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Julien Langrand

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Thèse de Doctorat

*Mention sciences agronomiques et écologiques
Spécialité biologie des organismes, biotechnologies animales, végétales et
microbiennes*

présentée à l'École Doctorale en Sciences Technologie et Santé (ED 585)

de l'Université du Littoral Côte d'Opale

par

Julien Langrand

pour obtenir le grade de Docteur de l'Université du Littoral Côte d'Opale

***Optimisation de la filière plantes aromatiques/huiles essentielles
dans un contexte de phytomanagement :
impact sur le microbiote des sols pollués par les éléments traces***

Soutenue le 3 juillet 2024, après avis des rapporteurs, devant le jury d'examen :

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Remerciements

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Liste des abréviations

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ADEME : Agence de l'environnement et de la maîtrise de l'énergie

ADN : Acide Désoxyribonucléique

ARN : Acide Ribonucléique

CE₅₀ : Concentration effectrice médiane

CI₅₀ : Concentration inhibitrice médiane

CMA : Champignon mycorhizien à arbuscules

DMSO : Diméthylsulfoxyde

dNTP : Désoxyribonucléotides triphosphate

DO : Densité optique

ET : Éléments traces

GC : Gas Chromatography – Chromatographie en phase gazeuse

GC-MS : Gas Chromatography-Mass Spectrometry – Chromatographie en phase gazeuse couplée à la spectrométrie de masse

HE : Huiles essentielles

ICP : Inductively Coupled Plasma spectrometry - Spectrométrie à plasma à couplage inductif

ICP-MS : Inductively Coupled Plasma Mass Spectrometry - Spectrométrie de masse à plasma à couplage inductif

OTU : Operational Taxonomic Unit - Unité taxonomique opérationnelle

PCR : Polymerase Chain Reaction – Réaction de polymérisation en chaîne

PPAM : Plantes à parfum aromatiques et médicinales

SAFIR : Sites Ateliers Français pour l'Innovation et la Recherche

SSP : Sites et sols pollués

β -CD : β -cyclodextrine

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De nos jours, la pollution des sols représente une problématique majeure à la fois sur le plan sanitaire et environnemental (Melebarry, 2023). On recense actuellement 10 millions de sites et sols pollués (SSP) à l'échelle planétaire, dont près de 3 millions en Europe (Gong et al., 2018; Mench et al., 2018). En France, 7 200 sites pollués sont répertoriés, dont près de 14 % sont localisés dans les Hauts-de-France, notamment à cause de son histoire industrielle et son passif minier (Raveau, 2020). En effet, les industries minières et sidérurgiques ont largement contribué par leurs rejets atmosphériques à la pollution des sols (Douay et al., 2013; Sterckeman et al., 2007). Parmi les polluants les plus fréquemment détectés dans les sites pollués, les éléments traces (ET) occupent une place importante (37% des SSP - Panagos *et al.*, 2013; Mench *et al.*, 2018).

Les ET sont omniprésents dans l'écosystème. Ils peuvent être d'origine naturelle (éruptions volcaniques, altération des roches, incendies de forêt ou érosion éolienne - Ali *et al.*, 2019; Melebarry, 2023) et principalement anthropique (processus industriels, activités agricoles, rejets d'eaux usées, exploitation minière, émissions de cheminées et des moteurs - Deng *et al.*, 2004; Khalid *et al.*, 2017). Les ET les plus abondants dans l'environnement sont le cuivre (Cu), le chrome (Cr), le plomb (Pb), le nickel (Ni), le mercure (Cd), le cadmium (Cd), l'arsenic (As) et le fer (Fe) (Rashid et al., 2023). En raison de leur non-biodégradabilité, de leur toxicité, et de leur bioaccumulation dans la chaîne alimentaire, les ET sont responsables de divers troubles chez l'Homme, notamment de maladies cardiovasculaires, de cancers, de troubles cognitifs, d'anémie chronique, de lésions des reins, du système nerveux, de la peau et des os (Gong et al., 2018; Järup, 2003). La pollution des sols par les ET menace non seulement la santé humaine, mais elle pose également des problèmes majeurs en termes d'équilibre des écosystèmes, de durabilité des systèmes agricoles et de sécurité alimentaire, en réduisant les rendements des cultures et en les rendant impropres à la consommation humaine ou animale.

