocontrol factors, which play an important role in destroying plant pathogens and in the bioremediation process [77,78].

Table 3. Unique bacterial taxa in individual experimental variants in 2019. (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

R1	R2	R3	R4	R5
Number of Unique Taxa				
10	0	9	18	28
Anaerococcus	–	*Chlamydia*	*Citricoccus*	*Antarctobacter*
Dorea		*Desulfonatronovibrio*	*Cryobacterium*	*Aureispira*
Eggerthella		*Desulfotalea*	*Dehalobacterium*	*Bulleidia*
Enhydrobacter		*Flectobacillus*	*Desulfomicrobium*	*Butyricimonas*

Fructobacillus	*Helcococcus*	*Entomoplasma*	*Coprococcus*
Mitsuokella	*Muricauda*	*Fusobacterium*	*Haliscomenobacter*
Paraprevotella	*Roseateles*	*Jeotgalicoccus*	*Lachnobacterium*
Sarcina	*Sinomonas*	*Kibdelosporangium*	*Limnothrix*
Thiocystis	*Spirochaeta*	*Kytococcus*	*Luteococcus*
Verminephrobacter		*Octadecabacter*	*Nevskia*
		Odoribacter	*Parabacteroides*
		Peptostreptococcus	*Porphyromonas*
		Roseococcus	*Propionigenium*
		Saccharomonospora	*Pseudanabaena*
		Salinivibrio	*Psychroflexus*
		Sporanaerobacter	*Rhodothalassium*
		Thermococcus	*Roseburia*
		Xenococcus	*Roseiflexus*
			Roseivivax
			Salinimicrobium
			Snowella
			Streptomonospora
			Sulfuricurvum
			Sulfuritalea
			Teredinibacter
			Terriglobus
			Thermacetogenium
			Zobellia

Table 4. Unique bacterial taxa in individual experimental variants in 2020. (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

R1	R2	R3	R4	R5
Number of Unique Taxa				
1	4	5	4	3
Amycolatopsis	*Haliscomenobacter* *Novibacillus* *f_Enterobacteriaceae;* Other *Hafnia-* *Obesumbacterium*	f_*Nostocaceae;*Other *Archangium* *Aetherobacter* f_Polyangiaceae;g_ uncultured *Leptospira*	*Vicingus* *Blastopirellula* *Chitinimona* f_*Parachlamydiaceae;* g_uncultured	o_Babeliales; Other; Lactococcus OM60(NOR5)_clade

Table 5. Unique bacterial taxa in individual experimental variants in 2021. (R1—agricultural soil; R2—replanted soil; R3—replanted soil with *Tagetes patula* L. foregut; R4—replanted soil with *Sinapis alba* foregut; R5—replanted soil with *Raphanus sativus* var. *oleifera* foregut).

R1	R2	R3	R4	R5
Number of Unique Taxa				
1	1	2	16	0
Amycolatopsis	*Rahnella1*	f_*Myxococcaceae;*Other *Candidatus_Falkowbacteria*	*Paludibacter* WCHB1-32 *Vitellibacter* *Bacteriovorax* *Pseudarcobacter* *Candidatus_Megaira* *Tolumonas*	-

	Idiomarina
	Shewanella
	Noviherbaspirillum
	Candidatus_Accumulibacter
	f_*Methylococcaceae*;g_uncultured
	Methylophaga
	Halomonas
	Alkanindiges
	f_*Criblamydiaceae*;g_uncultured

4. Conclusions

The use of metagenomics (functional analysis of genetic material isolated from the soil) as a tool for assessing soil biodiversity in the nursery after replantation proved to be a sensitive and precise method of assessment of the soil microbiome in the nursery. The analyses of the microbiome composition showed that biofumigation with phytosanitary plants—French marigold (*Tagetes patula* L.), white mustard (*Sinapis alba*), and oil radish (*Raphanus sativus* var. *oleifera*)—changed the structure and count of bacteria in the replanted soil in the fruit tree nursery. The phytosanitary plants increased the abundance of operational taxonomic units (OTU) of the *Proteobacteria*, *Bacteroidota*, *Patescibacteria*, *Chloroflexi*, *Fatescibacteria*, and *Verrucomicrobiota* phyla, but decreased the abundance of the *Firmicutes*, *Acidobacteriota*, and *Actinobacteriota* phyla. The biofumigation also increased the content of some dominant bacterial genera in the replanted soil, such as *Flavobacterium*, *Massila*, *Sphingomonas*, *Arenimonas*, and *Devosia*. These genera are considered crucial in promoting plant growth and inducing plant systemic immunity, which may indicate the regeneration of replanted soil.

Studies have shown that regardless of the species of phytosanitary plants used, there was an increase in the abundance of beneficial microbiomes. In practice, when planning production plantings, it is recommended to use a one-year break during which phytosanitary plants will be cultivated.

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References

1. Winkelmann, T.; Smalla, K.; Amelung, W.; Baab, G.; Grunewaldt-Stöcker, G.; Kanfra, X.; Meyhöfer, R.; Reim, S.; Schmitz, M.; Vetterlein, D. Apple replant disease: Causes and Mitigation Strategies. *Curr. Issues Mol. Biol.* **2019**, *30*, 89–106. https://doi.org/10.21775/cimb.030.089.
2. Mazzola, M.; Manici, L.M. Apple replant disease: Role of microbial ecology in cause and control. *Annu. Rev. Phytopathol.* **2012**, *50*, 45–65. https://doi.org/10.1146/annurev-phyto-081211-173005.
3. Volk, G.M.; Chao, C.T.; Norelli, J.; Brown, S.K.; Fazio, G.; Peace, C.; Mc Ferson, J.; Zhong, G.Y.; Bretting, P. The vulnerability of US apple (Malus) genetic resources. *Genet. Resour. Crop Evol.* **2015**, *62*, 765–794. https://doi.org/10.1007/s10722-014-0194-2.
4. Israel, D.W.; Giddens, J.E.; Powell, W.W. The toxicity of peach tree roots. *Plant Soil* **1973**, *39*, 103–112. https://doi.org/10.1007/BF00018049
5. Benizri, E.; Piutti, S.; Verger, S.; Pagès, L.; Vercambre, G.; Poessel, J.L.; Michelot, P. Replant diseases: Bacterial community structure and diversity in peach rhizosphere as determined by metabolic and genetic fingerprinting. *Soil Biol. Biochem.* **2005**, *37*, 1738–1746. https://doi.org/10.1016/j.soilbio.2005.02.009.

6. Mai, W.F.; Abawi, G.S. Determining the cause and extent of apple, cherry and pear replant diseases under controlled conditions. *Phytopathology* **1978**, *68*, 1540–1544. https://doi.org/10.1094/Phyto-68-1540.
7. Reginato, G.; Córdova, C.; Mauro, C. Evaluation of rootstock and management practices to avoid cherry replant diesease in Chile. *Acta Hortic.* **2008**, *795*, 357–362. https://doi.org/10.17660/ActaHortic.2008.795.52.
8. Spethmann, W.; Otto, G. Replant problems and soil sickness. In *Encyclopedia of Rose Science*; Roberts, A.V., Debener, T., Gudin, S., Eds.; Elsevier: Oxford, UK, 2003; pp. 169–180. https://doi.org/10.1016/B0-12-227620-5/00080-X.
9. Baumann, A.; Yim, B.; Grunewaldt-Stöcker, G.; Liu, B.; Beerhues, L.; Sapp, M.; Nesme, J.; Sørensen, S.J.; Smalla, K.; Winkelmann, T. Rose replant disease: Detailed analyses of plant reactions, root endophytes and rhizosphere microbial communities. *Acta Hortic.* **2020**, *1283*, 97–104. https://doi.org/10.17660/ActaHortic.2020.1283.14.
10. Deal, D.; Mail, W.; Boothroyd, C. A survey of biotic relationships in grape replant situations. *Phytopathology* **1972**, *62*, 503–507. https://doi.org/10.1094/Phyto-62-503.
11. Westphal, A.; Browne, G.T.; Schneider, S. Evidence for biological nature of the grape replant problem in California. *Plant Soil* **2002**, *242*, 197–203. Available online: https://www.jstor.org/stable/24122574 (access date 20.02.2010).
12. Elmer, W. Asparagus decline and replant problem: A look back and a look forward at strategies for mitigating losses. *Acta Hortic.* **2018**, *1223*, 195–204. https://doi.org/10.17660/ActaHortic.2018.1223.27.
13. Wu, L.; Wang, J.; Huang, W.; Wu, H.; Chen, J.; Yang, Y.; Zhang, Z.; Lin, W. Plant-microbe rhizosphere interactions mediated by *Rehmannia glutinosa* root exudates under consecutive monoculture. *Sci. Rep.* **2015**, *5*, 15871. https://doi.org/10.1038/srep15871.
14. Long, F.; Lin, Y.M.; Hong, T.; Wu, C.Z.; Li, J. Soil sickness in horticulture and forestry: A review. *Allelopathy J.* **2019**, *47*, 57–72. https://doi.org/10.26651/allelo.j/2019-47-1-1219.
15. Mai, W.F.; Merwin, I.A.; Abawi, G.S. Diagnosis, etiology and management of replant disorders in New York cherry and apple orchards. *Acta Hortic.* **1994**, *363*, 33–41. https://doi.org/10.17660/ACTAHORTIC.1994.363.5.
16. Mai, W.F.; Abawi, G.S. Controlling replant diseases of pome and stone fruits in Northeastern United States by preplant fumigation. *Plant Dis.* **1981**, *65*, 859–864. https://doi.org/10.1094/PD-65-859.
17. Weller, D. M., Raaijmakers, J. M., Gardener, B. B. M., Thomashow, L. S. (2002). Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol*, **2002**, 40(1), 309-348. https://doi.org/10.1146/annurev.phyto.40.030402.110010
18. Laurent, A.S.; Merwin, I.A.; Thies, J.E. Long-term orchard groundcover management systems affect soil microbial communities and apple replant disease severity. *Plant Soil* **2008**, *304*, 209–225. https://doi.org/10.1007/s11104-008-9541-4.
19. Manici, L.; Kelderer, M.; Franke-Whittle, I.; Rühmer, T.; Baab, G.; Nicoletti, F.; Caputo, F.; Topp, A.; Insam, H.; Naef, A. Relationship between root-endophytic microbial communities and replant disease in specialized apple growing areas in Europe. *Appl. Soil Ecol.* **2013**, *72*, 207–214. https://doi.org/10.1016/j.apsoil.2013.07.011.
20. Atucha, A.; Emmett, B.; Bauerle, T.L. Growth rate of fine root systems influences rootstock tolerance to replant disease. *Plant Soil* **2014**, *376*, 337–346. https://doi.org/10.1007/s11104-013-1977-5.
21. Yin, C.; Xiang, L.; Wang, G.; Wang, Y.; Shen, X.; Chen, X.; Zhiquan Mao, Z. How to plant apple trees to reduce replant disease in apple orchard: A study on the phenolic acid of the replanted apple orchard. *PLoS ONE* **2016**, *11*, e0167347. https://doi.org/10.1016/j.hpj.2018.05.003.
22. Sobiczewski, P.; Treder, W.; Bryk, H.; Klamkowski, K.; Krzewinska, D.; Mikicinski, A.; Berczynski, S.; Tryngiel-Gac, A. The impact of phytosanitary treatments in the soil with signs of fatigue on the growth of apple seedlings and populations of bacteria and fungi. *Pol. J. Agron.* **2018**, *34*, 11–22. https://doi.org/10.26114/pja.iung.361.2018.34.02.
23. Grunewaldt-Stöcker, G.; Mahnkopp, F.; Popp, C.; Maiss, E.; Winkelmann, T. Diagnosis of apple replant disease (ARD): Microscopic evidence of early symptoms in fine roots of different apple rootstock genotypes. *Sci. Hortic.* **2019**, *243*, 583–594. https://doi.org/10.1016/J.SCIENTA.2018.09.014.
24. Liu, E.T.; Wang, G.S.; Li, Y.Y.; Shen, X.; Chen, X.S.; Song, F.H.; Wu, S.J.; Che, Q.; Mao, Z.Q. Replanting affects the tree growth and fruit quality of Gala apple. *J. Integr. Agric.* **2014**, *13*, 1699–1706. https://doi.org/10.1016/S2095-3119(13)60620-6.
25. Henfrey, J.L.; Baab, G.; Schmitz, M. Physiological stress responses in apple under replant conditions. *Sci. Hortic.* **2015**, *194*, 111–117. https://doi.org/10.1016/j.scienta. 2015.07.034.
26. Lucas, M.; Balb´ın-Suarez, A.; Smalla, K.; Vetterlein, D. Root growth, function and rhizosphere microbiome analyses show local rather than systemic effects in apple plant response to replant disease soil. *PLoS ONE* **2018**, *13*, e0204922. https://doi.org/10.1371/journal.pone.0204922.
27. Nicola, L.; Insam, H.; Pertot, I.; Stres, B. Reanalysis of microbiomes in soils affected by apple replant disease (ARD): Old foes and novel suspects lead to the proposal of extended model of disease development. *Appl. Soil Ecol.* **2018**, *129*, 24–33. https://doi.org/10.1016/j.apsoil.2018.04.010.
28. Balb´ın-Suarez, A.; Lucas, M.; Vetterlein, D.; Sørensen, S.; Winkelmann, S.; Smalla, K.; Jacquiod, S. Exploring prokaryotic determinants of apple replant disease (ARD): A microhabitat approach under split-root design. *FEMS Microbiol. Ecol.* **2020**, *96*, fiaa211. https://doi.org/10.1093/femsec/fiaa211.
29. Manici, L.M.; Ciavatta, C.; Kelderer, M.; Erschbaumer, G. Replant problems in South Tyrol: Role of fungal pathogens and microbial population in conventional and organic apple orchards. *J. Plant Soil* **2003**, *256*, 315–324. https://doi.org/10.1023/A:1026103001592.
30. Zhao, L.; Jiang, W.; Chen, R.; Wang, H.; Duan, Y.; Chen, X.; Shen, X.; Yin, C.; Mao, Z. Quicklime and Superphosphate Alleviating Apple Replant Disease by Improving Acidified Soil. *ACS Omega* **2022**, *7*, 7920–7930. https://doi.org/10.1021/acsomega.1c06876.

31. Spath, M.; Insam, H.; Peintner, U.; Kelderer, M.; Kuhnert, R.; Franke-Whittle, I.H. Linking soil biotic and abiotic factors to apple replant disease: A greenhouse approach. *J. Phytopathol.* **2015**, *163*, 287–299. https://doi.org/10.1111/jph.12318.
32. Tilston, E.L.; Deakin, G.; Bennett, J.; Passey, T.; Harrison, N.B.; O'Brien, F.; Fernandez, F.; Xu, X.M. Candidate causal organisms for apple replant disease in the UK. *Phytobiomes J.* **2018**, *2*, 261–274. https://doi.org/10.1094/PBIOMES-11-18-0050-R.
33. Yin, C.; Duan, Y.N.; Xiang, L.; Wang, G.; Zhang, X.; Shen, X.; Chen, X.; Zhang, M.; Mao, Z. Effects of phloridzin, phloretin and benzoic acid at the concentrations measured in soil on the root proteome of Malus hupehensis Rehd Seedlings. *Scientia Hort.* **2018**, *228*, 10–17. https://doi.org/10.1016/j.scienta.2017.09.044.
34. Cabos, R.Y.M.; Hara, A.H.; Tsang, M.M.C. Hot water drench treatment for control of reniform nematodes in potted dracaena. *Nematropica* **2012**, *42*, 72–79.
35. Yim, B.; Winkelmann, T.; Ding, G.-C.; Smalla, K. Different bacterial communities in heat and gamma irradiation treated replant disease soils revealed by 16S rRNA gene analysis—Contribution to improved aboveground apple plant growth? *Front. Microbiol.* **2015**, *6*, 1224. https://doi.org/10.3389/fmicb.2015.01224.
36. Nicola, L.; Turco, E.; Albanese, D.; Donati, C.; Thalheimer, M.; Pindo, M.; Insam, H.; Cavalieri, D.; Pertot, I. Fumigation with dazomet modifies soil microbiota in apple orchards affected by replant disease. *Appl. Soil Ecol.* **2017**, *113*, 71–79. https://doi.org/10.1016/j.apsoil.2017.02.002.
37. Nyoni, M.; Mazzola, M.; Wessels, J.P.B.; McLeod, A. The efficacy of semiselective chemicals and chloropicrin/1,3-dichloropropene–containing fumigants in managing apple replant disease in South Africa. *Plant Dis.* **2019**, *103*, 1363–1373. https://doi.org/10.1094/PDIS-10-18-1844-RE.
38. Rudolph, R.E.; Zasada, I.A.; Hesse, C.; DeVetter, L.W. *Brassicaceous* seed meal, root removal, and chemical fumigation vary in their effects on soil quality parameters and *Pratylenchus penetrans* in a replanted floricane raspberry production system. *Appl. Soil Ecol.* **2019**, *133*, 44–51. https://doi.org/10.1016/j.apsoil.2018.08.024.
39. Hewavitharana, S.S.; Mazzola, M. Carbon source-dependent efects of aaerobic soil disinfestation on soil microbiome and suppression of Rhizoctonia solani AG-5 and Pratylenchus penetrans. *Phytopathology* **2016**, *106*, 1015–1028. https://doi.org/10.1094/PHYTO-12-15-0329-R.
40. Browne, G.; Ott, N.; Poret-Peterson, A.; Gouran, H.; Lampinen, B. Efficacy of anaerobic soil disinfestation for control of Prunus replant disease. *Plant Dis.* **2018**, *102*, 209–219. https://doi.org/10.1094/PDIS-09-16-1392-RE.
41. Forge, T.; Neilsen, G.; Neilsen, D. Organically acceptable practices to improve replant success of temperate tree-fruit crops. *Sci. Hort.* **2016**, *200*, 205–214. https://doi.org/10.1016/j.scienta.2016.01.002.
42. Franke-Whittle, I.H.; Juárez, M.F.-D.; Insam, H.; Schweizer, S.; Naef, A.; Topp, A.-R.; Kelderer, M.; Rühmer, T.; Baab, G.; Henfrey, J.; et al. Performance evaluation of locally available composts to reduce replant disease in apple orchards of central Europe. Renew. *Agric. Food Syst.* **2018**, *34*, 543–557. https://doi.org/10.1017/S1742170518000091.
43. De Corato, U. Disease-suppressive compost enhances natural soil suppressiveness against soil-borne plant pathogens: A critical review. *Rhizosphere* **2020**, *13*, 100192. https://doi.org/10.1016/j.rhisph.2020.100192.
44. Gimsing, A.L.; Kirkegaard, J.A. Glucosinolates and biofumigation: Fate of glucosinolates and their hydrolysis products in soil. *Phytochem. Rev.* **2009**, *8*, 299–310.
45. Hamaguchi, T.; Sato, K.; Vicente, C.S.L.; Hasegawa, K. Nematicidal actions of the marigold exudate -terthienyl: Oxidative stress-inducing compound penetrates nematode hypodermis. *Biol. Open* **2019**, *8*, bio038646. https://doi.org/10.1242/bio.038646.
46. Kumar, G.N.K.; Jayasudha, S.M.; Kirankumar, K.C. Disease management by Biofumigation in organic farming system. *J. Pharmacogn. Phytochem.* **2018**, *7*, 676–679. Available online: http://www.phytojournal.com/archives/2018/vol7issue4/PartK/7-3-654-578.pdf (access date 11.04.2022).
47. Gupta, P.; Vasudeva, N. In vitro antiplasmodial and antimicrobial potential of Tagetes erecta roots. *Pharm. Biol.* **2010**, *48*, 1218–1223. https://doi.org/10.3109/13880201003695142.
48. Padalia, H.; Chanda, S. Antimicrobial efficacy of different solvent extracts of Tagetes erecta L. flower, alone and in combination with antibiotics. *Appl. Microbiol.* **2015**, *1*, 106. https://doi.org/10.4172/2471-9315.1000106.
49. Wang, X.; Li, K.; Xu, S.; Duan, Y.; Wang, H.; Yin, C.; Chen, X.; Mao, Z.; Xiang, K. Effects of Different Forms of Tagetes erecta Biofumigation on the Growth of Apple Seedlings and Replanted Soil Microbial Environment. *Horticulturae* **2022**, *8*, 633. https://doi.org/10.3390/ horticulturae8070633.
50. Gałązka, A.; Grządziel, J.; Gałązka, R.; Ukalska-Jaruga, A.; Strzelecka, J.; Smreczak, B. Genetic and functional diversity of bacterial microbiome in soils with long term impacts of petroleum hydrocarbons. *Front. Microbiol.* **2018**, *9*, 1923. https://doi.org/10.3389/fmicb.2018.01923.
51. Rawat, N.; Joshi, G.K. Bacterial community structure analysis of a hot spring soil by next generation sequencing of ribosomal RNA. *Genomics* **2019**, *111*, 1053–1058. https://doi.org/10.1016/j.ygeno.2018.06.008.
52. Finley, S.J.; Benbow, M.E.; Javan, G.T. Potential applications of soil microbial ecology and next-generation sequencing in criminal investigations. *Appl. Soil Ecol.* **2015**, *88*, 69–78. https://doi.org/10.1016/j.apsoil.2015.01.001.
53. Furtak, K.; Grządziel, J.; Gałązka, A.; Niedźwiecki, J. Prevalence of unclassified bacteria in the soil bacterial community from floodplain meadows (fluvisols) under simulated flood conditions revealed by a metataxonomic approachss. *Catena* **2020**, *188*, 104448. https://doi.org/10.1016/j.catena.2019.104448.
54. Mahnkopp-Dirks, F.; Radl, V.; Kublik, S.; Gschwendtner, S.; Schloter, M.; Winkelmann, T. Molecular barcoding reveals the genus Streptomyces as associated root endophytes of apple (Malus domestica) plants grown in soils affected by apple replant disease. *Phytobiomes J.* **2021**, *5*, 177–189. https://doi.org/10.1094/PBIOMES-07-20-0053-R.