C'est le cas du site Metaleurop, appartenant au réseau SAFIR (Sites Ateliers Français pour l'Innovation et la Recherche), sur lequel l'ancienne fonderie de plomb Metaleurop Nord située à Evin - Malmaison (62) a provoqué la contamination d'environ 730 ha de terres agricoles. En activité pendant plus d'un siècle, jusqu'à sa fermeture en 2003, la fonderie a émis des quantités considérables de poussières chargées en ET, qui se sont par la suite déposées, et ont contribué à la pollution, notamment des sols agricoles environnants (Douay et al., 2013; Sterckeman et al., 2002). Sur ce site trois ET sont majoritairement rencontrés : le Cd, le Pb ainsi que le Zn (Sterckeman et al., 2002). La pollution est d'autant plus préoccupante pour la population et les autorités qu'elle s'étend sur une grande surface, et touche près de 60 000 habitants. En outre, les ET sont sujet à la dispersion dans l'environnement notamment via des phénomènes d'érosion éolienne (envol de poussières de sol pollué) et de lixiviation (contamination des eaux de surface et/ou de la nappe d'eau souterraine), ce qui accroît le risque

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d'exposition directe ou indirecte (via la contamination de la chaîne alimentaire) pour les populations (Kumpiene et al., 2019).

La zone géographique de Metaleurop fait également l'objet d'arrêtés préfectoraux, encadrant la mise sur le marché des productions agricoles en fonction de leur degré de contamination. Trois zones sont ainsi définies autour de l'ancienne fonderie de la plus polluée à la moins polluée. Dans la zone 1 (> 1000 ppm de Pb ou > 20 ppm de Cd), qui représente environ 30 ha, toute activité agricole est interdite (Figure 1).



Figure 1 : Site expérimentale d'Evin Malmaison

Dans les zones 2 (500 à 1000 ppm de Pb et 4 à 10 ppm de Cd) et 3 (200 à 500 ppm de Pb ou entre 4 et 10 ppm de Cd), qui représentent environ 700 ha, les cultures sont soumises à analyse avant leur mise sur marché. Les productions dépassant strictement les seuils de contaminations réglementaires (en fonction des cultures pour l'alimentation humaine de 0,1 à 10 ppm de Pb, et de 0,1 à 1 ppm pour le Cd) sont déclassées ou détruites (Genet et al., 2018). Le coût des analyses, qui jusqu'alors était assumé par l'état, est depuis quelques années pris en charge par les agriculteurs concernés, ce qui représente un enjeu économique important pour le monde agricole. En outre, la zone est soumise à une forte pression démographique, qui combinée à la raréfaction des sols agricoles, n'a jamais rendu aussi urgente la question de la requalification des SSP.

Parmi les nombreuses méthodes de requalifications des SSP, les phytotechnologies, peuvent constituer une alternative intéressante d'un point de vue environnemental, socio-économique et technique (Gong et al., 2018). Contrairement aux techniques physico-chimiques, qui sont coûteuses, difficilement applicables *in situ* sur de larges surfaces, et destructrice pour toute forme de vie dans les sols, les phytotechnologies s'appliquent parfaitement à la gestion de grands sites (zones agricoles, urbaines, industrielles et minières) et permettent la préservation ou la réhabilitation des fonctions

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écologiques du sol comme support de culture, réservoir de la biodiversité et/ou source de production de biomasse non alimentaire (Lenoir et al., 2016). Parmi ces phytotechnologies, la phytostabilisation, basée sur l'utilisation de plantes tolérantes aux ET, ne transférant pas ou peu les ET dans leurs parties aériennes, et capables d'immobiliser les polluants au niveau rhizosphérique (Burgess et al., 2018; Mench et al., 2010), pourrait s'avérer appropriée à la gestion des sols pollués par les ET (Raveau, 2020; Raveau et al., 2021a). Afin d'augmenter l'efficacité de la phytostabilisation, celle-ci peut être assistée par des amendements organiques, minéraux, ou biologiques. Pléthore d'amendements biologiques ou encore minéraux et organiques sont actuellement disponibles suscitant un intérêt grandissant (Gong et al., 2018; Kumpiene et al., 2019; Siebielec et al., 2021). En effet, il a été montré que certains amendements améliorent l'installation du couvert végétal, la croissance des plantes et leurs capacités phytostabilisatrices (Wang *et al.*, 2018; Ma *et al.*, 2019; Kumar *et al.*, 2022; Qian *et al.*, 2023; Ndour *et al.* 2023, *cf.* partie revue bibliographique). Ils peuvent également contribuer à réduire la biodisponibilité, et la toxicité des ET (Gómez-Sagasti et al., 2018; Gong et al., 2018; Kumpiene et al., 2019). Par exemple, certains amendements minéraux basiques, tels que les laitiers sidérurgiques augmentent le pH du sol, entraînant ainsi une immobilisation des ET, tandis que les amendements organiques tels que les composts, peuvent modifier la mobilité des ET par chélation ou adsorption grâce à leur richesse en matière organique (Agnello et al., 2018; Branca and Colla, 2012; Cicatelli et al., 2014; Ferrol et al., 2016; González-Chávez et al., 2004; Kumpiene et al., 2008; Ning et al., 2016). En outre, les laitiers sidérurgiques ont été décrites comme augmentant la biomasse microbienne, les activités enzymatiques du sol et le nombre de taxons bactériens copiotrophes (Das et al., 2020). Aussi, ces modifications peuvent améliorer durablement la fonctionnalité du sol et par conséquent les services écosystémiques rendus par celui-ci (Antoniadis et al., 2017; Gong et al., 2018).