55. Fierer, N.; Bradford, M.A.; Jackson, R.B. Toward an ecological classification of soil bacteria. *Ecology* **2007**, *88*, 1354–1364. https://doi.org/10.1890/05-1839.
56. Swędrzyńska, D.; Małecka-Jankowiak, I. The Impact of Tillaging Spring Barley on Selected Chemical, Microbiological, and Enzymatic Soil Properties. *Pol. J. Environ. Stud.* **2017**, *26*, 303–313. https://doi.org/10.15244/pjoes/64911.
57. Niewiadomska, A.; Majchrzak, L.; Borowiak, K.; Wolna-Maruwka, A.; Waraczewska, Z.; Budka, A.; Gaj, R. The Influence of Tillage and Cover Cropping on Soil Microbial Parameters and Spring Wheat Physiology. *Agronomy* **2020**, *10*, 200. https://doi.org/10.3390/agronomy10020200.
58. Wang, M.; Pendall, E.; Fang, C.; Li, B.; Nie, M. A global perspective on agroecosystem nitrogen cycles after returning crop residue. *Agric. Ecosyst. Environ.* **2018**, *266*, 49–54. https://doi.org/10.1016/j.agee.2018.07.019.
59. Liang, B.; Ma, C.; Fan, L.; Wang, Y.; Yuan, Y. Soil amendment alters soil physicochemical properties and bacterial community structure of a replanted apple orchard. *Microbiol. Res.* **2018**, *216*, 1–11. https://doi.org/10.1016/j.micres.2018.07.010.
60. Zheng, G.; Wang, T.; Niu, M.; Chen, X.; Liu, C.; Wang, Y.; Chen, T. Biodegradation of Nonylphenol during Aerobic Composting of Sewage Sludge under Two Intermittent Aeration Treatments in a Full-Scale Plant. *Environ. Pollut.* **2018**, *238*, 783–791. https://doi.org/10.1016/j.envpol.2018.03.112.
61. Tang, Z.; Zhang, L.; He, N.; Gong, D.; Gao, H.; Ma, Z.; Fu, L.; Zhao, M.; Wang, H.; Wang, C.; et al. Soil bacterial community as impacted by addition of rice straw and biochar. *Sci. Rep.* **2021**, *11*, 22185. https://doi.org/10.1038/s41598-021-99001-9.
62. Chamizo, S.; Mugnai, G.; Rossi, F.; Certini, G.; De Philippis, R. Cyanobacteria inoculation improves soil stability and fertility on different textured soils: Gaining insights for applicability in soil restoration. *Front. Environ. Sci.* **2018**, *6*, 49. https://doi.org/10.3389/fenvs.2018.00049.
63. Fazi, S.; Amalfitano, S.; Pernthaler, J.; Puddu, A. Bacterial communities associated with benthic organic matter in headwater stream microhabitats. *Environ. Microbiol. Niem* **2005**, *7*, 1633–1640. https://doi.org/10.1111/J.1462-2920.2005.00857.X.
64. Wolińska, A.; Kuźniar, A.; Zielenkiewicz, U.; Banach, A.; Błaszczyk, M. Indicators of arable soils fatigue–Bacterial families and genera: A metagenomic approach. *Ecol. Indic.* **2018**, *93*, 490–500. https://doi.org/10.1016/j.ecolind.2018.05.033.
65. Ding, Y.; Chen, Y.; Lin, Z.; Tuo, Y.; Li, H.; Wang, Y. Differences in soil microbial community composition between suppressive and root rot-conducive in tobacco fields. *Curr. Microbiol.* **2021**, *78*, 624–633. https://doi.org/10.1007/s00284-020-02318-3.
66. Wu, S.; Xue, S.; Iqbal, Y.; Xing, H.; Jie, Y. Seasonal nutrient cycling and enrichment of nutrient-related soil microbes aid in the adaptation of ramie (*Boehmeria nivea* L.) to nutrient-deficient conditions. *Front. Plant Sci.* **2021**, *12*, 644904. https://doi.org/10.3389/fpls.2021.644904.
67. Grady, E.N.; MacDonald, J.; Liu, L.; Richman, A.; Yuan, Z.C. Current knowledge and perspectives of Paenibacillus: A review. *Microb Cell Fact.* **2016**, *15*, 203. https://doi.org/10.1186/s12934-016-0603-7.
68. Gao, W.; Gao, K.; Guo, Z.; Liu, Y.; Jiang, L.; Liu, C.; Wang, G. Different responses of soil bacterial and fungal communities to 3 years of biochar amendment in an alkaline soybean soil. *Front. Microbiol.* **2021**, *12*, 630418. https://doi.org/10.3389/fmicb.2021.630418.
69. Numan, M.; Khan, A.L.; Al-Harrasi, A. Sphingomonas: From diversity and genomics to functional role in environmental remediation and plant growth. *Crit. Rev. Biotechnol.* **2020**, *40*, 138–152. https://doi.org/10.1080/07388551.2019.1709793.
70. Talwar, C.; Nagar, S.; Kumar, R.; Scaria, J.; Lal, R.; Negi, R.K. Defining the environmental adaptations of genus Devosia: Insights into its expansive short peptide transport system and positively selected genes. *Sci. Rep.* **2020**, *10*, 1151. https://doi.org/10.1038/s41598-020-58163-8.
71. Semenov, M.V.; Krasnov, G.S.; Semenov; VM; van Bruggen, A.H.C. Long-term fertilization rather than plant species shapes rhizosphere and bulk soil prokaryotic communities in agroecosystems. *Appl. Soil Ecol.* **2020**, *154*, 103641. https://doi.org/10.1016/j.apsoil.2020.103641).
72. Lee, H.; Oh, S.Y.; Lee, Y.M.; Jang, Y.; Jang, S.; Kim, C.; Kim, J.J. Successional variation in the soil microbial community in Odaesan National Park, Korea. *Sustainability* **2020**, *12*, 4795. https://doi.org/10.3390/su12114795.
73. Kraut-Cohen, J.; Shapiro, O.H.; Dror, B.; Cytryn, E. Pectin Induced Colony Expansion of Soil-Derived Flavobacterium Strains. *Front. Microbiol.* **2021**, *12*, 651891. https://doi.org/10.3389/fmicb.2021.651891.
74. Kanfra, X.; Wrede, A.; Mahnkopp-Dirks, F.; Winkelmann, T.; Heuer, H. Networks of free-living nematodes and co-extracted fungi, associated with symptoms of apple replant disease. *Appl. Soil Ecol.* **2022**, *172*, 104368. https://doi.org/10.1016/j.apsoil.2021.104368.
75. Hanschen, F.S.; Winkelmann, T. Biofumigation for fighting replant disease-A Review. *Agronomy* **2020**, *10*, 425. https://doi.org/10.3390/agronomy10030425.
76. Yi, S.; Li, F.; Wu, C.; Ge, F.; Feng, C.; Zhang, M.; Lu, H. Co-transformation of HMs-PAHs in rhizosphere soils and adaptive responses of rhizobacteria during whole growth period of rice (*Oryza sativa* L.). *J. Environ. Sci.* **2023**, *132*, 71–82. https://doi.org/10.1016/j.jes.2022.07.017.

Publikacja 3

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Biofumigation Treatment Using *Tagetes patula, Sinapis alba* and *Raphanus sativus* Changes the Biological Properties of Replanted Soil in a Fruit Tree Nursery

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Abstract: Apple replant disease (ARD) may cause significant losses both in commercial orchards and in fruit tree nurseries. The negative effects of ARD may be limited by using biofumigation. The aim of the study was to assess the influence of this treatment on the biological properties of replanted soil in a tree nursery. In two-year experiment, apple trees of the 'Golden Delicious' cultivar were used. The trees were planted into soil from two sites. The soil from one site had not been used in a nursery before (crop rotation soil). The other soil had been used for the production of apple trees (replanted soil). Three species of plants were used in the replanted soil as a forecrop: French marigold (*Tagetes patula*), white mustard (*Sinapis alba*), and oilseed radish (*Raphanus sativus* var. oleifera). The following parameters were assessed in the experiment: the enzyme and respiratory activity of the soil, the total count of bacteria, fungi, oomycetes and actinobacteria in the soil, as well as the count and species composition of soil nematodes. The vegetative growth parameters of the apple trees were also assessed. The biological properties of the replanted soil were worse than those of the crop rotation soil. In the replanted soil, the organic matter content, enzyme and respiratory activity as well as the count of soil microorganisms were lower. The biofumigants, used as a forecrop on the replanted soil, significantly increased its enzyme activity and respiratory activity. Dehydrogenase activity increased more than twofold. Growth parameters of the trees were significantly improved. The height of the trees increased by more than 50%, and the leaf area, weight and total length of side shoots were higher as well. The density of nematodes in the replanted soil after biofumigation was significantly reduced, with a larger reduction in the marigold fumigated soil. Eight of the eleven nematode species were completely reduced in the first year after biofumigation treatment.

Keywords: replanted soil; biofumigation; enzyme and respiratory activity; vegetative growth; nematodes; plant trees

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1. Introduction

Intensification is a prerequisite for high profitability of fruit production. It involves growing a large number of low-vigorous trees, which requires a large amount of high-quality nursery material. Due to changing market requirements for the selection of cultivars, nurserymen have to change plantings frequently and search for new areas for nursery plantations. New nurseries may be established in areas used as nurseries before because of the high risks due to replanting disease [1], soil fatigue [2] or, usually, apple replant disease (ARD). The occurrence of ARD is most common in apple plantations. Research on the effects of replant disease is also usually conducted in apple plantations, but rather rarely in fruit tree nurseries. Regardless of the crop type, researchers generally

agree on the effects of ARD. When establishing new orchards in the place of old ones, the production properties of the soil may decrease due to its low enzyme activity and respiratory activity [3,4]. As a result, the content of soil nutrients decreases [5,6]. In consequence of decreased productive properties of the soil affected by ARD, the vegetative growth of plants is poorer [7–11], and the yield and quality of fruit are lower [1,12,13].

Owing to the progressive intensification of fruit cultivation, the incidence of ARD is expected to increase, especially in regions where the regular replacement of land is limited due to its intensive use. Thus, it is necessary to find an effective method of mitigating the consequences of the disease. First, it is necessary to clearly identify the causes of ARD, which is not an easy task. As results from numerous studies show, ARD may be caused by abiotic or biotic factors. The former include soil acidification, high salinity, and phenolic compounds formed after the decomposition of root residues [14]. The latter are bacteria of the *Bacillius* and *Pseudomonas* genera [15,16], fungi/oomycetes of the *Cylindrocarpon*, *Rhizoctonia Alternaria*, *Phytophtora*, and *Pythium* genera [17,18], and nematodes [19–21]. The fact that the fumigation of replanted soil significantly reduces the consequences of ARD may indicate the decisive role of biological factors in its development. This treatment is particularly effective against nematodes [12,19,21]. It is estimated that there are several hundred to several million species of nematodes in the environment, but only a small group of them are considered to be plant parasites. The largest population of soil nematodes are non-pathogenic species [22], which are bioindicators of soil quality. Parasitic nematodes have been best investigated, especially the *Pratylenchidae* family [17]. They damage plant roots as they move through the soil in search of food [16]. Kanfra et al. [21] observed that when nematodes extracted from ARD soil were added to the soil in a nursery, they inhibited the growth of the root system of apple rootstocks. Available reference publications on nematodes include some results that do not confirm the relationship between parasitic nematodes and the occurrence of ARD [10,23]. Moreover, the symptoms of nematodes feeding on plants are rather non-specific, which makes it difficult to identify the loss they cause [22].

It may take a dozen years or so to restore the fertility of soil with ARD symptoms. According to Long et al. [24], the structure of soil microorganisms in replanted soil becomes imbalanced. In consequence, pathogenic organisms gain an advantage. Therefore, the primary goal should be to restore the balance of the species composition in the soil microflora, which involves reducing the count of pathogenic organisms in replanted soil. This can be performed by thermal sterilisation or chemical fumigation. Currently, dazomet, sodium methane, or chloropicrin are mainly used in the latter procedure [25,26]. Both methods very effectively reduce the count of biological perpetrators of ARD, including the *Fusarium* spp. [27] and *Phytophtora* spp. [28]. However, both thermal sterilisation of the substrate [29] and chemical fumigation [30] may also reduce the soil microbiota completely.

The undesirable effects of ARD can also be reduced through soil biofumigation. This involves using some plant species, mainly as forecrops, to reduce the count of harmful nematodes, pathogenic bacteria or fungi and oomycetes in the soil. The most common of these plants are marigold (*Tagetes* L.), white mustard (*Sinapis alba*), oilseed radish (*Raphanus sativus* var. oleifera), spring rapeseed (*Brassica napus*), and rye (*Secale cereale* L.) [31]. Plants used for biofumigation produce specific compounds—secondary metabolites (e.g., glucosinolates) and thiophene compounds, which have fungicidal, nematocidal, insecticidal, and antiviral effects [32]. Such plant properties make it possible to reduce the number of pathogenic organisms in the soil, including oomycetes: *Phytophthora cactorum* [33], and fungi as *Fusarium oxysporum* [34]. One of the best-investigated effects of biofumigation is the nematocidal effect [35]. Plants from the Asteraceae family (*Asteraceae* Dum) are also highly effective in combating nematodes, thanks to thiophene compounds released by their roots [36]. The highest content of these compounds was found in *Tagetes patula* and *Tagetes erecta*. Their cultivation causes significantly increase the vegetative growth of apple trees in an orchard on soil with ARD [37].

The aim of the study was to assess the influence of three plant species—French marigold (*Tagetes patula*), white mustard (*Sinapis alba*), and oilseed radish (*Raphanus sativus* var. oleifera) on improving the biological properties of replanted soil measured by its microbial activity in a fruit tree nursery.

2. Materials and Methods

2.1. Experiment Design

Between 2019 and 2021, an experiment was conducted on apple trees growing in a commercial nursery in western Poland (52°25'49.10" N 17°11'34.08" E). The trees were planted in containers with soil taken from two sites. The soil from one site had been used for agricultural crops (crop rotation soil—CRS). The other one came from a nursery where apple trees had been produced for two seasons (replanted soil—RS). At the first site, crops had been cultivated in a rotation system for 10 years. No treatments had been applied to improve the productive properties of the soil in the other site. The physicochemical properties of the soil from both sites are shown in Table 1. The soils from the two sites differed significantly in the content of nutrients. The content of P, K, Ca, Mg, and Zn in the replanted soil was lower than in the crop rotation soil. The humus content was also much lower (4.08% and 1.70%, respectively), but the acidity was higher (Table 1).

Table 1. Physicochemical and biological properties of the soil (CRS = crop rotation soil; RS = replanted soil).

Properties of the Soil	CRS	RS
pH (H_2O)	7.2	5.8
Bulk density (kg m^{-3})	1600	1830
Salinity (g NaCl dm^{-3})	0.23	0.23
Humus content (%)	4.88	1.70
Content of macro- and microelements (mg dm^{-3})		
$N\text{-}NO_3$	11	9
P	127	30
K	229	89
Ca	1333	240
Mg	188	38
Zn	6.20	3.03
Cu	2.10	1.27
Mn	34.38	111.63
Fe	170.60	380.85

Three species of plants were used in the experiment: French marigold (*Tagetes patula*), white mustard (*Sinapis alba*), and oilseed radish (*Raphanus sativus* var. oleifera). They were sown in the autumn of 2019 at the site with replanted soil. In the next year, in early spring, the plants were comminuted, mixed with the soil, and put into 8 L plastic containers. Apple trees of the 'Golden Delicious' cultivar, grafted on M9 rootstock, were planted in the containers.

Five treatments in the experiment were used: crop rotation soil (control variant—1), replanted soil (variant 2), replanted soil with a French marigold forecrop (*Tagetes patula*) (variant 3), replanted soil with a white mustard forecrop (*Sinapis alba*) (variant 4), and replanted soil with an oilseed radish forecrop (*Raphanus sativus* var. oleiformis) (variant 5). There were twenty containers (replicates) in each variant.

In 2020 and 2021, the containers with the plants were systematically weeded and watered with a drip irrigation system. The plants were protected in accordance with the recommendations for such crops. In each growing season, the plants were fertilised with a multi-component fertiliser (NPK 16+8+12+ micronutrients) at a dose of 3 g dm^{-3}.

The analysis of the climatic conditions at the site of the experiment included the average annual temperature and the amount of rainfall. Both of these parameters were higher (temperature) or lower (rainfall) than the long-term average values (1982–2016) (Appendix A).

2.2. Soil Analyses

The following soil analyses were conducted in the experiment: the enzyme activity and respiratory activity, the content of available form of macro- and micronutrients, the total counts of bacteria, oomycetes, fungi, and actinobacteria, and the number and species composition of soil nematodes. Soil samples for the analysis of the content of macronutrients (N-NO_3, P, K, Mg, Ca) and micronutrients (Zn, Cu, Mn, Fe) were collected in September 2021. A small amount of soil was collected from each container (replicate) with a laboratory spoon. Each soil sample representing one experimental variant was mixed and had a total weight of 900 g. The N-NO_3 content was measured by microdistillation, the P content—colorimetrically, the K and Ca content—photometrically, the Mg content—by atomic absorption spectrometry (AAS). Lindsay's Solution was used to extract micronutrients from the soil and their content was measured by AAS. The potentiometric method was applied to measure the soil acidity, Tiurins method for the determination of humus content, weight method for bulk density, and conductivity method for the determination of salinity.

Soil samples for the analysis of biological properties were collected in 2020 and 2021 in spring, summer, and autumn. In each container, samples were collected from the rhizosphere, and after their mixing, one 0.5 kg sample representative of the treatment was obtained. The protease activity in the soil was measured with the spectrophotometric method developed by Ladd and Butler [38]. The measurements were made after one-hour incubation of the samples at 50 °C and at a wavelength of 578 nm. The dehydrogenase activity was measured with a spectrophotometer and 1% TTC solution, according to the methodology developed by Thalman. The measurements were made after 24 h incubation of the samples at 30 °C at a wavelength of 485 nm (TTC test). The absorption method according by Gołębiowska and Pędziwilk [39] was applied to measure the soil respiratory activity. It was based on the amount of CO_2 released (CO_2 mg kg^{-1} 48 h^{-1}). All analyses were quadruplicated.

Soil samples for microbial abundance analyses were collected once—in autumn 2021. The microbial analysis was based on serial dilution—the method used in soil microbial ecology research. A selective agar was used to determine the counts of colony-forming units of bacteria, actinobacteria, oomycetes and fungi. The total count of bacteria (CFU 10^5 g^{-1} d.m.) was measured on standard Merck agar after five days of incubation at 28 °C; the count of actinobacteria (CFU 10^5 g^{-1} d.m.)—on Pochon agar after seven days of incubation at 24 °C (according to Grabińska-Łoniewska [40]); the count of oomycetes and fungi (CFU 10^4 g^{-1} d.m.)—on Martin agar after five days of incubation at 24 °C [41]. The cultures were quintuplicated.

The number and species composition of soil nematodes were analysed twice—in 2020 and 2021. Soil samples were collected with a soil sampler (diameter—30 mm) directly from the rhizosphere of the trees. The samples were collected from each container. Then, they were mixed, and a pooled sample was prepared (500 mL) for each variant. The quantitative analysis of nematodes was conducted at the Department of Nematology, Plant Protection Institute, Poznań, Poland. Existing keys were used to initially identify the species of nematodes [42,43]. The identifications based on morphology were confirmed by sequencing of molecular markers (D2-D3 28S rDNA).

2.3. Growth Strength Measurements

In autumn 2021, the strength of vegetative growth of two-year-old trees was measured. The following parameters were measured: the height of trees (cm), the number and total growth of side shoots (cm), the leaf weight (g) and area (cm^2). The height of all trees in each variant was measured from the root collar to the top of the main shoot. At the end of the growing season, 40 leaves were randomly collected from each variant and weighed. After weighing, the leaves were scanned and their area was measured with the DigiShape 1.9 software. The measurements were quadruplicated.

2.4. Statistical Analyses

The results were analysed statistically with the analysis of variance and Duncan's test, using the STATISTICA 12.1 program. The significance of differences was set at $\alpha = 0.05$.

3. Results and Discussion

3.1. Physicochemical and Biological Properties of Soil

The quality of soil was assessed on the basis of its enzyme activity and respiratory activity, the content of micro- and macronutrients, humidity, pH, and the count of microorganisms. Our experiment revealed significant differences in the physicochemical parameters of the soil, which depended on its earlier use. The replanted soil (RS) was more acidic than the crop rotation soil (CRS) (pH 4.8 and 5.0, respectively) and contained almost three times less organic matter (0.8% and 2.17%) (Table 2). The quality of replanted soil was significantly better in the variants with biofumigation. The soil pH increased from 4.8 to 5.0 (French marigold) and 5.5 (oilseed radish). The humus content also increased significantly and was the highest in the variant with white mustard. It was over 50% higher than in the variant with replanted soil without forecrops (RS), i.e., 0.8% and 1.09% (Table 2).

Table 2. Physicochemical and biological properties of the soil in 2021 (CRS = crop rotation soil; RS = replanted soil).

Treatments	pH (H_2O)	Bulk Density (kg m^{-3})	Salinity (g NaCl dm^{-3})	Humus Content (%)
CRS	5.0 ± 0.34 b	1660 ± 37 c	0.13 ± 0.04 c	2.17 ± 0.09 e
RS	4.8 ± 0.71 a	1790 ± 45 e	0.97 ± 0.06 a	0.80 ± 0.11 a
French marigold forecrop	5.0 ± 0.27 b	1610 ± 26 a	0.12 ± 0.04 b	1.19 ± 0.06 c
White mustard forecrop	5.3 ± 0.32 c	1640 ± 41 b	0.18 ± 0.03 e	1.24 ± 0.07 d
Oilseed radish forecrop	5.5 ± 0.28 d	1740 ± 35 d	0.14 ± 0.03 d	1.09 ± 0.06 b

Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

Humus is a basic source of nutrients available to plants. The rate of its mineralisation depends on the efficiency of the activity of soil microorganisms, which can be measured with the activity of soil enzymes [44]. It is believed that the assessment of quality of soil should be based on the activity of soil enzymes and other properties of soil [45]. Soil microorganisms (mainly bacteria) and root debris are basic sources of soil enzymes. One of the most important soil enzymes are oxidoreductases (dehydrogenases) and hydrolases (protease, urease). Our experiment showed significant differences in the activity of soil enzymes, which depended on the earlier use of the soil. The biggest differences were found in the dehydrogenase activity (0.56 and 1.22 cm^{-3} H_2 24 h^{-1} kg^{-1} DM, respectively) (Table 3). These enzymes are considered very sensitive indicators of changes in soil properties [46].

Table 3. Enzymatic and respiratory activity of the soil (average for years 2020–2021) (CRS = crop rotation soil; RS = replanted soil).

Treatments	Dehydrogenase Activity (in cm^{-3} H_2 24 h^{-1} kg^{-1} DM)	Protease Activity (in mg Tyrosine h^{-1} kg^{-1} DM)	Respiratory Activity (CO_2 in mg kg^{-1} 48 h^{-1})
CRS	1.22 ± 0.13 bc	2.96 ± 0.28 b	27.70 ± 1.03 b
RS	0.56 ± 0.08 a	1.97 ± 0.13 a	19.25 ± 4.97 a
French marigold forecrop	1.30 ± 0.17 c	3.88 ± 0.36 c	32.22 ± 1.94 d
White mustard forecrop	1.03 ± 0.03 b	3.04 ± 0.39 b	29.62 ± 3.63 c
Oilseed radish forecrop	1.31 ± 0.05 c	3.36 ± 0.25 b	32.61 ± 1.22 d

Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

The amount of CO_2 released from soil indicates the respiratory activity of soil microorganisms [47]. The earlier method of soil use significantly influenced the values of this parameter. The respiratory activity of the replanted soil (RS)—19.25 CO_2 in mg kg^{-1} 48 h^{-1} was significantly lower than that of the crop rotation soil (CRS)—27.70 CO_2 in mg kg^{-1} 48 h^{-1}.

The worse biological properties of the replanted soil, manifested by its enzyme activity and respiratory activity, were also observed in earlier studies [48]. The decrease in the enzyme activity and respiratory activity of replanted soil may result from its higher acidity. Järvan et al. [49] indicated that the count and activity of soil bacteria are reduced in an acidic environment. As a result, the dehydrogenase activity also decreases. An increase in the soil pH increases the activity of this enzyme [50,51].

A significant increase in both the enzyme activity and respiratory activity of the replanted soil in the variants with biofumigation were noted. On average, over the two years of the research, the dehydrogenase activity in the replanted soil in the variants with forecrops of French marigold (*Tagetes patula*) and oilseed radish (*Raphanus sativus* var. oleifera) was more than two times greater than in the soil without forecrops (1.3 and 0.56 cm^{-3} H_2 24 h^{-1} kg^{-1} DM, respectively) (Table 3). The analysis of the soil respiratory activity led to a similar conclusion (32.6 and 19.25 CO_2 mg kg^{-1} 48 h^{-1}). The protease activity in the replanted soil was the highest in the variant with French marigold. It is noteworthy that both the enzyme activity and respiratory activity of the replanted soil in the variants with biofumigation were significantly higher than in the control treatment with the crop rotation soil (CRS).

Plants used for biofumigation provide the soil with a large amount of organic matter. In consequence, there are a sufficient amount of nutrients for microorganisms, which produce more enzymes. These are the reasons for the increased enzyme activity and respiratory activity of the replanted soil in the variants with biofumigation treatment. Other researchers indicate a positive correlation between the content of organic matter in the soil and its enzyme activity [52,53].

The activity of soil enzymes depends on the physicochemical properties of soil (pH, organic matter content, heavy metal contamination), climate, and cultivation system [53,54]. According to Weaver [55], an insufficient amount of water in the soil may significantly limit the enzyme activity. In our experiment, the enzyme activity and respiratory activity of the of soil were analysed in spring, summer, and autumn. There were differences in the results depending on the vegetation period. The activity of soil dehydrogenases and proteases was the highest in autumn but the lowest in spring (Table 4).

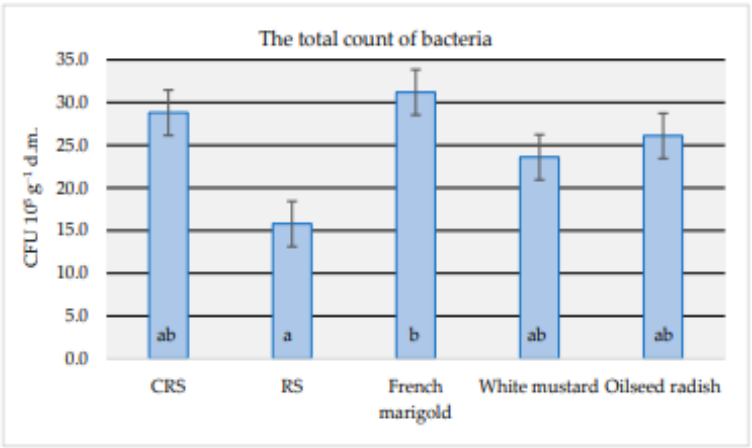
Table 4. The enzymatic and respiratory activity of the of soil during the vegetation period (average for years 2020–2021).

Vegetation Period	Dehydrogenase Activity (in cm^{-3} H_2 24 h^{-1} kg^{-1} DM)	Protease ACTIVITY (in mg tyrosine h^{-1} kg^{-1} DM)	Respiratory Activity (CO_2 in mg kg^{-1} 48 h^{-1})
Spring	0.89 ± 0.08 a	2.31 ± 0.72 a	31.53 ± 2.01 b
Summer	1.00 ± 0.07 a	3.17 ± 0.25 b	32.24 ± 2.12 b
Autumn	1.36 ± 0.09 b	3.66 ± 0.29 c	21.07 ± 1.94 a

Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

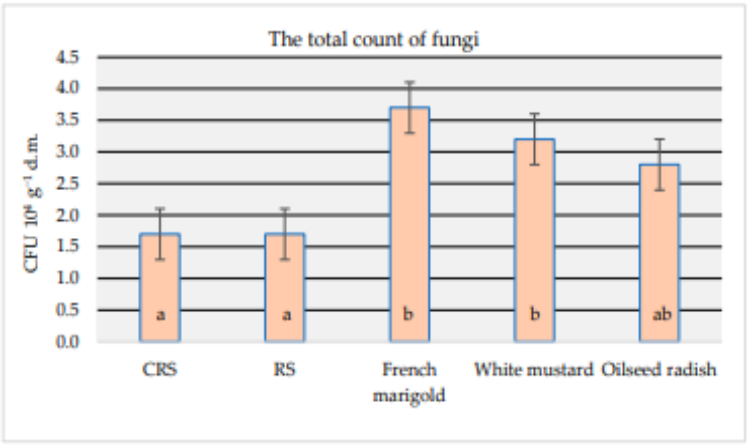
The high dehydrogenase activity in the soil in autumn was also observed by Yuan and Yue [52] and Zydlik et al. [6]. There was a different relationship in the soil respiratory activity. On average, in 2020 and 2021, it was the lowest at the end of the growing season. In autumn, the soil humidity is usually high and there is optimal temperature for the development of soil microorganisms. The availability of water considerably influences the activity of soil enzymes because increased moisture facilitates the solution of organic matter in the soil [56]. Sardans et al. [57] observed that a 10% decrease in the soil moisture caused the protease activity to drop by 15–66%. Results from the analysis of the weather conditions at the site of the experiment show that in September 2020 and 2021, the monthly rainfall was higher than the long-term average (Appendix A).

The high enzyme activity and respiratory activity of soil depends on the count and diversity of microbial communities. The analysis of the count of soil microorganisms conducted in our experiment confirmed the positive effect of biofumigation treatment on this parameter. The most effective plant was French marigold (*Tagetes patula*). The total bacterial count in the French marigold variant was almost two times greater than in the variant without it (Figure 1A).–



A

B



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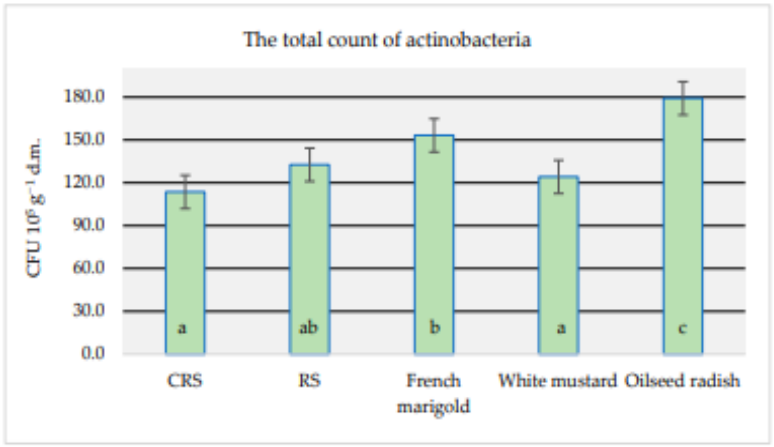

Figure 1. Microbial abundance in the soil (CRS = crop rotation soil; RS = replanted soil). Means marked with the same letters do not differ significantly at = 0.05. (**A**) The total count of bacteria; (**B**) The total count of fungi; (**C**) The total count of actinobacteria.

Hanschen and Winkelmann [31] also observed an increase in the count of plant growth-promoting bacteria in the replanted soil after the application of *Brassica juncea* and *Sinapis alba*. The analysis of the count of oomycetes and fungi gave similar results. The total count of oomycetes and fungi in the variants with French marigold and white mustard was more than two times greater than in the replanted soil without forecrops (Figure 1B). There was a slightly different result in the count of actinobacteria. The total count of these microorganisms (17.92 CFU 10^5 g^{-1} d.m.) was the highest in the oilseed radish variant (*Raphanus sativus* var. oleifera) (Figure 1C).

The increase in the soil enzyme activity increased the rate of mineralisation of organic matter, and consequently, the amount of macro- and micronutrients available to plants. In our experiment, significant differences in the content of macro- and micronutrients in the soil, depending on its earlier use, were noted. The content of all macro- and micronutrients (except Mn) in the replanted soil was significantly lower than in the crop rotation soil (Table 5). Other researchers also observed the low content of nutrients in replanted soil [5,6].

Table 5. Content of macro- and microelements (mg dm^{-3}) in the soil in 2021 (CRS = crop rotation soil; RS = replanted soil).

Mineral Elements	CRS	RS	French Marigold Forecrop	White Mustard Forecrop	Oilseed Radish Forecrop
N-NO_3	98.1 ± 8.62 c	83.2 ± 10.4 a	91.3 ± 9.77 b	132.6 ± 11.2 e	124.5 ± 10.7 d
P	310.3 ± 16.4 c	249.2 ± 21.7 a	275.0 ± 26.6 b	318.5 ± 30.9 d	440.0 ± 36.5 e
K	247.0 ± 20.6 b	190.2 ± 19.8 a	256.4 ± 22.3 d	251.4 ± 19.6 c	290.8 ± 21.9 e
Ca	117.5 ± 10.3 c	101.1 ± 9.4 a	105.0 ± 9.90 b	128.6 ± 11.2 d	127.4 ± 13.6 d
Mg	15.0 ± 1.34 a	16.0 ± 1.72 a	19.0 ± 1.63 b	16.0 ± 1.45 a	19.0 ± 1.9 b
Zn	7.7 ± 0.67 d	4.37 ± 0.74 a	6.17 ± 0.56 b	7.3 ± 0.60 c	8.3 ± 0.49 e
Cu	2.2 ± 0,11 c	1.56 ± 0.09 a	1.9 ± 0.08 b	2.3 ± 0.10 d	2.3 ± 0.94 d
Mn	50.8 ± 2.34 e	28.2 ± 1.96 b	20.4 ± 1.36 a	41.1 ± 0.45 d	40.1 ± 2.17 c
Fe	106.1 ± 10.3 d	96.9 ± 9.75 b	79.7 ± 8.32 a	111.6 ± 9.69 e	107.3 ± 9.73 d

Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

The increase in the microbial activity manifested by the higher enzyme activity and respiratory activity of the soil in the variants with forecrops of three species of biofumigants translated into an increase in the content of soil macro- and micronutrients. There was a significant increase in the content of N, P, K, Zn, Cu, and Fe in the replanted soil with the phytosanitary plants, especially in the variant with *Tagetes patula*. The content of minerals in the oilseed radish variant (*Raphanus sativus* var. oleifera) was from about 9% (Fe) to about 90% (Zn) greater than in the replanted soil (RS) without forecrops (Table 5).

3.2. *Growth Strength of Apple Trees*

The worse biological properties of the replanted soil, manifested by its enzyme activity and respiratory activity, resulted in a weaker vegetative growth of the apple trees. The plants in the RS variant were significantly shorter than those in the CRS treatment (117.3 and 138.7 cm, respectively). They had a smaller number of side shoots, and their total length was shorter (Table 6).

Table 6. Vegetative growth of the apple trees (CRS = crop rotation soil; RS = replanted soil).

Treatments	Height (cm)	Number of Side Shoots	Total Length of Shoots (cm)
CRS	138.75 ± 15.22 c	4.0 ± 1.26 b	25.24 ± 13.74 b
RS	117.30 ± 19.25 a	2.0 ± 0.87 a	6.87 ± 4.27 a
French marigold forecrop	123.22 ± 12.46 ab	3.0 ± 1.20 ab	24.26 ± 4.20 b
Whight mustard forecrop	125.02 ± 16.77 b	3.0 ± 1.41 ab	20.36 ± 7.37 b
Oilseed radish forecrop	121.05 ± 20.22 ab	3.0 ± 1.19 ab	24.11 ± 11.75 b

Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

The values of the other parameters of apple tree leaves under analysis, i.e., the weight and, especially, the surface area, were also several dozen per cent lower (Figure 2). Weiß et al. [9] also observed poor vegetative growth of apple rootstocks growing under ARD conditions in their experiment. Sobiczewski et al. [10] found that the leaf area of apple trees growing under ARD conditions was smaller. One of the reasons for the poor growth of plants growing under ARD conditions is the limited growth of their root system. According to Grunewaldt-Stöcker et al. [58], ARD causes the necrosis of root cells and inhibits the growth of hairy roots. In consequence, the uptake of water and nutrients by plants was significantly reduced.

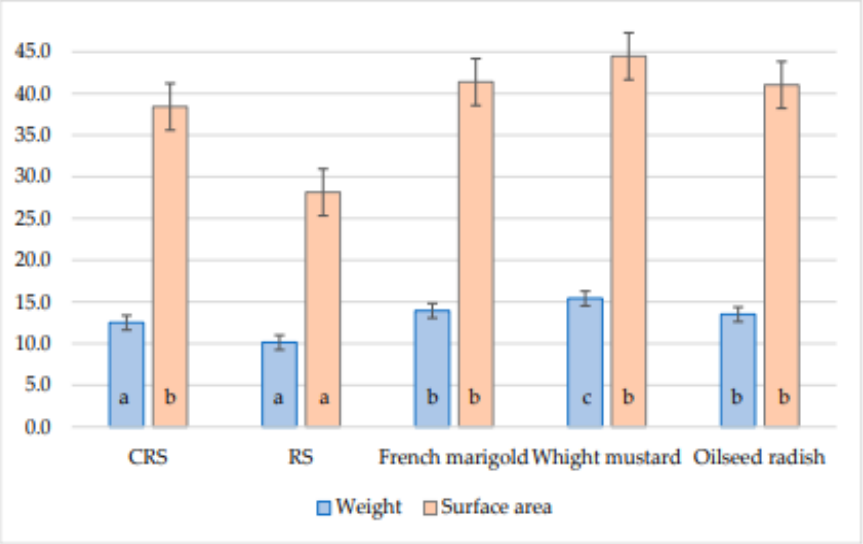

Figure 2. Biometric parameters of apple tree leaves (CRS = crop rotation soil; RS = replanted soil). Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

Three species of biofumigants used in our experiment improved the growth parameters of the apple trees cultivated on the replanted soil. The best effect was observed in the white mustard variant (*Sinapis alba*), where the weight of apple tree leaves was over 50% greater than in the replanted soil without forecrops—RS (15.1 and 10.1 g, respectively) (Figure 2). There were smaller differences between the French marigold and oilseed radish variants and the replanted soil without forecrops. The leaf area in the variants with the phytosanitary plants was about 50% greater than in the RS variant. The apple trees in the variants with the forecrops of phytosanitary plants were significantly taller than in the variant without forecrops (RS). The biggest differences were observed in the variants with *Sinapis alba* and *Raphanus sativus* var. oleifera (125, 126 and 177 cm, respectively) (Table 6).