Parmi les amendements biologiques, les inoculums microbiens et notamment ceux à base de champignons mycorhiziens arbusculaires (CMA) ont été proposés en phytostabilisation aidée (Israel et al., 2022; Raveau et al., 2021a). En effet, capables d'établir une relation symbiotique avec 80 % des plantes terrestres, les CMA permettent en outre aux plantes hôtes de croître vigoureusement dans des conditions de stress, en particulier dans les sols pollués par les ET (Cicatelli et al., 2014; Smith and Read, 2008). Ils contribuent de manière significative à l'absorption des nutriments du sol, en particulier le phosphore (Javot et al., 2007), mais aussi l'azote, le calcium, le soufre, le potassium et le zinc, augmentant ainsi la biomasse végétale et améliorant la tolérance des plantes à la pollution du sol (Meier et al., 2012; Smith and Read, 2008). En outre, les CMA produisent de la glomaline, une glycoprotéine insoluble, mais aussi de la mélanine et de la chitine, qui réduisent la biodisponibilité des ET en les immobilisant dans les hyphes ou le sol (Cicatelli et al., 2014; Ferrol et al., 2016; González-Chávez et al., 2004).

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À l'heure actuelle, l'un des principaux freins conditionnant le développement de la phytostabilisation aidée repose sur la démonstration de ses performances et le développement de filières de valorisation de la biomasse produite sur ces sols pollués (Kumpiene et al., 2019). Dans ce contexte, le phytomanagement qui combine à la fois la phytoremédiation des sols pollués et la valorisation de la biomasse produite sur ces sites pollués présente un intérêt certain. Initialement assimilée à un déchet, cette biomasse est désormais utilisée comme une ressource valorisable, permettant d'abaisser les coûts globaux de gestion et/ou de requalification des SSP tout en redonnant une valeur économique à ces sites, souvent délaissés (Bert et al., 2013). C'est pourquoi, le développement de filières de production de molécules à hautes valeurs ajoutées issues de la biomasse végétale récoltée sur des parcelles polluées permettrait de répondre aux attentes environnementales et socio-économiques. Jusqu'alors, cette biomasse végétale était majoritairement valorisée dans les bioénergies, ou la production d'éco-matériaux (Evangelou et al., 2015; Garbisu et al., 2020). Cependant, d'autres filières pourraient présenter une rentabilité importante (Bernal et al., 2019; Bert et al., 2017; Chalot et al., 2020; Mench et al., 2010; Zgorelec et al., 2020). C'est le cas de la production d'huiles essentielles (HE) issues de la culture de plantes à parfums aromatiques et médicinales (PPAM) (Raveau et al., 2021a). En effet, la demande mondiale en produits biosourcés tels que les HE ne cesse de croître, et le marché mondial des HE devrait atteindre 40,12 milliards de dollars d'ici 2030 (Grand View Research, n.d.). Pour répondre à cette forte demande, la culture des PPAM pour la production d'HE sur les SSP, pourrait constituer une option prometteuse (Raveau et al., 2021a). Enfin, grâce à leur richesse en principes actifs et à leurs innombrables propriétés biologiques, les HE peuvent être valorisées dans de nombreux domaines non alimentaires, tels que l'industrie cosmétique et pharmaceutique, ou comme alternative aux produits phytosanitaires (De Clerck et al., 2020; Muchembled et al., 2018; Raveau et al., 2022).