In comparison with the RS variant, the total length of side shoots in the other variants increased significantly, regardless of the used plant species. The measured values were similar to those recorded in the control variant (CRS). There were no significant variant-dependent differences in the number of side shoots of the apple trees. As results from earlier studies show, biofumigation has a positive effect on the vegetative growth of plants growing under ARD conditions. This effect was observed in fruit trees growing on soil with French marigold [12,34] and in trees growing in a nursery [59]. The positive effect of phytosanitary plants on vegetative growth of fruit trees may result from the fact that they reduce pathogenic soil microorganisms responsible for the development of ARD, e.g., *Fusarium oxysporum* [34].

3.3. Species Composition and Number of Nematodes in Soil

Nematodes are among the biological causative agents responsible for the development of ARD [21]. In our experiment, eleven species of nematodes were identified in the soil (Table 7). In the control variant (CRS), there were four species of nematodes, mostly *Ecumenicus monohystera* (about 34 individuals in 100 cm^3 of soil). The population of *Mesorhabditis spiculigera* was much smaller. The replanted soil (RS) had the most nematodes of the *Mesorhabditis spiculigera* and *Tylenchorhynchus dubius* species (79 and 60 individuals in 100 cm^3 of soil, respectively) (Table 7).

Table 7. Average for years 2020–2021 nematode abundance in the soil (in 100 cm^{-3} of soil) (CRS = crop rotation soil; RS = replanted soil).

Species	CRS	RS	French Marigold Forecrop	Whight Mustard Forecrop	Oilseed Radish Forecrop
Cephalobus persegnis	4.1 ± 0.9 a	44.9 ± 9.2 b	0.0 ± 0.0 a	2.9 ± 1.0 a	1.0 ± 0.0 a
Cuticularia oxycerca	0.0 ± 0.0 a	3.3 ± 1.1 a	12.8 ± 3.4 b	3.3 ± 1.6 a	13.2 ± 3.1 a
Ecumenicus monohystera	33.9 ± 8.0 c	49.6 ± 5.6 c	44.2 ± 6.0 c	0.0 ± 0.0 a	42.7 ± 1.6 c
Geocenamus nothus	0.0 ± 0.0 a	16.5 ± 5.4 b	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
Mesorhabditis spiculigera	19.2 ± 5.2 b	79.2 ± 8.3 c	39.5 ± 4.9 b	9.9 ± 2.2 a	75.9 ± 14.8 c
Mononhoides sp.	6.6 ± 2.0 a	13.2 ± 6.7 c	6.6 ± 1.1 b	0.0 ± 0.0 a	9.9 ± 3.3 b
Panagralaimus rigidus	0.0 ± 0.0 a	56.2 ± 9.2 a	249.2 ± 24.5 b	240.9 ± 27.3 b	211.2 ± 28.1 b
Pratylenhus penetrans	0.0 ± 0.0 a	33.5 ± 5.8 c	0.2 ± 0.0 a	7.3 ± 2.0 b	7.2 ± 2.2 b
Prismatolaimus sp.	0.0 ± 0.0 a	49.5 ± 7.7 c	6.6 ± 1.0 a	19.5 ± 5.8 b	13.2 ± 3.5 a
Terrtocephalus terrestris	0.0 ± 0.0 a	21.3 ± 4.3 b	16.1 ± 3.7 b	36.0 ± 9.7 b	0.0 ± 0.0 b
Tylenchorhynchus dubius	0.0 ± 0.0 a	60.1 ± 8.8 c	3.7 ± 1.2 a	25.3 ± 4.4 b	25.1 ± 5.3 b

Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

According to Dutta et al. [35], the nematocidal properties of plants used for biofumigation have been well investigated. In our experiment, all the three species of biofumigants effectively reduced the number of nematodes, especially in the replanted soil. The French marigold (*Tagetes patula*) was highly effective, because in the variant with it, the number of nematodes of the *Pratylenhus penetrans* species was reduced from about 33 individuals in 100 cm^3 of soil to zero. This species belongs to the *Pratylenchidae* family, which is considered one of the most important pests in orchards as well as plantations of vegetables and ornamental plants. The *Pratylenhus penetrans* feed on roots, where they cause necrotic spots, which significantly reduce the active surface of the roots. The experiments conducted by Weerakoon et al. [60], Mazolla et al. [61], and Wang et al. [62] showed that the use of plants from the Brassicaceae family (*Brassica juncea*, *Sinapis alba*) or radish (*Raphanus sativus*) [16] reduced the number of phytopathogenic nematodes of the *Pratylenhus penetrans* species in replanted soil. In comparison with the variant without forecrops (RS), the forecrop of French marigold reduced the population of *Tylenchorhynchus dubius* several dozen times, the populations of *Prismatolaimus* sp. and *Geocenamus nothus*—about a dozen times, and the population of *Mononhoides* sp.—several times (Table 7). It is noteworthy that the population of *Tylenchorhynchus dubius* (another internal plant parasite after *Pratylenhus penetrans* that feeds on roots) in the replanted soil was significantly reduced. *Tylenchorhynchus dubius* is able to survive and develop in various environmental conditions. It occurs in the root zone of over one hundred plant species. The populations of species such as *Ecumenicus monohystera* and *Mononhoides* sp. in the soil with the white mustard forecrop (*Sinapis alba*) were completely reduced. The populations of *Cephalobus persegnis* and *Geocenamus nothus* were also reduced several times. There were no significant variant-dependent differences in the population of *Cuticularia oxycerca*. Oilseed radish was relatively the least effective in reducing the number of nematodes in the replanted soil. In the variant with the forecrop of this plant, the number of nematodes of the *Geocenamus nothus* and *Terrtocephalus terrestris* species in the soil was reduced to zero. However, the populations of *Ecumenicus monohystera* and *Mononhoides* sp. did not change.

The analysis of the populations of soil nematodes in individual years of the research showed that the nematocidal effect of the plants used for biofumigation was noticeable as early as one year after their application. The populations of eight of the eleven species of nematodes identified in 2020 decreased significantly in the following year of the research (Figure 3). These were mostly *Geocenamus nothus*, *Mesorhabditis spiculigera*, *Mononhoides* sp., *Pratylenhus penetrans*, and *Prismatolaimus* sp. In the second year of the experiment, no nematodes of these species were found in the soil.

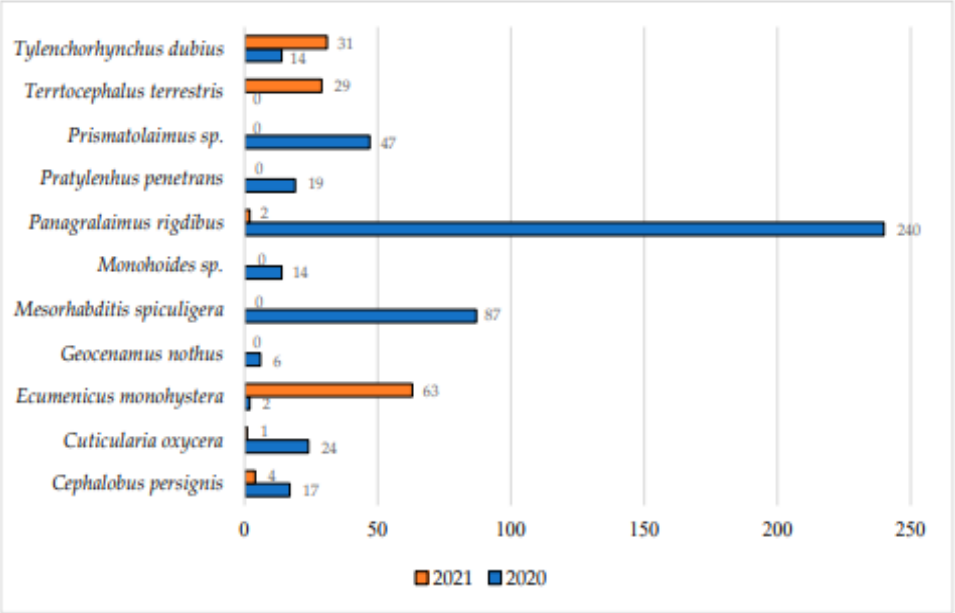

Figure 3. Nematode abundance in the replanted soil after biofumigation treatment (in 100 cm^{-3} of soil) in 2020 and 2021.

4. Conclusions

The results of our experiment confirmed the fact that the replanted soil was characterised by a lesser production value. It contained fewer minerals than the crop rotation soil and had worse biological parameters manifested by the enzyme activity and respiratory activity. The apple trees grew worse in such conditions. It was possible to improve the biological properties of the replanted soil by using three species of plants as forecrops. A more than double increase in the content of humus, as well as significant higher enzyme activity and respiratory activity in the replanted soil, in the variants with French marigold, white mustard, and oilseed radish was noted. Compared to the treatments without biofumigation, there was also a significant increase in the number of bacteria in the soil, especially in the variant with the use of *Tagetes patula*. This significantly improved the growth strength of the apple trees. The leaves of the trees in the variants with the biofumigation treatment had a larger surface area and weight (increase by 50%) than those from the trees growing on the replanted soil without forecrops. Also, the trees were taller and had a greater total growth of side shoots. The experiment also confirmed the nematocidal effect of the three species of the biofumigants, especially French marigold. The biofumigation treatment with its use made it possible to reduce the population of nematode *Pratylenhus penetrans* species from 33 to 0 individuals in 100 cm^{-3} of soil.

Biofumigation treatments using *Tagetes patula*, *Sinapis alba* and *Raphanus sativus* on replanted soil should be considered a safer and futuristic alternative to thermal disinfection and chemical fumigation, because it improves the biological properties of replanted soil and reduces the number of parasitic nematodes feeding on plants. It restores the balance of soil microorganisms and improves the growth strength of fruit trees in nurseries.

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Appendix A

Table A1. The course of temperatures and precipitation in the 2019–2021 growing seasons.

Months	Total Precipitation (mm)				Average Temperature (°C)			
	1982–2016	2019	2020	2021	1982–2016	2019	2020	2021
I	30.9	43.8	1.2	43.8	−0.8	−0.1	3.2	−0.6
II	24.4	13.0	36.6	24.4	0.1	2.8	4.6	−0.7
III	34.3	41.4	30.4	16.2	3.5	6.1	4.7	4.9
IV	29.8	6.6	4.6	35.2	9.3	10.3	8.9	7.1
V	49.5	74.4	49.4	94.6	14.5	12.1	11.6	11.9
VI	62.5	6.0	48.6	24.2	1.2	22.5	18.2	19.9
VII	78.2	34.8	78.2	29.8	19.5	19.4	18.5	20.7
VIII	60.0	35.4	57.4	60.6	18.9	20.6	20.3	18.9
IX	40.9	45.0	41.6	24.2	14.1	14.1	15.1	15.6
X	34.2	29.0	53.2	18.3	9.0	10.0	10.5	9.8
XI	37.5	34.7	11.4	21.2	3.9	8.9	5.9	7.7
XII	37.0	2.9	25.8	19.1	0.6	3.1	2.3	2.4
Total/Average	516.3	367.2	438.4	411.6	9.1	10.8	10.3	9.9

References

1. Nicola, L.; Insam, H.; Pertot, I.; Stres, B. Reanalysis of microbiomes in soils affected by apple replant disease (ARD): Old foes and novel suspects lead to the proposal of extended model of disease development. *Appl. Soil Ecol.* **2018**, *129*, 24–33. https://doi.org/10.1016/j.apsoil.2018.04.010.
2. Wolińska, A.; Kuźniara, A.; Zielenkiewicz, U.; Banach, A.; Błaszczykc, M. Indicators of arable soils fatigue–bacterial families and genera: A metagenomic approach. *Ecol. Indic.* **2018**, *93*, 490–500. https://doi.org/10.1016/j.ecolind.2018.05.033.
3. Zydlik, Z.; Zydlik, P. Effect of a preparation containing humic acids on selected physico-chemical and biological properties of replanted soil. *J. Elem.* **2020**, *25*, 993–1004. https://doi.org/10.5601/jelem.2020.25.2.2002.
4. Zydlik, Z.; Zydlik, P.; Kleiber, T. The effect of the mycorrhization on the content of macroelements in the soil and leaves of blueberry cultivated after replantation. *Zemdirb. Agric.* **2019**, *106*, 345–350. https://doi.org/10.13080/z-a.2019.106.044.
5. Cesarano, G.; Zotti, M.; Antignani, V.; Marra, R.; Scala, F.; Bonanomi, G. Soil sickness and negative plant-soil feedback: A reappraisal of Hypotheses. *J. Plant Pathol.* **2017**, *99*, 545–570. https://doi.org/10.4454/jpp.v99i3.3960.
6. Zydlik, Z.; Zydlik, P.; Wieczorek, R. The Effects of Bioinoculants Based on Mycorrhizal and Trichoderma spp. Fungi in an Apple Tree Nursery under Replantation Conditions. *Agronomy* **2021**, *11*, 2355. https://doi.org/10.3390/agronomy11112355.
7. Atucha, A.; Emmett, B.; Bauerle, T.L. Growth rate of fine root systems influences rootstock tolerance to replant disease. *Plant Soil* **2014**, *376*, 337–346. https://doi.org/10.1007/s11104-013-1977-5.
8. Emmett, B.; Nelson, E.B.; Kessler, A.; Bauerle, T.L. Fine-root system development and susceptibility to pathogen colonization. *Planta* **2014**, *239*, 325–340. https://doi.org/10.1007/s00425-013-1989-7.
9. Weiß, S.; Liu, B.; Reckwell, D.; Beerhues, L.; Winkelmann, T. Impaired defense reactions in apple replant disease-affected roots of *Malus domestica* 'M26'. *Tree Physiol.* **2017**, *37*, 1672–168.. https://doi.org/10.1093/treephys/tpx108.
10. Sobiczewski, P.; Treder, W.; Bryk, H.; Klamkowski, K.; Krzewińska, D.; Mikiciński, A.; Berczyński, S.; Tryngiel-Gać, A. The impact of phytosanitary treatments in the soil with signs of fatigue on the growth of apple seedlings and populations of bacteria and fungi. *Pol. J. Agron.* **2018**, *34*, 11–22. https://doi.org/10.26114/pja.iung.361.2018.34.02.
11. Zydlik, Z.; Zydlik, P. The Effect of a Preparation Containing Humic Acids on the Growth, Yield, and Quality of Strawberry Fruits (*Fragaria* x *ananassa* (Duchesne ex Weston) Duchesne ex Rozier). *Agronomy* **2023**, *13*, 1872. https://doi.org/10.3390/agronomy13071872.
12. Yim, B.; Hanschen, F.S.; Wrede, A.; Nitt, H.; Schreiner, M.; Smalla, K.; Winkelmann, T. Effects of biofumigation using *Brassica juncea* and *Raphanus sativus* in comparison to disinfection using Basamid on apple plant growth and soil microbial communities at three field sites with replant disease. *Plant Soil* **2016**, *406*, 389–408. https://doi.org/10.1007/s11104-016-2876-3.

13. Lucas, M.; Balbín-Suárez, A.; Smalla, K.; Vetterlein, D. Root growth, function and rhizosphere microbiome analyses show local rather than systemic effects in apple plant response to replant disease soil. *PLoS ONE*, **2018**, *13*, e0204922. https://doi.org/10.1371/journal.pone.0204922.g00.
14. Hofmann, A.; Wittenmayer, L.; Arnold, G.; Schieber, A.; Merbach, W. Root exudation of phlorizin by apple seedlings (*Malus x domestica Borkh.)* with symptoms of apple replant disease. *J. Appl. Bot. Food Qual.* **2012**, *82*, 193–198.
15. Utkhede, R.S. Soil sickness, replant problem or replant disease and its integrated control. *Allelopath. J.* **2006**, *18*, 23–38.
16. Mazzola, M.; Manici, L.M. Apple replant disease: Role of microbial ecology in cause and control. *Annu. Rev. Phytopathol.* **2012**, *50*, 45–65.
17. Kelderer, M.M.; Manici, L.; Caputo, F.; Thalheimer, M. Planting in the 'inter-row' to overcome replant disease in apple orchards: A study on the effectiveness of the practice based on microbial indicators. *Plant Soil* **2012**, *357*, 381–393. https://doi.org/10.1007/s11104-012-1172-0.
18. Manici, L.M.; Kelderer, M.; Caputo, F.; Mazzola, M. Auxin-mediated relationships between apple plants and root inhabiting fungi: Impact on root pathogens and potentialities of growth-promoting populations. *Plant Pathol.* **2015**, *64*, 843–851. https://doi.org/10.1111/ppa.12315.
19. Spath, M.; Insam, H.; Peintner, U.; Kelderer, M.; Kuhnert-Finkernagel, R.; Franke-Whittle, I.H. Linking soil biotic and abiotic factors to apple replant disease: A greenhouse approach. *J. Phytophatol.* **2015**, *163*, 287–299.
20. Yin, C.; Xiang, L.; Wang, G.; Wang, Y.; Shen, X.; Chen, X. How to Plant Apple Trees to Reduce Replant Disease in Apple Orchard: A Study on the Phenolic Acid of the Replanted Apple Orchard. *PLoS ONE*, **2016**, *11*, e01673470 https://doi.org/10.1371/journal.pone.0167347.
21. Kanfra, X.; Liu, B.; Beerhues, L.; Sørensen, S.J.; Heuer, H. Free-Living Nematodes Together With Associated Microbes Play an Essential Role in Apple Replant Disease. *Front. Plant Sci.* **2018**, *9*, 1666. https://doi.org/10.3389/fpls.2018.01666.
22. Siddique, S.; Grundler, F.M. Parasitic nematodes manipulate plant development to establish feeding sites. *Curr. Opin. Microbiol.* **2018**, *46*, 102–108. https://doi.org/10.1016/j.mib.2018.09.004.
23. Manici, L.M.; Kelderer, M.; Caputo, F.; Saccà, M.L.; Nicoletti, F.; Topp, A.R.; Mazzola, M. Involvement of *Dactylonectria* and *Ilyonectria* spp. in tree decline affecting multi-generation apple orchards. *Plant Soil* **2018**, *425*, 217–230. https://doi.org/10.1007/s11104-018-3571-3.
24. Long, F.; Lin, Y.M.; Hong, T.; Wu, C.Z.; Li, J. Soil sickness in horticulture and forestry: A review. *Allelopath. J.* **2019**, *47*, 57–72. https://doi.org/10.26651/allelo.j/2019-47-1-1219.
25. Nyoni, M.; Mazzola, M.; Wessels, J.P.B.; McLeod, A. The efficacy of semiselective chemicals and chloropicrin/1,3-dichloropropene–containing fumigants in managing apple replant disease in South Africa. *Plant Dis.* **2019**, *103*, 1363–1373.
26. Rudolph, R.E.; Zasada, I.A.; Hesse, C.; DeVetter, L.W. *Brassicaceous* seed meal, root removal, and chemical fumigation vary in their effects on soil quality parameters and *Pratylenchus penetrans* in a replanted floricane raspberry production system. *Appl. Soil Ecol.* **2019**, *133*, 44–51.
27. Li, J.; Huang, B.; Wang, Q.; Li, Y.; Fang, W.; Yan, D.; Guo, M.; Cao, A. Effect of fumigation with chloropicrin on soil bacterial communities and genes encoding key enzymes involved in nitrogen cycling. *Environ. Pollut.* **2017**, *227*, 534–542. https://doi.org/10.1016/j.envpol.2017.03.076.
28. Li, Q.; Zhang, D.; Cheng, H.; Song, Z.; Ren, L.; Hao, B.; Zhu, J.; Fang, W.; Yan, D.; Li, Y.; et al. Chloropicrin alternated with dazomet improved the soil's physicochemical properties, changed microbial communities and increased strawberry yield. *Ecotoxicol. Environ. Saf.* **2021**, *220*, 112362. https://doi.org/10.1016/j.ecoenv.2021.112362.
29. Cabos, R.Y.M.; Hara, A.H.; Tsang, M.M.C. Hot water drench treatment for control of reniform nematodes in potted dracaena. *Nematropica* **2012**, *42*, 72–79.
30. Fang, W.; Yan, D.; Wang, X. Responses of nitrogen-cycling microorganisms to dazomet fumigation. *Front. Microbiol.* **2018**, *9*, 2529. https://doi.org/10.3389/fmicb.2018.02529.
31. Hanschen, F.S.; Winkelmann, T. Biofumigation for fighting replant disease—A Review. *Agronomy* **2020**, *10*, 425. https://doi.org/10.3390/agronomy10030425.
32. Karakas, M.; Bolukbasi, E. A review: Using marigolds (*Tagetes* spp.) as an alternative to chemical nematicides for nematode management. *Int. J. Adv. Eng. Manag. Sci.* **2019**, *5*, 556–560. https://doi.org/10.22161/ijaems.59.3.
33. Barrau, C.; Porras, M.; Romero, E.; Zurera, K.; Ramos, N.; Soares, C.; Neto, E.; Marreiros, A.; Entrudo, J.; Romero, F. *Brassica carinata* for control of *Phytophthora* spp. in straw-berry field crops, *Rev. Ciências Agrárias 2009*, *32*, 135–138.
34. Wang, X.; Li, K.; Xu, S.; Duan, Y.; Wang, H.; Yin, C.; Chen, X.; Mao, Z.; Xiang, K. Effects of Different Forms of *Tagetes erecta* Biofumigation on the Growth of Apple Seedlings and Replanted Soil Microbial Environment. *Horticulturae* **2022**, *8*, 633. https://doi.org/10.3390/horticulturae8070633.
35. Dutta, T.K.; Khan, M.R.; Phani, V. Plant-parasitic nematode management via biofumigation using brassica and non-brassica plants: Current status and future prospects. *Curr. Plant Biol.* **2019**, *17*, 17–32. https://doi.org/10.1016/j.cpb.2019.02.001.
36. Hamaguchi, T.; Sato, K.; Vicente, C.S.L.; Hasegawa, K. Nematocidal actions of the marigold exudate -terthienyl: Oxidative stress-inducing compound penetrates nematode hypodermis. *Biol. Open* **2019**, *8*, bio038646.
37. Yim, B.; Nitt, H.; Wrede, A.; Jacquiod, S.; Sørensen, S.J.; Winkelmann, T.; Smalla, K. Effects of soil pre-treatment with Basamid®granules, *Brassica juncea*, *Raphanus sativus*, and *Tagetes patula* on bacterial and fungal communities at two apple replant disease sites. *Front. Microbiol.* **2017**, *8*, 1604. https://doi.org/10.3389/fmicb.2017.01604.