Mon projet de thèse s'inscrit dans ce contexte général. Il constitue une partie du projet DEPHYTOP (AAP Graine 2019, ADEME), qui découle lui-même d'un précédent projet, PHYTEO (AAP Graine, 2016), dont le principal objectif était de développer un mode de gestion des sols pollués par les ET reposant sur la phytostabilisation aidée en cultivant des PPAM dans une perspective de production d'HE. Ces projets ont été réalisés, notamment en collaboration avec un partenaire privé, producteur d'HE situé dans la région Hauts-de-France (FERRANT PHE). Trois espèces végétales ont été testées lors du projet PHYTEO: la coriandre, la sauge sclarée et l'angélique.

Les essais exploratoires *in situ* réalisés dans le cadre de ce projet ont permis d'obtenir des résultats encourageants en démontrant la faisabilité technique de ce mode de gestion et son bien-fondé, notamment en mettant en évidence les capacités phytostabilisatrices de la sauge, un transfert limité

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des ET dans les HE, les propriétés biocides de ces HE et par conséquent leur potentielle valorisation dans la protection des cultures (Raveau, 2020; Raveau et al., 2021a). Cependant, un transfert de Cd dans les parties aériennes de la coriandre a été mis en évidence, ce qui nécessite une optimisation des pratiques culturales visant à limiter son transfert. Quant à l'angélique, espèce de PPAM ayant une importance commerciale pour notre partenaire privé dans les domaines de la parfumerie, son développement a été fortement impacté par les épisodes récurrents de sécheresse de 2017 et 2018.

Ainsi, le projet DEPHYTOP, dans lequel s'inscrit directement mon travail de thèse, a pour ambition, outre la pérennisation des démonstrateurs *in situ* mis en place à grande échelle dans le cadre du projet PHYTEO, de proposer un développement et une optimisation de la filière PPAM/HE en (i) poursuivant les essais de mise en culture de l'angélique, (ii) améliorant les rendements de biomasse et du processus de phytostabilisation en testant différents amendements minéraux, issus de l'économie circulaire (utilisation de sous-produits issus de certaines activités locales comme les laitiers sidérurgiques) et biologiques à base d'inoculum mycorhiziens arbusculaires, (iii) en étudiant les propriétés biologiques des HE issues de la biomasse végétale produite sur un sol pollué afin de proposer des voies de valorisation dans le domaine de la protection des cultures. Enfin, il s'agira également (iv) d'étudier l'effet de la phytostabilisation aidée par les PPAM sur les fonctions écologiques des sols pollués, notamment microbiennes via la caractérisation de leur microbiote.

Après une **introduction générale** mettant en lumière le contexte général, la problématique et les objectifs de mon projet de thèse, le manuscrit et l'articulation des différentes parties qui le compose s'organiseront de la façon suivante :

- La **première partie** de ce manuscrit consiste en une synthèse bibliographique structurée autour des points suivants, incluant trois revues, dont une publiée :
 - Contribution des amendements dans la phytoremédiation des sols pollués, ce point est traité dans le cadre de deux revues :
 - **Revue 1 : Papa Mamadou Sitor Ndour, Julien Langrand, Joël Fontaine and Anissa Lounès-Hadj Sahraoui** « Exploring the significance of different amendments for boosting phytoremediation efficiency: focus on soil ecosystem services », *soumise dans Environmental Science and Pollution Research*
 - **Revue 2 : Abir Israel, Julien Langrand, Joël Fontaine et Anissa Lounès-Hadj Sahraoui** « Significance of Arbuscular Mycorrhizal Fungi in Mitigating Abiotic Environmental Stress in Medicinal and Aromatic Plants: A Review », *publiée dans Foods (2022)*.
 - Apport de l'étude du microbiote du sol dans la phytoremédiation traité dans :

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- **Revue 3 : Amandine Ducouso-Détrez, Julien Langrand, Anissa Lounès-Hadj Sahraoui, and Joël Fontaine** « Environmental DNA metabarcoding and shotgun metagenomics inside polluted soil microbiomes to assist phytomanagement : potentialities, limitations and prospects. A review », à soumettre dans *Science of the Total Environment*.
- Les **principales questions scientifiques posées**, auxquelles ce projet de thèse tentera d'apporter des réponses, sont ensuite abordées.
- La **partie 2** décrit le **site d'étude** utilisé dans le cadre de ce travail
- La **partie 3** présente les **résultats** obtenus et leurs **discussions**, organisés en 4 publications :
 - **Publication 1 : Julien Langrand, Joël Fontaine, Anthony Verdin, Frédéric Laruelle, Natacha Facon, Benoît Tisserant, Hervé Flanquart, et Anissa Lounès-Hadj Sahraoui** « Evaluation of a technico-economic viability of the essential oil production chain on trace element-aged polluted soil vegetated with angelica: A three-years field study », à soumettre dans *Science of the Total Environment*.
 - **Publication 2 : Julien Langrand, Joël Fontaine, Anthony Verdin, Frédéric Laruelle, Natacha Facon, Sophie Fourmentin, et Anissa Lounès-Hadj Sahraoui** « Biocide, antioxidant and anti-inflammatory properties of essential oils distilled from angelica grown on polluted soils » à soumettre dans *Industrial crops and products*.
 - **Publication 3 : Julien Langrand, Anissa Lounès-Hadj Sahraoui, Frédéric Laruelle, Jérôme Duclercq, Natacha Facon, et Joël Fontaine** « Phytomanagement of trace elements-polluted soil, contribution of angelica cultivation and mycorrhizal inoculum on soil microbiota: a three-year field study », à soumettre dans *Environmental Science*
 - **Publication 4 : Julien Langrand, Anissa Lounès-Hadj Sahraoui, Jérôme Duclercq, Robin Raveau, Frédéric Laruelle, Valérie Bert, Natacha Facon, Benoît Tisserant et Joël Fontaine** « Coriander (*Coriandrum sativum*) Cultivation Combined with Arbuscular Mycorrhizal Fungi Inoculation and Steel Slag Application Influences Trace Elements-Polluted Soil Bacterial Functioning », publiée dans *Plants* (2023).
- La **partie 4** constitue une discussion générale qui met en relation et discute les principaux résultats obtenus dans les différentes expérimentations réalisées.
- Ce manuscrit s'achève avec une **conclusion générale**, ouvrant sur les perspectives envisagées pour la poursuite de ce travail.

Introduction générale

Les **références bibliographiques** citées dans la synthèse bibliographique (hors revues et articles) ainsi que dans la discussion générale sont citées à la fin du manuscrit.

Les différentes valorisations et communications auxquelles j'ai participé durant mes années de thèse seront également présentées en **annexe**.

Partie 1 : Synthèse bibliographique

Synthèse bibliographique

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I. Contribution des amendements dans la phytoremédiation des sols pollués

La pollution des sols, due à l'industrialisation et à l'urbanisation rapide, est une préoccupation mondiale. Les polluants proviennent principalement des déchets, des activités industrielles et des résidus de mines, et comprennent des éléments toxiques comme le cadmium, le plomb et le zinc, ainsi que des polluants organiques persistants tels que les hydrocarbures aromatiques polycycliques ou les polychlorobiphényles. Les sites pollués industriels présentent souvent une multi-pollution, rendant l'évaluation des risques et les stratégies de réhabilitation complexes. À l'heure actuelle, différentes approches de réhabilitation ont été développées, incluant des méthodes hors-site nécessitant l'excavation et le traitement *ex-situ* des sols pollués, des méthodes physico-chimiques, et des méthodes sur site utilisant des micro-organismes et/ou des plantes. Parmi elles, la phytoremédiation, utilisant des plantes et leur microbiote rhizosphérique comprend plusieurs stratégies : la phytostabilisation, qui immobilise les polluants dans la rhizosphère, limitant leur biodisponibilité ; la phytoextraction, qui mobilise les polluants pour leur absorption et translocation vers les parties aériennes des plantes ; et la phyto/rhizodégradation, qui permet la dégradation des polluants organiques par les plantes ou par la stimulation du microbiote du sol.

Les friches industrielles sont souvent caractérisées par des contaminations variables tant au niveau des concentrations et des types de polluants (multi-pollution à la fois organique et inorganique), et sont généralement peu fertiles et fortement anthropisés (compaction, présence de gravats, ...). Il est souvent nécessaire d'améliorer leurs propriétés en travaillant le sol et en introduisant, dans le respect des principes de l'économie circulaire, différents types d'amendements afin de redonner à ces sols leurs fonctions premières : le support de culture et le maintien de la biodiversité. Des amendements minéraux (cendres volantes, laitiers sidérurgiques,...), organiques (fumier, BRF, ...) ou biologiques (champignons mycorhiziens arbusculaires, PGPR, ...) sont généralement utilisés pour accroître la croissance des plantes, modifier la mobilité ou la biodisponibilité des polluants dans le sol, mais aussi assurer sa requalification écologique. En effet, la qualité biologique des sols qui fait référence à l'abondance, à la diversité et à l'activité des organismes vivants est généralement très affectée dans les sols fortement anthropisés. Or la fraction biotique du sol, et en particulier les microorganismes telluriques jouent un rôle fondamental dans le fonctionnement des sols et en particulier dans la dégradation de la matière organique, le recyclage des nutriments (carbone, azote, phosphore...), mais

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également la rhizodégradation des polluants organiques ou encore la phytostabilisation des polluants inorganiques.