38. Ladd, N.; Butler, J.H.A. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biol. Biochem.* **1972**, *4*, 19–30.
39. Gołębiowska, J.; Pędziwilk, Z. CO_2 release as on index of biological activity of cultivated soils. *Acta Microbiol. Pol.* **1984**, *33*, 249–256.
40. Grabińska-Łoniewska, A. *Laboratory Classes in General Microbiology*, 2nd ed.; OWPW: Warsaw, Poland, 1999; ISBN 83-7207-136-5.
41. Martin, J.P. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* **1950**, *69*, 215–232. https://doi.org/10.1097/00010694-195003000-00006.
42. Brzeski, M.W. *Nematodes of Tylenchida in Poland and Temperate Europe*; Muzeum i Instytut Zoologii, Polska Akademia Nauk: Warsaw, Poland, 1998; 396p.
43. Andrássy, I. Free-living nematodes of Hungary (*Nematoda errantia*), II. In *Pedozoologica Hungarica*; Csuzdi, C., Mahunka, S., Eds.; Hungarian Natural History Museum: Budapest, Hungary, 2007; Volume 4, 496p. ISBN 963 7093 98 2.
44. Wolińska, A.; Rekosz-Burlagar, H.; Goryluk-Salmonowicz, A.; Błaszczyk, M.; Stępniewska, Z. Bacterial Abundance and Dehydrogenase Activity in Selected Agricultural Soils from Lublin Region. *Pol. J. Environ. Stud.* **2015**, *24*, 2677–2682. https://doi.org/10.15244/pjoes/59323.
45. Furtak, K.; Gałązka, A. Enzymatic activity as a popular parameter used to determine the quality of the soil environment. *Pol. J. Agron.* **2019**, *37*, 22–30. https://doi.org/10.26114/pja.iung.385.2019.37.04.
46. Gałązka, A.; Gawryjołek, K.; Perzyński, A.; Gałązka, R.; Księżak, J. Changes in Enzymatic Activities and Microbial Communities in Soil under Long-Term Maize Monoculture and Crop Rotation. *Pol. J. Environ. Stud.* **2017**, *26*, 39–46. https://doi.org/10.15244/pjoes/64745.
47. Meena, A.; Rao, K.S. Assessment of soil microbial and enzyme activity in the rhizosphere zone under different land use/cover of a semiarid region, India. *Ecol. Process.* **2021**, *10*, 16.
48. Zydlik, Z.; Zydlik, P. The effect of microbiological products on soil properties in the conditions of replant disease. *Zemdirb. - Agric.* **2013**, *100*, 19–24.
49. Järvan, M.; Edesi, L.; Adamson, A.; Võsa, T. Soil microbial communities and dehydrogenase activity depending on farming systems. *Plant Soil Environ.* **2014**, *10*, 459–463. https://doi.org/10.17221/410/2014-PSE.
50. Rodriguez-Loinaz, G.; Onaindia, M.; Amezaga, I.; Mijangos, I.; Garbisu, C. Relationship between vegetation diversity and soil functional diversity in native mixed-oak forests. *Soil Biol. Biochem.* **2008**, *40*, 49–60. https://doi.org/10.1016/j.soilb io.2007.04.015.
51. Kompała-Bąba, A.; Bierza, W.; Sierka, E.; Błońska, A.; Besenyei, L.; Woźniak, G. The role of plants and soil properties in the enzyme activities of substrates on hard coal mine spoil heaps. *Sci. Rep.* **2021**, *11*, 5155. https://doi.org/10.1038/s41598-021-84673-0.
52. Yuan, B.; Yue, D. Soil Microbial and Enzymatic Activities Across a Chronosequence of Chinese Pine Plantation Development On The Loess Plateau of China. *Pedosphere* **2012**, *22*, 1–12.
53. Zhan, J.; Sun, Q. Development of microbial properties and enzyme activities in copper mine wasteland during natural restoration. *Catena* **2014**, *116*, 86–94.
54. Błońska, E.; Lasota, J.; Januszek, K. Aktywność enzymatyczna leśnych gleb gruntowoglejowych. *Rocz. Glebozn.* **2012**, *63*, 3–8. (In Polish)
55. Weaver, M.; Zablotowicz, R.; Krutz, L.; Bryson, C.; Locke, M. Microbial and Vegetative Changes Associated With Development of a Constructed Wetland. *Ecol. Indic.* **2012**, *13*, 37–45.
56. Geisseler, D.; Horwath, W.; Scow, K. Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. *Pedobiologia* **2011**, *54*, 71–78. https://doi.org/10.1016/j.pedob i.2010.10.001.
57. Sardans, J.; Peñuelas, J.; Ogaya, R. Drought decreases soil enzyme activity in a Mediterranean Quercus ilex L. Forest. *Soil Biol. Biochem.* **2005**, *37*, 455–461. https://doi.org/10.1016/j.soilbio.2004.08.004.
58. Grunewaldt-Stöcker, G.; Mahnkopp, F.; Popp, C.; Maiss, E.; Winkelmann, T. Diagnosis of apple replant disease (ARD): Microscopic evidence of early symptoms in fine roots of different apple rootstock genotypes. *Sci. Hortic.* **2019**, *243*, 583–594 https://doi.org/10.1016/j.scienta.2018.09.014.
59. Kanfra, X.; Obawolu, T.; Wrede, A.; Strolka, B.; Winkelmann, T.; Hardeweg, B.; Heuer, H. Alleviation of Nematode-Mediated Apple Replant Disease by Pre-Cultivation of Tagetes. *Horticulturae* **2021**, *7*, 433. https://doi.org/10.3390/horticulturae7110433.
60. Weerakoon, D.M.N.; Reardon, C.L.; Paulitz, T.C.; Izzo, A.D.; Mazzola, M. Long-term suppression of *Pythium abappressorium* induced by *Brassica juncea* seed meal amendment is biologically mediated. *Soil Biol. Biochem.* **2012**, *51*, 44–52.
61. Mazzola, M.; Hewavitharana, S.S.; Strauss, S.L. Brassica seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen reinfestation. *Phytopathology* **2015**, *105*, 460–469.
62. Wang, Y.; Wei, K.; Han, X.; Zhao, D.; Zheng, Y.; Chao, J.; Gou, J.; Kong, F.; Zhang, C.S. The antifungal effect of garlic essential oil on *Phytophthora nicotianae* and the inhibitory component involved. *Biomolecules* **2019**, *9*, 632.

Publikacja 4

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The Response of the Mycobiome to the Biofumigation of Replanted Soil in a Fruit Tree Nursery

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Abstract: In a long-term monoculture with fruit trees and tree nurseries, it is necessary to regenerate the soil due to the risk of apple replant disease (ARD). The occurrence of ARD is manifested in the structure of the mycobiome. The assumption of our experiment was that the use of oil radish (*Raphanus sativus* var. *oleifera*), white mustard (*Sinapis alba*), and marigold (*Tagetes patula* L.) as phytosanitary plants for biofumigation would provide crops with nutrients, improve soil physicochemical properties, and influence the diversity of microbiota, including fungal networks, towards a beneficial mycobiome. Metagenomic analysis of fungal populations based on the hypervariable ITS1 region was used for assessing changes in the soil mycobiome. It showed that biofumigation, mainly with a forecrop of marigold (*Tagetes patula* L.) (R3), caused an improvement in soil physicochemical properties (bulk density and humus) and the highest increase in the abundance of operational taxonomic units (OTUs) of the *Fungi* kingdom, which was similar to that of agriculturally undegraded soils, and amounted to 54.37%. In this variant of the experiment, the most OTUs were identified at the phylum level, for *Ascomycota* (39.82%) and *Mortierellomycota* beneficial fungi (7.73%). There were no such dependencies in the soils replanted with forecrops of oilseed radish (*Raphanus sativus* var. *oleifera*) and white mustard (*Sinapis alba*). Biofumigation with marigold and oil radish contributed to a reduction in the genus *Fusarium*, which contains several significant plant-pathogenic species. The percentages of operational taxonomic units (OTUs) of *Fusarium* spp. decreased from 1.57% to 0.17% and 0.47%, respectively.

Keywords: biodiversity; fungal networks; *Mortierellomycota*; Eurotiales; physicochemical properties of soil

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1. Introduction

Intensive farming affects the natural soil microbiome, including its mycobiome, due to the lack of nutrient circulation as well as the use of large amounts of pesticides and monocultures [1]. This influence can also be observed in orchards. The soil mycobiome, also known as the fungal microbiome, is one of the main components of the integrative microbiome, which includes various groups of microorganisms inhabiting agroecosystems [2]. So far, the soil mycobiome has not been thoroughly investigated in relative terms, as in recent studies researchers around the world have focused mainly on soil bacteria [3]. Although up to now there have been rather few studies on fungi, these microorganisms are widely known to support various processes occurring in the ecosystem. They are also absolutely necessary for the sustainable development of agriculture [4]. They are involved

in plant–soil interactions, the decomposition of organic matter [5], plant health promotion, and nutrition [6].

Nowadays the vast majority of agricultural land requires soil regeneration and restoration of biological processes [7], which includes the restoration of all components of the soil mycobiome. It is important to stress the fact that it is particularly necessary to regenerate the soil under fruit trees, including the nurseries producing such trees, due to long-term monoculture. Due to the scarcity of new areas, producers establish nurseries in the same places, which often causes apple replant disease (ARD). ARD is usually described as a harmfully disordered physiomorphological response of apple plants to soils whose microbiome has changed as a result of an earlier plantation of apple trees [8]. It is also described as dysbiosis of the soil microbiome [9–11]. This soil dysfunction is believed to be mainly caused by fungi (*Rhizoctonia*, *Fusarium*, *Phytium*, *Phyophtora* spp., and others) and bacteria (the *Actinobacteria* phylum and the *Pseudomonas* and *Bacillus* genera) [12], as well as nematodes. According to Manici et al. [11], the main causes of ARD may be imbalance in the soil microbiota structure and the accumulation of harmful microorganisms. Zhao et al. [13] found that the severity of ARD in apple plantations was related to the increase in soil acidification and, consequently, a shortage of available minerals. Due to the wide variety of factors responsible for the occurrence of ARD and the complexity of their interactions, it is difficult to reduce the negative consequences of this disease [14–16].

ARD usually occurs in orchards with apple trees [8,12]. It is so frequent in these settings because apple trees are some of the most commonly grown fruit species in the world. Driven by the intensification of fruit production and the introduction of novel, more desirable cultivars, fruit growers are increasingly replacing their orchards with new plantings. Scientific reports show that when a new orchard replaces an old one, the new trees yield low-quality fruit and their aerial parts grow shorter [17,18].

As the authors of reference publications indicate, ARD can be counteracted by chemical fumigation, which involves disinfecting the soil with chemicals. However, the chemicals used for soil fumigation are toxic. The primary fumigants employed are dazomet and sodium methyldithiocarbamate (both precursors to methyl isothiocyanate), along with the mixture of 1,3-dichloropropene and chloropicrin [19–22]. These chemicals act totally (non-selectively). Their residues remain in the soil environment for a very long time, which significantly reduces the microbial populations and often extends the soil regeneration time. Therefore, the need to limit the use of chemical crop-protection products in horticultural production, including nursery production, is more and more often advocated.

ARD can also be limited by thermal disinfection (50–100 °C) or gamma radiation, which also strongly reduces the total soil microbiota [23,24].

Another method of combating ARD in orchard soils is anaerobic soil disinfection (ASD). The process involves the application of fast-biodegradable organic carbon to the soil, followed by the hermetic sealing of the soil surface with transparent foil. Consequently, soil microorganisms decomposing organic substances use the supply of oxygen completely. Despite these anaerobic conditions, some organisms do not die. However, it is noteworthy that the decomposition of organic material leads to the production of free volatile fatty acids. The toxicity of these acids reduces the abundance of the soil microbiota, including facultative anaerobes. ARD can also be combatted by applying composts to change the biodiversity of the soil environment [25,26].

The problem of soil recultivation in orchards can be solved through environmentally friendly agriculture, also known as sustainable agriculture. The holistic approach combining agricultural production with an uncontaminated environment is known as regenerative agriculture [27]. Biofumigation, which is based on the phenomenon of allelopathy, is a promising method of limiting the negative influence of replantation. Biofumigation entails the release of volatile organic compounds with biocidal properties. The deployment of suitable forecrops, particularly phytosanitary plant species, which emit phytoncides into the environment, can effectively reduce populations of harmful nematodes, bacteria, and pathogenic fungi within the soil. Commonly utilized phytosanitary plants include marigold

(*Tagetes patula* L.), white mustard (*Sinapis alba*), oilseed radish (*Raphanus sativus* var. *oleifera*), spring rapeseed (*Brassica napus* L.), oats (*Avena sativa* L.), rye (*Secale cereale*), and asparagus (*Asparagus officinalis* L.) [28].

Plants of the *Brassicaceae* family, including *Sinapis alba* and *Raphanus sativus*, produce secondary metabolites known as glucosinolates. These compounds undergo hydrolysis to yield bioactive isothiocyanates, which can be classified as aromatic (e.g., benzyl isothiocyanate and 2-phenylethyl isothiocyanate) or aliphatic (e.g., allyl isothiocyanate) [29]. The application of fresh biomass is advocated due to its elevated glucosinolate content. Members of the *Asteraceae* family, particularly *Tagetes* spp., synthesize insecticidal and nematicidal compounds. These phytochemicals, released as root exudates by mature plants, encompass thiophene derivatives such as α-terthienyl [30]. When employing phytosanitary plants, it is imperative to ensure thorough maceration prior to soil incorporation at a depth of 15–20 cm [31].

The use of phytosanitary plants is a vital aspect of the environmentally friendly agricultural policy of the European Union and a significant agrotechnical measure. These plants are seen as elements that protect the soil from erosion and regenerate habitats. Moreover, they decrease greenhouse gas emissions and thus limit the causes of global warming.

The use of appropriate quantities and compositions of phytosanitary plants at the right time is assumed to provide crops with nutrients, influence biochemical changes occurring in the soil, and improve its physicochemical properties. Consequently, the treatment will improve soil fertility and, above all, the diversity of microbiota. As a result, the fungal network will be altered towards the beneficial mycobiome.

The objective of this study was to characterize the mycobiome composition of apple tree nursery soil affected by apple replant disease (ARD) and to evaluate the impact of phytosanitary plants (*Tagetes patula* L., *Sinapis alba*, and *Raphanus sativus* var. *oleifera*) on mycobiome dynamics and soil physicochemical properties.

2. Materials and Methods

2.1. Experimental Design

The experiment was carried out on stagnic luvisol soil (according to the World Reference Base for Soil Resources) at a commercial apple nursery in Puszczykowo Zaborze, western Poland (52°25′49.10″ N, 17°11′34.08″ E), from 2019 to 2021. Soil samples were obtained from two distinct sites. The first site comprised agricultural soil optimally prepared for apple tree cultivation (henceforth, agricultural soil). The second site featured soil under apple trees for three consecutive years, exhibiting symptoms of apple replant disease (ARD) (henceforth, replanted soil). Three phytosanitary plant species were employed: *Tagetes patula* L. (var. Honey Moon), *Sinapis alba* (var. Gracia), and *Raphanus sativus* var. *oleifera* (var. Romesa), sourced from the Legutko Seed Company, Poland. The experimental design included five treatments: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*). All phytosanitary plants were sown directly into the soil following the removal of apple trees. During the subsequent early spring (March), the plants were incorporated into the soil through crushing. In early May, 7.5 L containers were filled with the amended soil, and apple grafts were planted therein. The experimental material consisted of Golden Delicious apple cultivars grafted onto M.9 rootstocks. Grafting was performed manually during the preceding winter. Each experimental treatment included 30 replicate containers.

2.2. Soil Analyses

Soil mycobiome composition was assessed once, using samples collected in September 2021. A 30 g soil aliquot was obtained from each experimental unit using a sterile laboratory spoon. Subsequently, these aliquots were pooled to form a composite sample of 900 g.

2.2.1. Physicochemical Analyses of Soil

Before the experiment, the physicochemical properties of the replanted soil and the agricultural soil were analyzed. After the experiment, the physicochemical properties of the replanted soil were determined after the application of the phytosanitary plants: *Tagetes patula* L. (R3), *Sinapis alba* (R4), and *Raphanus sativus* var. *oleifera* (R5). The pH of the soil was measured with the potentiometric method in H_2O (1:2 m/v). Salinity was determined using the conductometric method, the percentage of humus and carbon content using the Tiurin method, and the bulk density using the picnometric method. The universal method developed by Nowosielski, with 0.03N CH_3COOH (1:10 m/v) as an extract, was used to measure the content of mineral components. The content of N-NO_3 was measured with an ion-selective electrode (ISE). Atomic Absorption Spectrometry (AAS) was applied to analyze the contents of mineral nutrients, such as K and Mg. The chloride content was measured using the nephelometric method, the Ca content using the photometric method, and the P content using the colorimetric method.