Cette première revue vise donc à actualiser les connaissances sur l'apport des amendements organiques, minéraux et microbiens sur la phytostabilisation, la phytoextraction et la phytodégradation des polluants, ainsi qu'à examiner leurs effets bénéfiques sur le microbiote du sol, le cycle des nutriments, la croissance des plantes et la séquestration du carbone.

***Revue 1 : Exploring the significance of different amendments for boosting
phytoremediation efficiency: focus on soil ecosystem services***

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Abstract:

Phytoremediation is now recognized as an environmentally, economically and socially efficient phytotechnology for the reclamation of polluted soils. To improve its efficiency, several strategies can be used including the optimization of agronomic practices, selection of high-performance plant species but also the application of amendments. Despite evidences of the benefits provided by different types of amendments on pollution control through several phytoremediation pathways, their contribution to other soil ecosystem functions remains sparsely documented. This current review aims at (i) updating the state of the art about the contribution of organic, mineral and microbial amendments in improving phytostabilization, phytoextraction and phytodegradation of inorganic and organic pollutants, and (ii) reviewing their potential beneficial effects on soil microbiota, nutrient cycling, plant growth and carbon sequestration. We pointed out some research questions that should be tackled for improving amendment-assisted phytoremediation strategies, and discussed different perspectives of using amendments to help phytomanagement projects to improve their economic sustainability.

Keywords: Amendments; Polluted soils; Phytoremediation; Potential toxic element; Persistent organic pollutant; Soil functioning; C sequestration

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1. Introduction

Soil pollution is now considered as a global environmental concern due to industrialization as well as the rapid and continuous urbanization (Sathishkumar et al. 2023). Soil pollutants are mainly originated from waste disposals, industrial activities and mine tailings. They comprise potential toxic elements (PTEs) such as Cd, Pb, Zn, Cu, As etc. (Palansooriya et al. 2020; Kumar et al. 2022a) and persistent organic pollutants (POPs) such as perfluoroalkyl substances, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB), dioxins/furans and various other petroleum hydrocarbons (PHs) originating from biological processes (brush and forest fires) and anthropogenic activities (industry, urbanization, mining and agriculture...) (Rathankumar et al. 2022; Anerao et al. 2022). Globally, in industrial polluted sites, it is common to find multi-pollution in soils with both inorganic and organic pollutants, which can interact and render risk assessment complex and remediation strategies more challenging (Deng et al. 2018; Cao et al. 2022; Sathishkumar et al. 2023). Therefore, different remediation approaches were developed including off-site methods which require soil excavation transportation and ex-situ treatment (thermal, chemical, physical treatment) and onsite methods which rely on the use of microorganisms and/or plants (Rehman et al. 2023). Amongst them, phytoremediation which uses plant species and their associated rhizosphere microbiota, is considered as promising eco-friendly remediation approach to repair and/or to recover polluted soils (Malik et al. 2022). Phytoremediation comprises various strategies that can be carried out according to the plant species and the nature of the contaminants with the final goal of avoiding the contact of these hazardous substances with humans, animals and the food-chain (Kumar et al. 2022b; Malik et al. 2022). This includes phytostabilization that is defined as the immobilization of contaminants (mainly inorganic) in rhizosphere through different mechanisms (adsorption, oxidation/reduction, complexation, ions exchange etc.) limiting their bioavailability, dispersion or leaching into the environment (Palansooriya et al. 2020). Conversely, phytoextraction strategy relies on the mobilization of inorganic contaminants by plant root followed by their uptake and translocation into aerial part which can be easily harvested (shoots, stems and fruits) (Garbisu and Alkorta 2001; Kumar et al. 2022a). Phytoremediation also includes phytodegradation which consists in degradation of organic pollutants even directly by plants through their root exudates and the release of harmless substances or indirectly, through stimulation of soil microbiota able to metabolize some organic pollutants. There is also phytovolatilization which involves extraction and volatilization of pollutants such as selenium and mercury (Garbisu and Alkorta 2001). Thanks to their lower adverse effects, phytoremediation approaches are recognized to be more cost-effective and sustainable compared to physicochemical techniques (thermal desorption, dig and haul, chemical treatment, solidification and stabilization) which can alter soil (Jayapal et al. 2022; Hou et al. 2023). Another advantage of phytoremediation is its possibility to be combined with the application of amendments (assisted phytoremediation) for a better efficiency of pollutant management along with beneficial effects on soil fertility, plant growth and biomass production (Fuke et al. 2021). Nevertheless, while reducing the risk associated with pollutants, the cultivation of plant species with economically valuable biomass during the phytoremediation could considerably improve its economic profitability and attractiveness (Cundy et al. 2016; Mench et al. 2018; Burges et al. 2018). In this perspective, the selection of productive plant species with high physiological performances on polluted soils and an economic value for valorization is considered paramount in phytomanagement (Yang et al. 2016; Burges et al. 2018), which is the ecological and economical management of polluted soils.