2.2.2. Metagenomic Analysis of Fungi in Soil

The fungal populations were analyzed metagenomically based on hypervariable region ITS1. The specific primer sequences ITS1FI2 and 5.8S were used to amplify the selected region and prepare the library. A Q5 Hot Start High-Fidelity 2× Master Mix was used for the polymerase chain reaction (PCR). The PCR was conducted according to the recommendations given by the device manufacturer. An MiSeq sequencer was used for sequencing, with paired-end (PE) technology, 2 × 300 nt, and an Illumina v3 kit. Detailed information is available on the websites of the reagent manufacturers. The MiSeq device and MiSeq Reporter (MSR) v2.6 software were used for automatic preliminary data analysis. There were two stages of the analysis: 1. automatic sample demultiplexing; 2. generating fastq files containing raw reads.

The UNITE v8 reference sequence databases and the QIIME v2 software package ensured the classification of reads in the bioinformatic analysis at the species level. There were six stages of the analysis: (1) removal of adapter sequences—the Cutadapt program; (2) analysis of the quality of reads and removal of low-quality sequences (quality < 20, minimum length: 30)—the Cutadapt program; (3) combining paired sequences—the fastq-join algorithm; (4) clustering based on the selected database of reference sequences—the uclust algorithm; (5) removal of sequence chimeras—the usearch61 algorithm; (6) assignment of taxonomy to the selected reference sequence database—the blast algorithm.

2.3. *Statistical Analysis*

A comparative analysis of genus composition between experimental variants was visualized using several types of plots [32]. Differences in the mean abundance of bacteria between the variants were calculated and visualized. All analyses were conducted using the statistical package GenStat version 23.1 [33].

3. Results and Discussion

3.1. *Soil Physicochemical Properties*

Physicochemical analysis showed significant differences in mineral composition, humus content, and pH between all soil variants. The transplanted soil (R2) showed a higher bulk density and a significantly lower humus content compared to agricultural soil (R1) and soils subjected to biofumigation with phytosanitary plants, namely, French marigold (R3), white mustard (R4), and oilseed radish (R5) (Table 1). In addition, R2 contained reduced concentrations of mineral components (P, K, Ca, and Mg) compared to R1, R3, R4, and R5. This means that the replanted soil was characterized by low fertility, and it may indicate the occurrence of ARD. It is important to stress the fact that after the application of *Tagetes patula* L. (R3), *Sinapis alba* (R4), and *Raphanus sativus* var. *oleifera* (R5) as phytosanitary plants in the replanted soil, the analysis of physicochemical properties of the soil revealed an increase in the humus content from 1.70% (R1) to 3.10% (R3), 2.80%

(R4), and 2.9% (R5), respectively. The soil salinity was at the same level in all experimental variants, but the content of mineral components (P, K, Ca, and Mg) increased (Table 1). The bulk density of the soil decreased, which means that it was more aerated and thus a better environment for the development of microorganisms, including populations of beneficial fungi. There were similar findings in the research conducted by Zhang et al. [34], who found a significant increase in soil NH_4^+-N, NO_3^--N, available P and K, and organic matter after the application of chicken manure in a strawberry plantation. Sennett et al. [35] observed that biofumigation with mustard residues significantly increased soil respiration by reducing bulk density.

Table 1. The chemical analysis of the soil: agricultural soil—R1, replanted soil before the experiment—R2, replanted soil with a forecrop of marigold—R3, replanted soil with a forecrop of white mustard—R4, replanted soil with a forecrop of oil radish—R5.

Soil Properties	R1	R2	R3	R4	R5
pH (H_2O)	7.6	5.8	6.9	6.7	6.8
Bulk density (g dm^{-3})	1600	1830	1610	1640	1740
Salinity (g Na Cl dm^{-3})	0.23	0.23	0.23	0.23	0.23
Humus content (%)	4.88	1.70	3.10	2.80	2.95
N-NO_3 (mg dm^{-3})	<3.9	<3.9	<3.9	<3.9	<3.9
P (mg dm^{-3})	127	30	115	105	98
K (mg dm^{-3})	229	89	180	167	156
Ca (mg dm^{-3})	1333	240	1200	1180	1100
Mg (mg dm^{-3})	188	38	160	166	140
Cl (mg dm^{-3})	<21.3	<21.3	<21.3	<21.3	<21.3

3.2. Mycobiome Structure after Application of Phytosanitary Plants

To investigate metapopulation dynamics of fungi within ARD soils subjected to biofumigation, the ITS1 hypervariable region was analyzed. Next-generation sequencing, a highly sensitive technique for characterizing soil mycobiome composition and diversity, has gained widespread adoption. This fact was confirmed by the findings of our experiment and data in reference publications [36–38]. The ITS analysis showed that in each of the soil samples, *Fungi* (18.94–54.19%) and *Viridiplantae* (14.13–18.69%) were the largest populations of organisms (Figure 1).

The percentage of operational taxonomic units (OTUs) representing fungi identified in our research showed that the share of OTUs in the agricultural soil amounted to 54.19%, whereas the share of OTUs in the ARD soil was only 25.65%. Biofumigation applied to the ARD soil, mainly with the forecrop of marigold (*Tagetes patula* L.) (R3), increased the abundance of fungi most. No such relationships were observed in the replanted soil with the forecrops of white mustard and oil radish, where the shares of OTUs representing fungi amounted to 31.38% and 18.94%, respectively. This effect may have been caused by the improved air conditions in the soil. In comparison with the soil variants of the other phytosanitary plants, the bulk density of the soil in which marigold had been applied decreased significantly (Table 1). Zhang et al. [34] and Sennett et al. [35] made similar observations in their studies.

A significant reduction in the abundance of fungal taxa is not always a beneficial phenomenon for the soil environment. It is important to remember that this group of *Eucariota* includes not only pathogens but also symbionts (mycorrhizal fungi) and decomposers. This kingdom plays a key role in biogeochemical cycles [39]. Some species of fungi inhabiting the soil contribute to its bioremediation by absorbing large amounts of pollutants from the environment. Moreover, the mycelium, which extends underground like blood vessels in the human body, carries water and nutrients from and to various plants. Fungi support numerous processes taking place in the ecosystem and their functions are essential for the sustainability of agriculture in the future [4]. They are responsible for interactions

between plants and soil, decomposition of organic matter [5], and plant health promotion and nutrition [6].

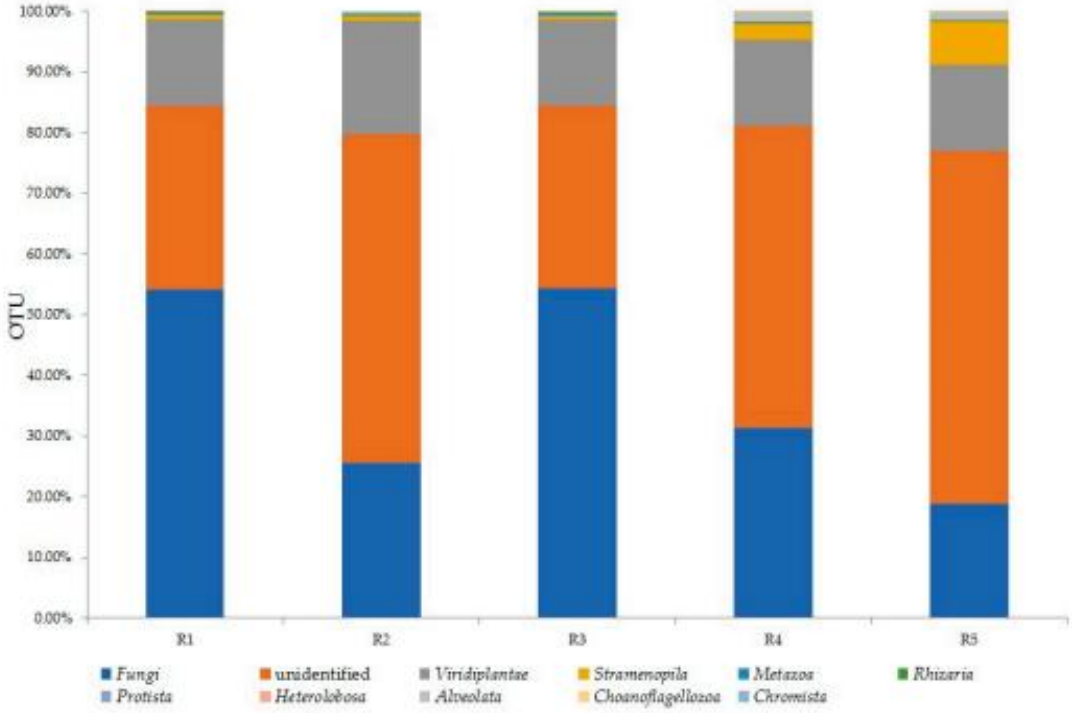

Figure 1. The percentages of operational taxonomic units (OTUs) of 11 eukaryotic organisms in five variants of the experiment: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*).

Apart from fungi, *Viridiplantae*, which occur in large numbers, are a clade of eukaryotic organisms with several hundred thousand species. According to reference publications, they also play an important role in both terrestrial and aquatic ecosystems [40]. The clade includes land plants (embryophytes), which evolved from green algae [41,42].

In our study, the highest percentage of OTUs belonging to this kingdom was found in the ARD soil (18.69%). There were smaller percentages of OTUs in the agricultural soil (14.18%) and in the replanted soils with the forecrops of marigold (14.23%), white mustard (14.28%), and oil radish (14.13%). Qiao et al. [43] observed similar dependencies in the reduction in *Viridiplantae*. The researchers conducted an experiment on a tomato plantation, where instead of biofumigation plants they treated the soil with beneficial microorganisms belonging to the PGPR (Plant-Growth-Promoting Rhizobacteria) group. After the treatment, the abundance of *Viridiplantae*, mainly organisms of the *Chlamydomonas* genus, was reduced. These microorganisms, isolated from fertile soils, secreted several antagonistic compounds, such as lipopeptide antibiotics, including surfactin, iturin, and fengycin, which significantly reduced the *Viridiplantae* population.

It is likely that in our study the plants used for biofumigation secreted secondary metabolites and reduced the abundance of the *Viridiplantae*, which was smaller than in the ARD soil. According to the data provided in reference publications, *Sinapis alba* and *Raphanus sativus*, which were used for biofumigation in our experiment, produce glucosinolates. They are hydrolyzed into bioactive isothiocyanate compounds—aromatic isothiocyanates, aliphatic allyl isothiocyanate, benzyl isothiocyanate, and 2–phenylethyl [29]. *Tagetes* L. produces thiophene compounds such as α–terthienyl [30].

The observations within the *Kingdom* category also revealed the presence of eukaryotic microorganisms which had not been classified before. Their shares amounted to 30.05% in the replanted soil with the forecrop of marigold (*Tagetes patula* L.) (R3), 30.28% in the agricultural soil (R1—control variant), up to 58.05% in the replanted soil with the oil radish forecrop, and 54.02% in the ARD soil (Figure 1).

According to Furtak et al. [38] and Wolna-Maruwka et al. [44], in data analysis, all obtained sequences should be taken into account, including those identified as unclassified or other. Unclassified taxa cannot be excluded or averaged and considered as a collective entity, as their contributions to the structure of soil eukaryotic and prokaryotic communities are substantial. Although this group of organisms have not been classified yet, they probably cause changes in environmental conditions and have a significant influence on changes occurring in the soil [45,46].

Apart from assessing and indicating the dominant kingdoms of eukaryotic organisms found in the soil samples, our research also included a relative analysis of differences in the number of operational taxonomic units (OTUs) between the ARD soil variant and the ARD soils subjected to biofumigation and the control soil variant: R2 vs. R1, R3 vs. R1, R4 vs. R1, and R5 vs. R1. The results are shown in Figure 2. The greatest differences were found in R5 vs. R1 for the fungi and for the unclassified microorganisms. The smallest differences were observed in R3 vs. R1. However, it is noteworthy that there were significant differences in all variants for the unclassified organisms.

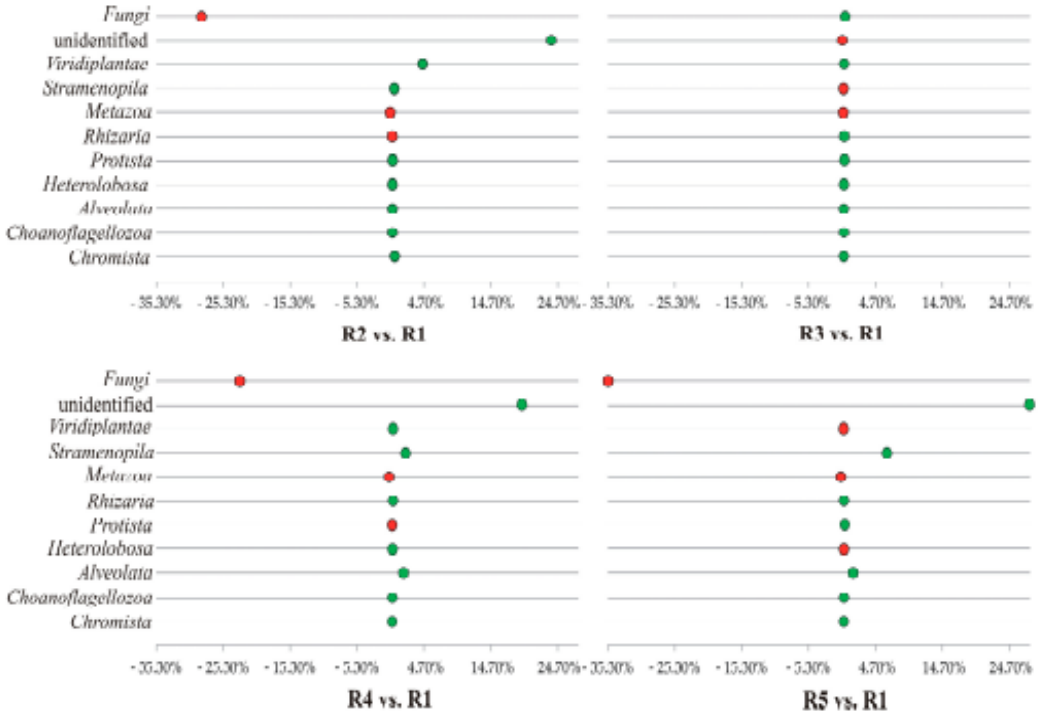

Figure 2. Relative abundance of 11 eukaryotic organisms in five variants of the experiment: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*). Green dots indicate positive differences; red dots indicate negative differences.

Metapopulation analysis targeting the hypervariable ITS1 region revealed that the prior utilization of soil in the nursery and the phytosanitary status of plants employed in the experiment impacted the richness (OTU count) of fungal taxa exclusively at the phylum

level (Figure 3). Depending on the soil variant, there were 7–11 phyla found in the kingdom of fungi (Figure 3).

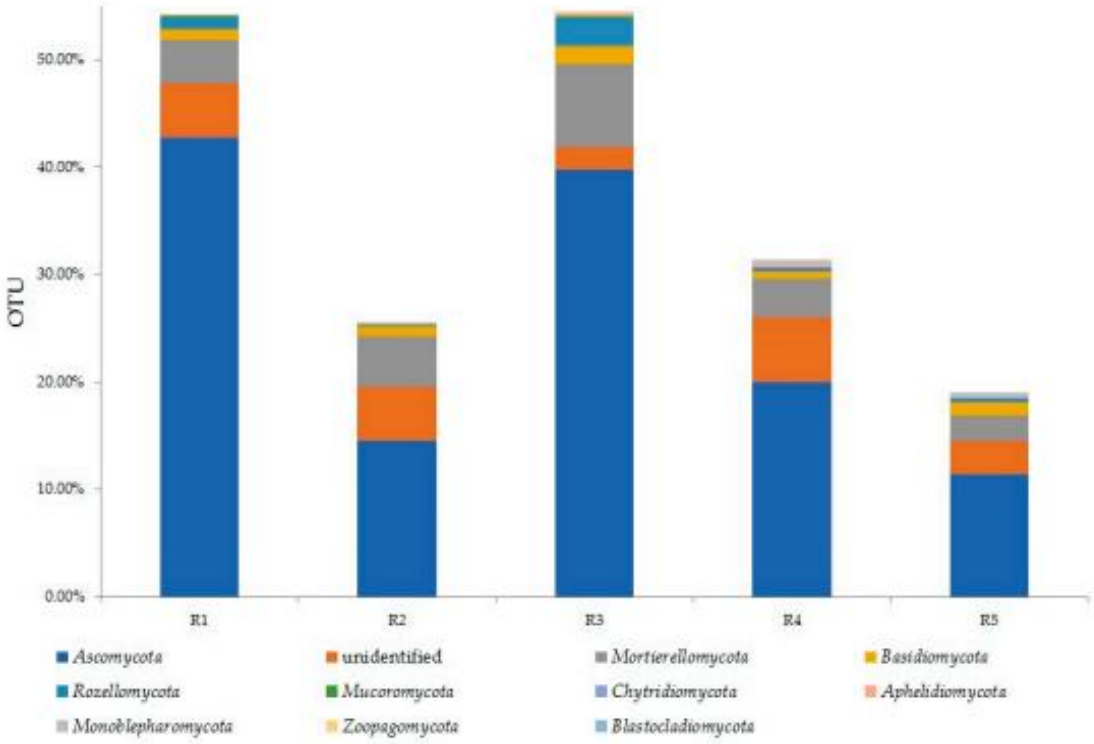

Figure 3. The percentages of operational taxonomic units (OTUs) of dominant phyla of fungi in five variants of the experiment: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*).

In all variants of the experiment, the largest number of OTUs within the phylum category was identified for *Ascomycota*. Their shares were much greater in the control soil and in the replanted soil with the forecrop of marigold (R3), i.e., 42.78% and 39.82%, respectively (Figure 3). By contrast, the number of OTUs was significantly reduced in the ARD soil, where their absolute value amounted to 14.48%.

According to the data provided in reference publications, *Ascomycota* commonly occur in agricultural soils. The microorganisms belonging to this group have various genes which make them resistant to the stress caused by agrotechnical treatments and, above all, environmental hypoxia. Our experiment showed that marigold (*Tagetes patula* L.) used as a forecrop (R3) in the ARD soil significantly increased the relative count of this group of fungi to a similar value to the one found in the agricultural soil.

Mortierellomycota was another dominant phylum in the soils under analysis. Its highest level was found in the replanted soil with the forecrop of marigold (*Tagetes patula* L.) (R3) (7.73%). The lowest level was in the replanted soil with the forecrop of oilseed radish (R5) (2.37%). In the R1 (control soil), the share of OTUs for *Mortierellomycota* was 4.07%, whereas in the ARD soil it was 4.68%.

According to the data provided in reference publications, members of the *Mortierellomycota* phylum are beneficial fungi. They support the production of phytohormones (e.g., gibberellins and indoleacetic acid) and provide symbiotic plants with nutrients, mainly phosphorus [47], by releasing various organic acids which dissolve recalcitrant inorganic forms of phosphorus [48].

The dominant members of the *Mortierellomycota* phylum play an important role, as they regulate the functional processes of the network ecosystem [49]. They are mainly involved in the soil phosphorus cycle [50]. However, further research is necessary to specifically determine how these species promote the soil phosphorus cycle.

The smallest absolute differences in the OTUs in the phyla identified in the analysis were found between R3 and R1, whereas the biggest difference was found between R5 and R1. For *Ascomycota*, the difference between all variants was greater than 2% (Figure 4). There was also a difference of over 2% between R2 and R1 and between R3 and R1 for the unclassified microorganisms and between R3 and R1 for *Mortierellomycota*.