Soil contributes considerably to provision of different ecosystem services through its various functions including primary production, carbon (C) storage, nutrient recycling, water purification, fertility maintenance, contamination

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control etc. (Millennium Ecosystem Assessment 2005). The primary goal in remediating soil pollutions is to limit their leaching into environment and to avoid their integration in the food chain. However, in the context of global reduction of cultivable area around the world coupled with climate change which impact considerably soils and their capabilities for delivering ecosystem services, more attention is devoted to other ecosystem functions in the soil phytoremediation (Burgess et al. 2018).

Application of amendments to polluted soils may improve soil quality indicators like pH, electric conductivity (EC), Soil organic matter (SOM) and nutrient contents but also may reduce their toxicity and ecotoxicity (Lwin et al. 2018) and therefore impact soil ecosystem services. Therefore, combining soil amendments with phytoremediation could be particularly interesting to enhance recover or to enhance ecosystem services in polluted soils. Different mineral, organic and microbial amendments have been demonstrated to assist the phytoremediation of polluted soils. Organic amendments including crop residues green wastes, manures, biosolids (sewage sludge) and biochar, have been reported to have positive effects on soil polluted by total petroleum hydrocarbon (TPHs) through an improvement of soil physical, chemical or microbiological parameters (Hoang et al. 2021; Qian et al. 2023). Different mineral amendments have demonstrated their abilities to reduce the availability of different PTEs and to improve their phytostabilization (Houben et al. 2012; Qayyum et al. 2017; Wang et al. 2018; Palansooriya et al. 2020). Similarly, microbial (bacterial and fungal) amendments are also used to improve the phytoremediation of both mineral and organic pollutants (Ma et al. 2019; Li et al. 2021; Lebrun et al. 2021a, b; Alves et al. 2022). Therefore, these above-mentioned findings have shown the big potential of amendments to increase the efficiency of soil phytoremediation.

Scientific interest on the role of amendments in assisting phytoremediation of polluted soils is growing as demonstrated by the growing number of reviews on the topic (**Fig. 1A**). Several review papers have discussed the impact of different soil amendments on the mobility of PTEs in brownfield soils (Khan et al. 2017; Palansooriya et al. 2020), the potential of some amendments to improve phytoremediation of mineral or organic contaminants (Chirakkara et al. 2016; Kumar et al. 2022a; Qian et al. 2023). However, comprehensive reviews considering both inorganic and organic pollutants (**Fig. 1B**) are lacking, as well as review summarizing the influence of amendment-assisted phytoremediation on other ecosystem functions (i.e., productivity and ecotoxicity, biomass valorization, microbial diversity and activities, carbon sequestration potential). Indeed, beyond amendments effect on pollutants fate, information on global ecosystem functioning is required to know the extent to which the different amendments could be useful in a phytomanagement-oriented perspective.

Thus, the aims of the current review were to critically explore the literature reporting the different amendments used to improve the efficiency of different phytoremediation strategies along with analyzing their effects on nutrient recycling, plant physiology and biomass production as well as C sequestration. Upcoming challenge toward an effective implementation on these aided-phytoremediation approaches are analyzed and the potential of amendments to sustainably improve biomass production and valorization for strengthening economic incomes during phytomanagement is also discussed.