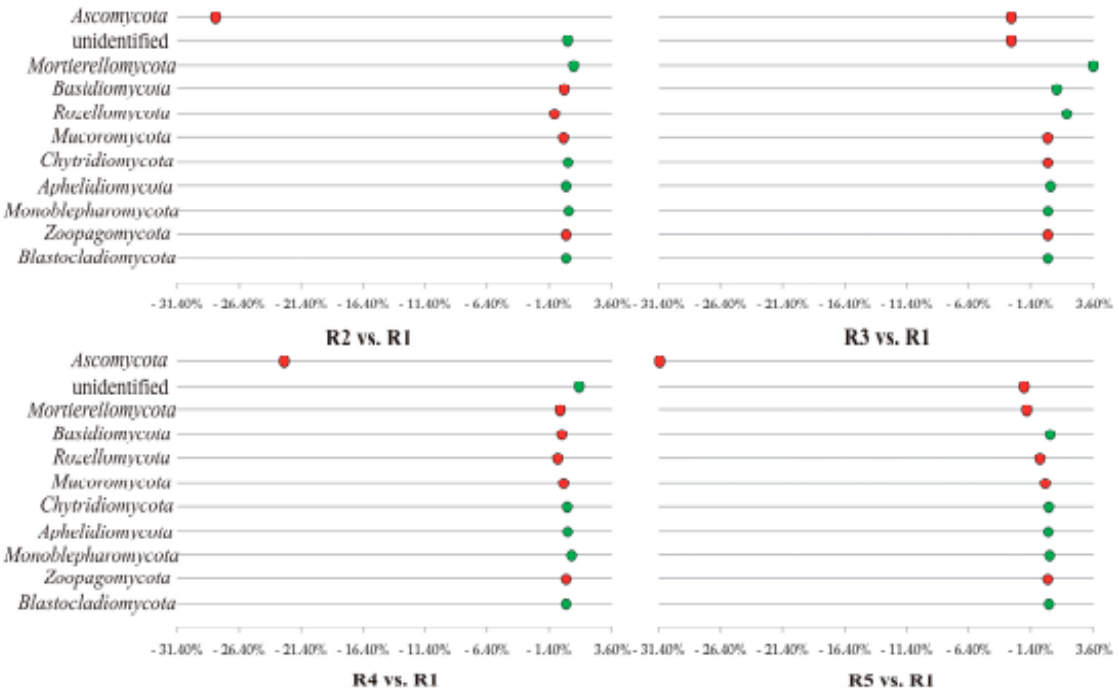

Figure 4. Relative abundance of dominant phyla of fungi in five variants of the experiment: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*). Green dots indicate positive differences; red dots indicate negative differences.

Further analyses involved assessment of the level of dominant classes within the mycobiome in the ARD soil; in the ARD soils with the forecrops of marigold, white mustard, and oil radish; and in the agricultural soil. As there was a large number of operational taxonomic units (OTUs) of fungi at the class level (29), only those whose share in at least one of the experimental variants exceeded 1% are shown in Figure 5. The observations of the metapopulation at the class level revealed the dominance of *Eurotiomycetes* in all variants (ranging from 6.10% in R5 to 37.78% in R1), except R4, where *Sordariomycetes* had the largest number of OTUs (9.29%). Generally, in all variants of our experiment, *Tremellomycetes* was the class with the lowest number of taxonomic units.

The greatest negative differences in OTUs at the class level between the ARD soil (R1), the ARD soils where biofumigation had been applied, and the control soil (R1) were observed for *Eurotiomycetes* (−31.68–−9.21%). The greatest positive difference (6.53% OTUs) was observed for the *Sordariomycetes* class between variants R4 and R1. Moreover, in four cases, the OTU difference between variants R3 and R1 was greater than 2% (the absolute

value): unclassified microorganisms (−2.9%), *Mortierellomycetes* (3.66%), *Sordariomycetes* (2.35%), and *Ascomycota*;c__unidentified (3.05%) (Figure 6).

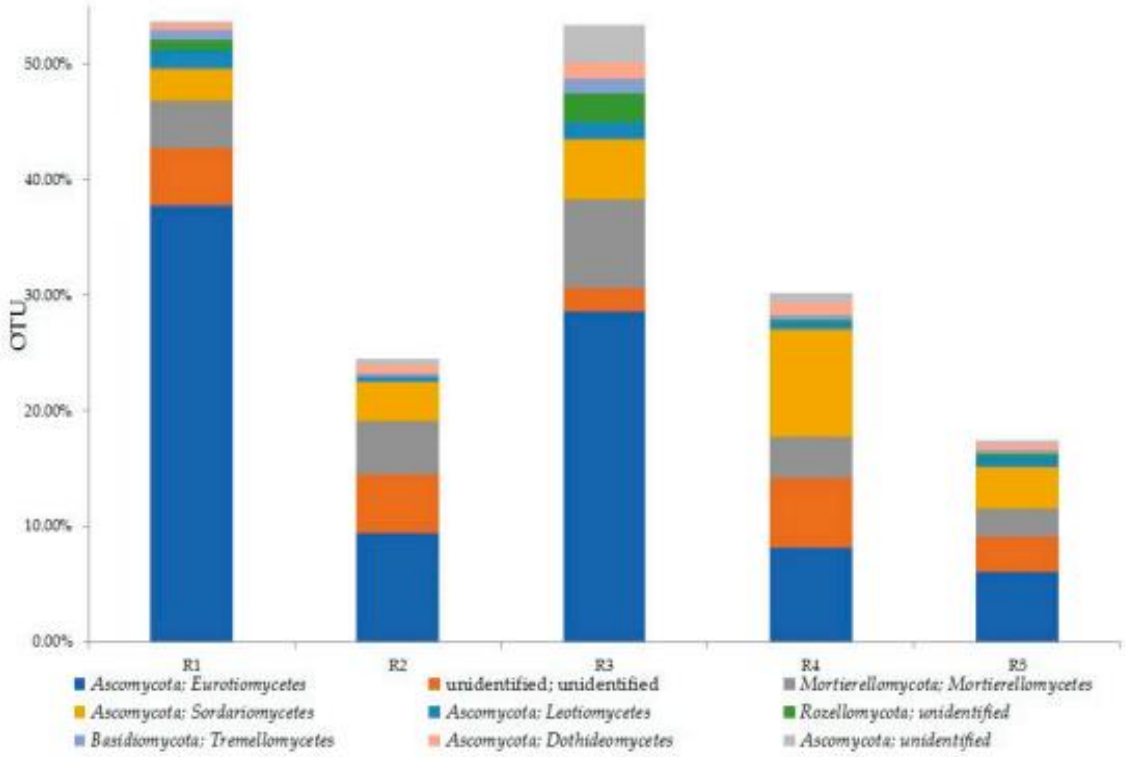

Figure 5. The percentages of operational taxonomic units (OTUs) of the dominant classes of fungi in five variants of the experiment: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*).

The results of our research showed that biofumigation positively affected the changes occurring in the replanted soils, especially in the variant with the forecrop of marigold (R3). *Eurotiomycetes* and *Sordariomycetes* were the dominant classes, depending on the biofumigation plant variant and in the agricultural soil. Other researchers observed similar correlations, i.e., the development and dominance of the *Eurotiomycetes* and *Sordariomycetes* classes of filamentous fungi in agricultural soils. These classes of fungi secrete antimicrobial proteins (AMPs), which play an important role in combating fungal diseases of crops and in soils with ARD. Hegedüs and Marx [51] as well as Meyer and Jung [52] demonstrated that AMPs function as defensive and/or signaling molecules within their host organisms. Fungal AMPs have been shown to exhibit potent activity against phytopathogenic fungi, including *Botrytis* spp. and *Fusarium* spp. [53]. Furthermore, certain fungal AMPs possess antiyeast properties and effectively inhibit the growth of pathogenic yeasts like *Candida albicans* at low micromolar concentrations [μM] [54–56]. According to Huber et al. [56], apart from inhibiting the growth of yeasts and filamentous fungi, some AMPs also have antiviral potential but do not exhibit any cytotoxic or hemolytic activity in mammalian cells. It is a serious challenge to control plant diseases caused by filamentous fungi due to the fact that species such as *Fusarium* spp. are responsible for enormous crop losses every year [57]. Therefore, researchers are continuing investigations to develop alternative antifungal treatments and strategies of treating fungal diseases. It is believed that new therapeutic compounds can be developed from AMPs. These small (~5.6–6.6 kDa), cysteine-rich, and amphipathic proteins are secreted into a culture supernatant and can be easily purified

by applying ion-exchange chromatography due to their net positive charge. Therefore, it would be easy and inexpensive to launch large-scale industrial production of AMPs [58,59].

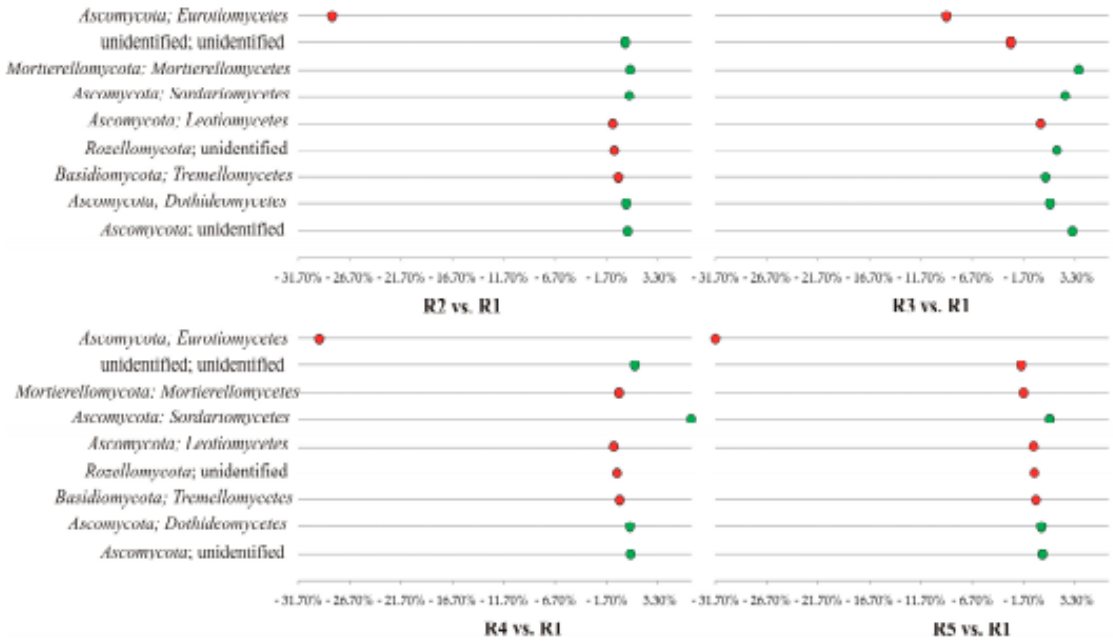

Figure 6. Relative abundance of the dominant classes of fungi in five variants of the experiment: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*). Green dots indicate positive differences; red dots indicate negative differences.

The relative number of OTUs of fungal orders was also estimated in our study. The analysis of the hypervariable ITS resulted in the identification of 61 orders. Similar to the analysis of the level of the classes of fungi, only those orders whose number in at least one of the tested variants exceeded 1% are shown in Figure 7.

Eurotiales was the dominant order in all variants of the experiment (ranging from 5.96% in R5 to 37.54% in R1). There was also a significant share of *Mortierellales* (ranging from 2.37% in R5 to 7.73% in R3) (Figure 7).

The analysis of differences in OTUs between the ARD soil, the ARD soils after biofumigation, and the control soil revealed the lowest negative values for *Eurotiales* (Figure 8). The difference in the OTUs between variants R4 and R1 for *Tremellales* was noteworthy, as it amounted to 6.02%. For R3 vs. R1, there were two differences of at least 2.5% observed: *Mortierellales* (3.66%) and unclassified microorganisms (−2.96%).

The analysis of fungal communities at the genus level showed that the biofumigation with marigold and oil radish reduced the population of the *Fusarium* genus, which includes several important plant-pathogenic species. The number of operational taxonomic units (OTUs) of *Fusarium* spp. decreased from 1.57% to 0.17% and 0.47%, respectively (Figure 9). The forecrop of white mustard did not have this effect. Wang et al. [60] also observed that selected phytosanitary plants caused various reductions in the population of *Fusarium* spp. fungi. Their study showed the highest reduction in a *Fusarium* spp. population, which included several important plant-pathogenic species, after the application of *Brassica juncea*. When *Allium fistulosum* and *Triticum aestivum* were used as phytosanitary plants, they reduced the population of these fungi to a much lesser extent.

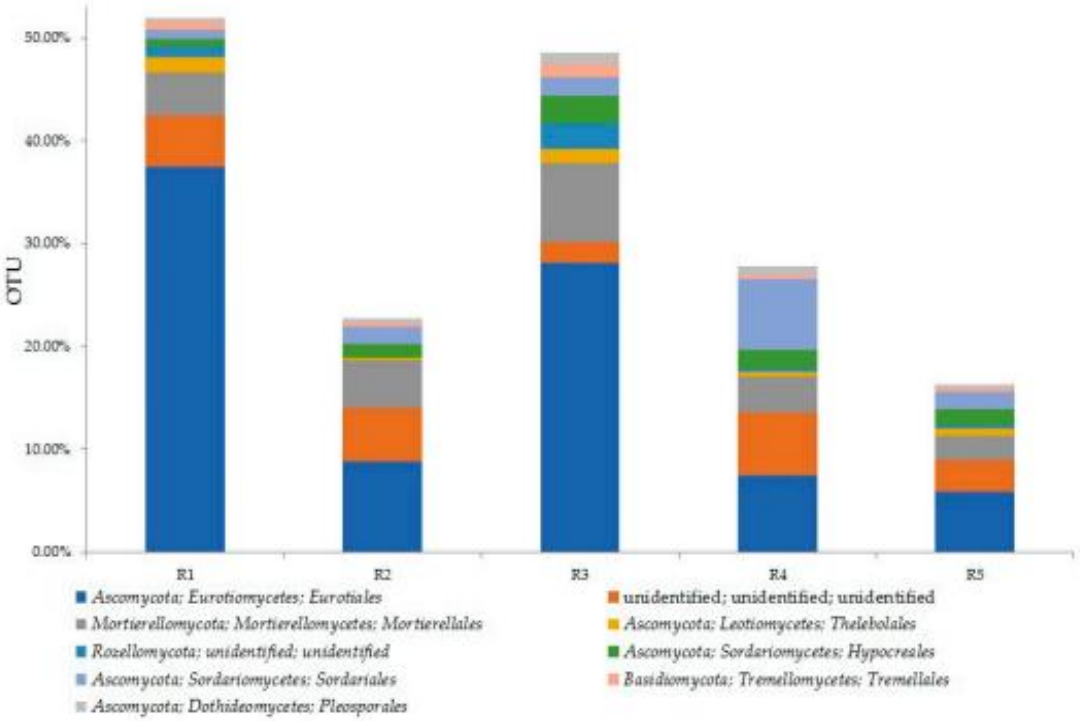

Figure 7. The percentages of operational taxonomic units (OTUs) of the dominant orders of fungi in five variants of the experiment: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*).

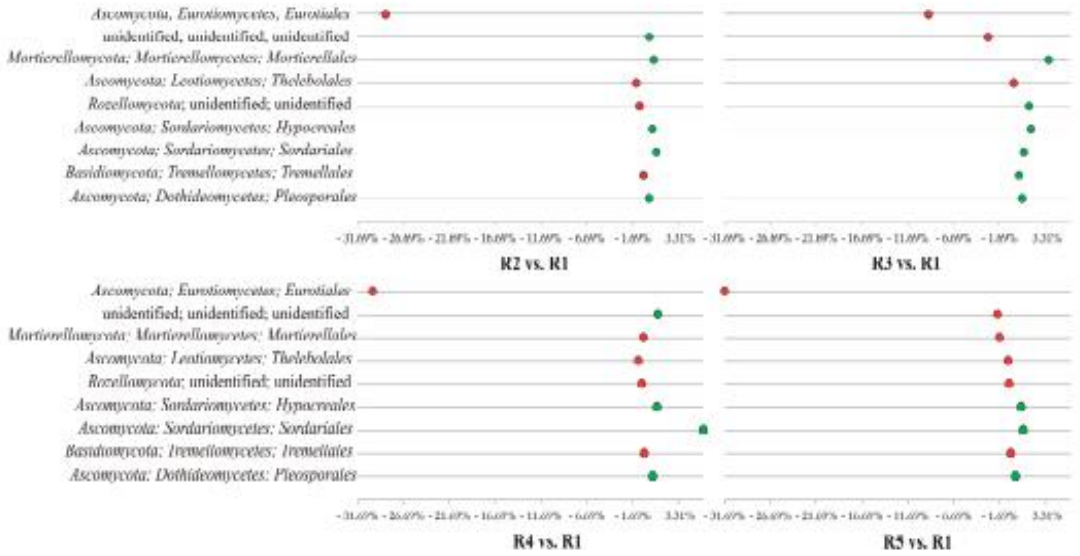

Figure 8. Relative abundance of the dominant orders of fungi in five variants of the experiment: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*). Green dots indicate positive differences; red dots indicate negative differences.

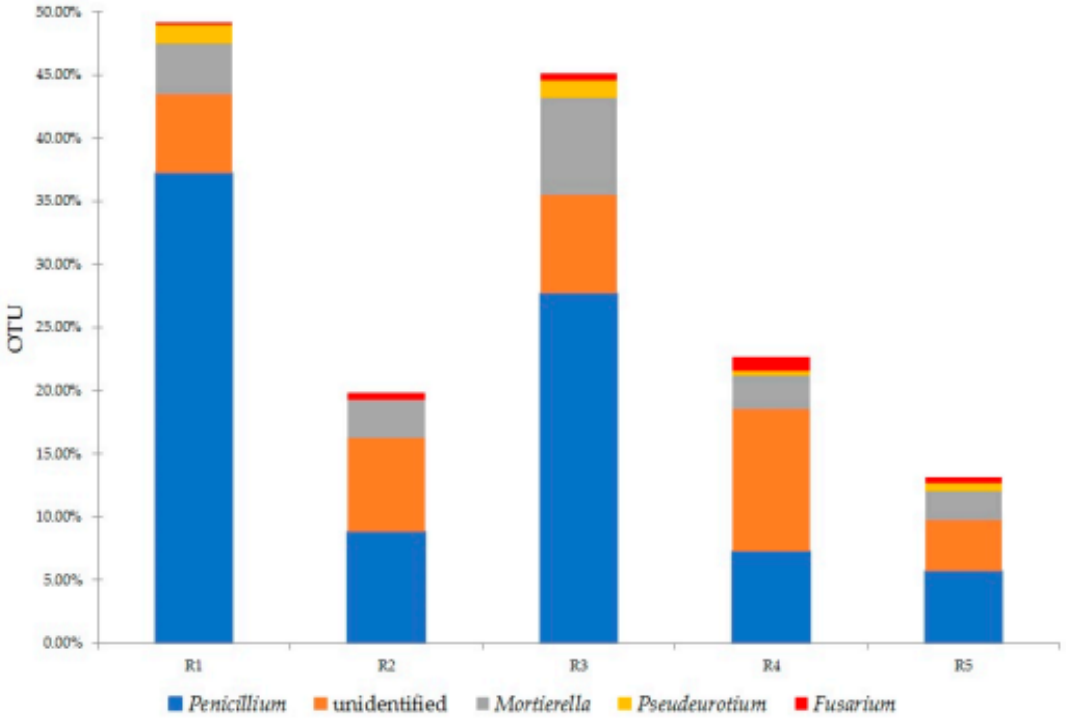

Figure 9. The percentages of operational taxonomic units (OTUs) of the dominant species of fungi in five variants of the experiment: R1—agricultural soil (control variant), R2—replanted soil, R3—replanted soil with a forecrop of marigold (*Tagetes patula* L.), R4—replanted soil with a forecrop of white mustard (*Sinapis alba*), and R5—replanted soil with a forecrop of oil radish (*Raphanus sativus* var. *oleifera*).

4. Conclusions

Metagenomic analysis, a functional assessment of soil-derived genetic material, was employed to evaluate alterations in the mycobiome following soil replantation in a fruit tree nursery. These analyses revealed that the implementation of phytosanitary plants, specifically marigold, white mustard, and oil radish, for biofumigation, significantly modified the fungal community structure and abundance in the replanted soil, which was also associated with an improvement in its physicochemical properties. This was particularly noticeable after the use of *Tagetes patula* L. The ITS analysis revealed an increase in the percentage of taxonomic units for the *Fungi* kingdom. Moreover, the number of OTUs for the *Ascomycota* phyla in this soil variant was a few times greater, and it was similar to the value in the control soil. The population of unclassified fungi in the replanted soil with the forecrop of marigold (*Tagetes patula* L.) was reduced. These fungi may have included some species which negatively affected the condition of trees in the nursery. Another dominant phylum in this experimental variant was *Mortierellomycota*. The analyses of the classes and orders showed that biofumigation, mainly with the marigold forecrop, resulted in the dominance of the *Eurotiomycetes* class and the *Eurotiales* order. This class of fungi plays an important role in combatting fungal diseases of plants, as well as in ARD soils. The use of white mustard and oil radish as forecrops did not have such strong analogous effects. Biofumigation with marigold and oil radish contributed to the reduction in fungi of the genus *Fusarium*, which includes several important plant-pathogenic species. The demonstration of the effect of biofumigation using phytosanitary plants as a tool for improving the physicochemical properties of the soil and the development of beneficial fungi and the reduction in pathogenic fungi of the genus *Fusarium* spp. is an important indicator for future scientific research and horticultural practice.

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References

1. Walker, B.A.; Powell, S.M.; Tegg, R.S.; Doyle, R.B.; Hunt, I.G.; Wilson, C.R. Ten years of green manuring and biofumigation alters soil characteristics and microbiota. *Appl. Soil Ecol.* **2023**, *187*, 104836. [CrossRef]
2. Geisen, S. The future of (soil) microbiome studies: Current limitations, integration, and perspectives. *mSystems* **2021**, *6*, e00613–e00621. [CrossRef] [PubMed]
3. Yadav, A.N.; Kour, D.; Kaur, T.; Devi, R.; Yadav, A.; Dikilitas, M.; Abdel-Azeem, A.M.; Ahluwalia, A.S.; Saxena, A.K. Biodiversity, and biotechnological contribution of beneficial soil microbiomes for nutrient cycling, plant growth improvement and nutrient uptake. *Biocatal. Agric. Biotechnol.* **2021**, 33, 102009. [CrossRef]
4. Fernandes, M.L.P.; Bastida, F.; Jehmlich, N.; Martinovič, T.; Větrovský, T.; Baldrian, P.; Delgado-Baquerizo, M.; Starke, R. Functional soil mycobiome across ecosystems. *J. Proteom.* **2022**, *252*, 104428. [CrossRef]
5. Větrovský, T.; Kohout, P.; Kopecký, M.; Machac, A.; Man, M.; Bahnmann, B.D.; Brabcová, V.; Choi, J.; Meszárošová, L.; Human, Z.R.; et al. A meta-analysis of global fungal distribution reveals climate-driven patterns. *Nat. Commun.* **2019**, *10*, 5142. [CrossRef]
6. Polme, S.; Abarenkov, K.; Nilsson, R.H.; Lindahl, B.D.; Clemmensen, K.E.; Kauserud, H.; Nguyen, N.; Kjøller, R.; Bates, S.T.; Baldrian, P.; et al. Fungal Traits: A user-friendly traits database of fungi and fungus-like stramenopiles. *Fungal Divers.* **2020**, *105*, 1–16. [CrossRef]
7. Gosnell, H. Regenerating Soil, Regenerating Soul: An Integral Approach to Understanding Agricultural Transformation. *Sustain. Sci.* **2022**, *17*, 603–620. [CrossRef]
8. Winkelmann, T.; Smalla, K.; Amelung, W.; Baab, G.; Grunewaldt-Stöcker, G.; Kanfra, X.; Meyhöfer, R.; Reim, S.; Schmitz, M.; Vetterlein, D.; et al. Apple replant disease: Causes and Mitigation Strategies. *Curr. Issues Mol. Biol.* **2019**, *30*, 89–106. [CrossRef]
9. Nicola, L.; Insam, H.; Pertot, I.; Stres, B. Reanalysis of microbiomes in soils affected by apple replant disease (ARD): Old foes and novel suspects lead to the proposal of extended model of disease development. *Appl. Soil Ecol.* **2018**, *129*, 24–33. [CrossRef]
10. Balbin-Suarez, A.; Lucas, M.; Vetterlein, D.; Sørensen, S.; Winkelmann, S.; Smalla, K.; Jacquiod, S. Exploring prokaryotic determinants of apple replant disease (ARD): A microhabitat approach under split-root design. *FEMS Microbiol. Ecol.* **2020**, *96*, fiaa211. [CrossRef]
11. Manici, L.M.; Ciavatta, C.; Kelderer, M.; Erschbaumer, G. Replant problems in South Tyrol: Role of fungal pathogens and microbial population in conventional and organic apple orchards. *J. Plant Soil* **2003**, *256*, 315–324. [CrossRef]
12. Mazzola, M.; Manici, L.M. Apple replant disease: Role of microbial ecology in cause and control. *Annu. Rev. Phytopathol.* **2012**, *50*, 45–65. [CrossRef]
13. Zhao, L.; Jiang, W.; Chen, R.; Wang, H.; Duan, Y.; Chen, X.; Shen, X.; Yin, C.; Mao, Z. Quicklime and Superphosphate Alleviating Apple Replant Disease by Improving Acidified Soil. *ACS Omega* **2022**, *7*, 7920–7930. [CrossRef]
14. Spath, M.; Insam, H.; Peintner, U.; Kelderer, M.; Kuhnert, R.; Franke-Whittle, I.H. Linking soil biotic and abiotic factors to apple replant disease: A greenhouse approach. *J. Phytopathol.* **2015**, *163*, 287–299. [CrossRef]
15. Tilston, E.L.; Deakin, G.; Bennett, J.; Passey, T.; Harrison, N.B.; O'Brien, F.; Fernandez, F.; Xu, X.M. Candidate causal organisms for apple replant disease in the UK. *Phytobiomes J.* **2018**, *2*, 261–274. [CrossRef]
16. Yin, C.; Duan, Y.N.; Xiang, L.; Wang, G.; Zhang, X.; Shen, X.; Chen, X.; Zhang, M.; Mao, Z. Effects of phloridzin, phloretin and benzoic acid at the concentrations measured in soil on the root proteome of *Malus hupehensis* Rehd Seedlings. *Sci. Hortic.* **2018**, *228*, 10–17. [CrossRef]
17. Liu, E.T.; Wang, G.S.; Li, Y.Y.; Shen, X.; Chen, X.S.; Song, F.H.; Wu, S.J.; Che, Q.; Mao, Z.Q. Replanting affects the tree growth and fruit quality of Gala apple. *J. Integr. Agric.* **2014**, *13*, 1699–1706. [CrossRef]
18. Henfrey, J.L.; Baab, G.; Schmitz, M. Physiological stress responses in apple under replant conditions. *Sci. Hortic.* **2015**, *194*, 111–117. [CrossRef]
19. Nicola, L.; Turco, E.; Albanese, D.; Donati, C.; Thalheimer, M.; Pindo, M.; Insam, H.; Cavalieri, D.; Pertot, I. Fumigation with dazomet modifies soil microbiota in apple orchards affected by replant disease. *Appl. Soil Ecol.* **2017**, *113*, 71–79. [CrossRef]
20. Nyoni, M.; Mazzola, M.; Wessels, J.P.B.; McLeod, A. The efficacy of semiselective chemicals and chloropicrin/1,3-dichloropropene–containing fumigants in managing apple replant disease in South Africa. *Plant Dis.* **2019**, *103*, 1363–1373. [CrossRef]

21. Rudolph, R.E.; Zasada, I.A.; Hesse, C.; DeVetter, L.W. *Brassicaceous* seed meal, root removal, and chemical fumigation vary in their effects on soil quality parameters and *Pratylenchus penetrans* in a replanted floricane raspberry production system. *Appl. Soil Ecol.* **2019**, *133*, 44–51. [CrossRef]
22. Hewavitharana, S.S.; Mazzola, M. Carbon source-dependent efects of aaerobic soil disinfestation on soil microbiome and suppression of *Rhizoctonia solani* AG-5 and *Pratylenchus penetrans*. *Phytopathology* **2016**, *106*, 1015–1028. [CrossRef] [PubMed]
23. Cabos, R.Y.M.; Hara, A.H.; Tsang, M.M.C. Hot water drench treatment for control of reniform nematodes in potted dracaena. *Nematropica* **2012**, *42*, 72–79.
24. Yim, B.; Winkelmann, T.; Ding, G.C.; Smalla, K. Different bacterial communities in heat and gamma irradiation treated replant disease soils revealed by 16S rRNA gene analysis—Contribution to improved aboveground apple plant growth? *Front. Microbiol.* **2015**, *6*, 1224. [CrossRef]
25. Franke-Whittle, I.H.; Juárez, M.F.D.; Insam, H.; Schweizer, S.; Naef, A.; Topp, A.R.; Kelderer, M.; Rühmer, T.; Baab, G.; Henfrey, J.; et al. Performance evaluation of locally available composts to reduce replant disease in apple orchards of central Europe. Renew. *Agric. Food Syst.* **2018**, *34*, 543–557. [CrossRef]
26. De Corato, U. Disease-suppressive compost enhances natural soil suppressiveness against soil-borne plant pathogens: A critical review. *Rhizosphere* **2020**, *13*, 100192. [CrossRef]
27. Newton, P.; Civita, N.; Frankel-Goldwater, L.; Bartel, K.; Johns, C. What Is Regenerative Agriculture? A Review of Scholar and Practitioner Definitions Based on Processes and Outcomes. *Front. Sustain. Food Syst.* **2020**, *4*, 577723. [CrossRef]
28. Wieczorek, R.; Zydlik, Z.; Wolna-Maruwka, A.; Niewiadomska, A.; Kayzer, D. The Effect of Biofumigation on the Microbiome Composition in Replanted Soil in a Fruit Tree Nursery. *Agronomy* **2023**, *13*, 2507. [CrossRef]
29. Ko, D.Y.; Seo, S.M.; Lee, Y.H.; Gil, C.S.; Lee, H.; Ku, K.M. Turning glucosinolate into allelopathic fate: Investigating allyl isothiocyanate variability and nitrile formation in eco-friendly *Brassica juncea* from South Korea. *Sci. Rep.* **2024**, *14*, 15423. [CrossRef]
30. Hamaguchi, T.; Sato, K.; Vicente, C.S.L.; Hasegawa, K. Nematicidal actions of the marigold exudate α-terthienyl: Oxidative stress-inducing compound penetrates nematode hypodermis. *Biol. Open* **2019**, *8*, bio038646. [CrossRef]
31. Kumar, G.N.K.; Jayasudha, S.M.; Kirankumar, K.C. Disease management by Biofumigation in organic farming system. *J. Pharmacogn. Phytochem.* **2018**, *7*, 676–679.
32. Kozak, M.; Bocianowski, J.; Sawkojć, S.; Wnuk, A. Call for more graphical elements in statistical teaching and consultancy. *Biom. Lett.* **2010**, *47*, 57–68.
33. *VSN International Genstat for Windows*, 23rd ed.; VSN International: Hemel Hempstead, UK, 2023.
34. Zhang, D.; Yan, D.; Cheng, H.; Fang, W.; Huang, B.; Wang, X.; Wang, X.; Yan, Y.; Ouyang, C.; Li, Y.; et al. Effects of multi-year biofumigation on soil bacterial and fungal communities and strawberry yield. *Environ. Pollut.* **2020**, *256*, 113415. [CrossRef]
35. Sennett, L.; Burton, D.L.; Goyer, C.; Zebarth, B.J. Influence of chemical fumigation and biofumigation on soil nitrogen cycling processes and nitrifier and denitrifier abundance. *Soil Biol. Biochem.* **2021**, *162*, 108421. [CrossRef]
36. Rawat, N.; Joshi, G.K. Bacterial community structure analysis of a hot spring soil by next generation sequencing of ribosomal RNA. *Genomics* **2019**, *111*, 1053–1058. [CrossRef] [PubMed]
37. Finley, S.J.; Benbow, M.E.; Javan, G.T. Potential applications of soil microbial ecology and next-generation sequencing in criminal investigations. *Appl. Soil Ecol.* **2015**, *88*, 69–78. [CrossRef]
38. Furtak, K.; Grządziel, J.; Gałązka, A.; Niedźwiecki, J. Prevalence of unclassified bacteria in the soil bacterial community from floodplain meadows (fluvisols) under simulated flood conditions revealed by a metataxonomic approachss. *Catena* **2020**, *188*, 104448. [CrossRef]
39. Frąc, M.; Hannula, E.S.; Bełka, M.; Salles, J.F.; Jedryczka, M. Soil mycobiome in sustainable agriculture. *Front. Microbiol.* **2022**, *13*, 1033824. [CrossRef]
40. Leebens-Mack, J.H.; Barker, M.S.; Carpenter, E.J.; Deyholos, M.K.; Gitzendanner, M.A.; Graham, S.W.; Grosse, I.; Li, Z.; Melkonian, M.; Mirarab, S.; et al. One Thousand Plant Transcriptomes and the Phylogenomics of Green Plants. *Nature* **2019**, *574*, 679–685.
41. Becker, B. Function and evolution of the vacuolar compartment in green algae and land plants (*Viridiplantae*). *Int. Rev. Cytol.* **2007**, *264*, 1–24.
42. Cocquyt, E.; Verbruggen, H.; Leliaert, F.; Zechman, F.W.; Sabbe, K.; De Clerck, O. Gain and loss of elongation factor genes in green algae. *BMC Evol. Biol.* **2009**, *9*, 39. [CrossRef]
43. Qiao, J.; Yu, X.; Liang, X.; Liu, Y.; Borriss, R.; Liu, Y. Addition of plant-growth-promoting *Bacillus subtilis* PTS-394 on tomato rhizosphere has no durable impact on composition of root microbiome. *BMC Microbiol.* **2017**, *17*, 131. [CrossRef]
44. Wolna-Maruwka, A.; Piechota, T.; Niewiadomska, A.; Kamiński, A.; Kayzer, D.; Grzyb, A.; Pilarska, A.A. The Effect of Biochar-Based Organic Amendments on the Structure of Soil Bacterial Community and Yield of Maize (*Zea mays* L.). *Agronomy* **2021**, *11*, 1286. [CrossRef]
45. Mhuantong, W.; Wongwilaiwalin, S.; Laothanachareon, T.; Eurwilaichitr, L.; Tangphatsornruang, S.; Boonchayaanant, B.; Limpiyakorn, T.; Pattaragulwanit, K.; Punmatharith, T.; McEvoy, J.; et al. Survey of microbial diversity in flood areas during Thailand 2011 Flood Crisis using high-throughput tagged amplicon pyrosequencing. *PLoS ONE* **2015**, *10*, e0128043. [CrossRef]
46. Delgado-Baquerizo, M. Obscure soil microbes and where to find them. *ISME J.* **2019**, *13*, 2120–2124. [CrossRef] [PubMed]

47. Gaiero, J.R.; Tosi, M.; Bent, E.; Boitt, G.; Khosla, K.; Turner, B.L.; Richardson, A.E.; Condron, L.M.; Dunfield, K.E. Soil microbial communities influencing organic phosphorus mineralization in a coastal dune chronosequence in New Zealand. *FEMS Microbiol. Ecol.* **2021**, *97*, fiab034. [CrossRef] [PubMed]
48. Zhang, J.; Feng, L.; Ouyang, Y.; Hu, R.; Xu, H.; Wang, J. Phosphate-solubilizing bacteria and fungi in relation to phosphorus availability under different land uses for some latosols from Guangdong, China. *Catena* **2020**, *195*, 104686. [CrossRef]
49. Li, B.B.; Roley, S.S.; Duncan, D.S.; Guo, J.; Quensen, J.F.; Yu, H.-Q.; Tiedje, J.M. Long-Term Excess Nitrogen Fertilizer Increases Sensitivity of Soil Microbial Community to Seasonal Change Revealed by Ecological Network and Metagenome Analyses. *Soil Biol. Biochem.* **2021**, *160*, 108349. [CrossRef]
50. Gao, W.H.; Gao, K.; Guo, Z.H.; Liu, Y.; Jiang, L.; Liu, C.; Liu, X.Y.; Wang, G.L. Different Responses of Soil Bacterial and Fungal Communities to 3 Years of Biochar Amendment in an Alkaline Soybean Soil. *Front. Microbiol.* **2021**, *12*, 630418. [CrossRef]
51. Hegedüs, N.; Marx, F. Antifungal Proteins: More than Antimicrobials? *Fungal Biol. Rev.* **2013**, *26*, 132–145. [CrossRef]
52. Meyer, V.; Jung, S. Antifungal Peptides of the AFP Family Revisited: Are These Cannibal Toxins? *Microorganisms* **2018**, *6*, 50. [CrossRef] [PubMed]
53. Marx, F. Small, basic antifungal proteins secreted from filamentous ascomycetes: A comparative study regarding expression, structure, function and potential application. *Appl. Microbiol. Biotechnol.* **2004**, *65*, 133–142. [CrossRef] [PubMed]
54. Lee, D.G.; Shin, S.Y.; Maeng, C.Y.; Jin, Z.Z.; Kim, K.L.; Hahm, K.S. Isolation and Characterization of a Novel Antifungal Peptide from *Aspergillus niger*. *Biochem. Biophys. Res. Commun.* **1999**, *263*, 646–651.
55. Tóth, L.; Kele, Z.; Borics, A.; Nagy, L.G.; Váradi, G.; Virágh, M.; Takó, M.; Vágvölgyi, C.; Galgóczy, L. NFAP2, a novel cysteine-rich anti-yeast protein from *Neosartorya fischeri* NRRL 181: Isolation and characterization. *AMB Express* **2016**, *6*, 75. [CrossRef]
56. Huber, A.; Hajdu, D.; Bratschun-Khan, D.; Gáspári, Z.; Varbanov, M.; Philippot, S.; Fizil, Á.; Czajlik, A.; Kele, Z.; Sonderegger, C.; et al. New Antimicrobial Potential and Structural Properties of PAFB: A Cationic, Cysteine-Rich Protein from *Penicillium chrysogenum* Q176. *Sci. Rep.* **2018**, *8*, 1751. [CrossRef] [PubMed]
57. Engalycheva, I.; Kozar, E.; Frolova, S.; Vetrova, S.; Tikhonova, T.; Dzhos, E.; Kostanchuk, Y. Fusarium Species Causing Pepper Wilt in Russia: Molecular Identification and Pathogenicity. *Microorganisms* **2024**, *12*, 343. [CrossRef]
58. Marx, F.; Binder, U.; Leiter, É.; Pócsi, I. The *Penicillium chrysogenum* antifungal protein PAF, a promising tool for the development of new antifungal therapies and fungal cell biology studies. *Cell. Mol. Life Sci.* **2008**, *65*, 445–454. [CrossRef]
59. Sonderegger, C.; Galgóczy, L.; Garrigues, S.; Fizil, Á.; Borics, A.; Manzanares, P.; Hegedüs, N.; Huber, A.; Marcos, J.F.; Batta, G. A *Penicillium Chrysogenum*-Based Expression System for the Production of Small, Cysteine-Rich Antifungal Proteins for Structural and Functional Analyses. *Microb. Cell Factories* **2016**, *15*, 192. [CrossRef]
60. Wang, H.Y.; Zhao, L.; Jiang, W.T.; Zhang, R.; Chen, R.; Mao, Y.F.; Chen, X.S.; Shen, H.; Yin, C.M.; Mao, Z.Q. Effects of *Allium fistulosum-Brassica juncea-Triticum aestivum* rotation a year on the soil microbial environment and the subsequent growth of young apple trees. *Sci. Hortic.* **2021**, *290*, 110549. [CrossRef]