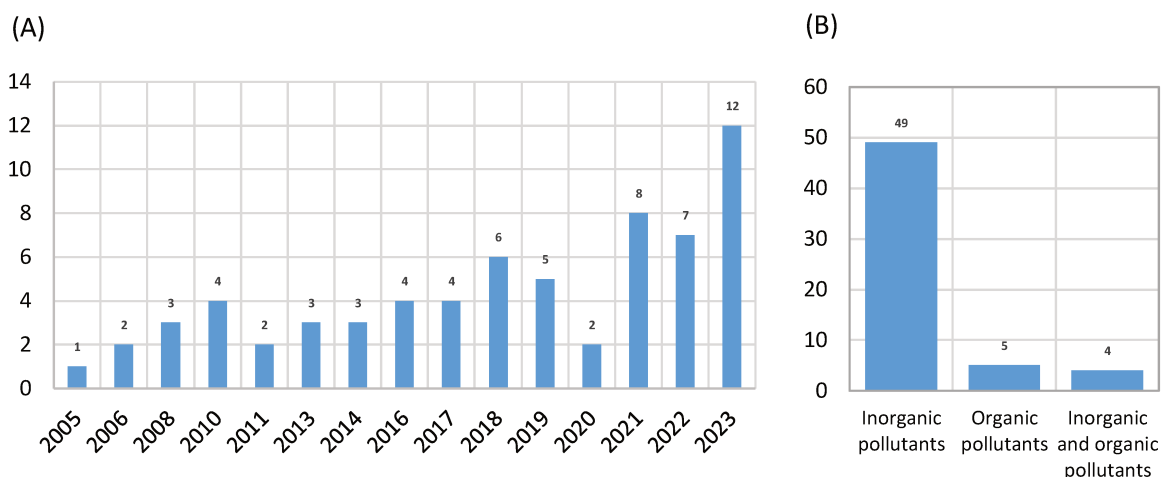


Figure 1: (A) Review articles treating the effect of amendments on phytoremediation of soils polluted between 2005 and 2023. (B) Type of pollutants considered in these articles. The data result from a search in Scopus database with “soil” AND “amendment” AND “phytoremediation” keywords and a screen to filter out scope reviews.

2. Role of organic amendments on the ecosystem services of polluted soils

Organic amendments may constitute an important sources of nutrients, can greatly improve soil structure and thus promote plant growth for a sustainable agricultural production (Trivedi et al. 2017). Different organic amendments including biochar, biosolid, animal wastes, plant residues and compost from these different materials are used in polluted soils reclamation (Park et al. 2011; Khan et al. 2017; Palansooriya et al. 2020). These organic amendments may impact the different ecosystem functions in polluted soils subject to phytoremediation (**Table 1**). Here, we fill focused on pollutant dynamic (mobility and fate), organic matter (OM) decomposition and nutrient recycling driving processes, biomass production and C sequestration.

2.1. How organic amendments affect the fate of soil pollutants during phytoremediation?

Organic amendments are rich in micro-and-macronutrients essential for plant growth (Kidd et al. 2015). Their effects on PTEs mobility and bioavailability could be versatile (**Fig. 2**) and depend on the nature of the organic matter, the pollutant concerned, soil type and properties (Clemente et al. 2005; Kumpiene et al. 2011). Globally, unstabilized OM (such as manure, biosolids, bone meal, etc) can increase metal mobility due to their high content of dissolved OM, which can complex metals and facilitate their movement through the soil (Tandy et al. 2009). For example, addition of manure compost in soil resulted in an increased bioconcentration and translocation of Cd and Zn in potherb mustard (*Brassica juncea*, Coss.) cultivated in polluted soil (Guo et al. 2019). Amendment with bone meal led to Cd increased bioaccumulation in ryegrass (*Lolium perenne*) planted in a polluted soil (Li et al. 2023b). Nevertheless, the relevance of this beneficial effect of organics amendments on PTEs phytoextraction need to be demonstrated at the field scale as all these studies were conducted in pot conditions under controlled conditions. Indeed, the scaling up as well as the climate conditions may result in unexpected results.

Biosolids have been reported to be effective in the phytostabilization of Zn, Cu, Fe, Cd in several studies (Jamal Khan and Jones 2008; Alvarenga et al. 2009; Madejón et al. 2014; Al-Lami et al. 2022). This capacity of biosolids

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to improve PTEs phytostabilization could be related to its structure and chemical composition which define its binding abilities. Nevertheless, Shaheen et al. (2017a) found that biosolids application effectively decreased available fractions of Cu and Pb when these pollutants are present in the soil at high concentrations (250-300 mg/L), whereas an opposite effect was noticed at low concentration in soil (<100 mg/L). Indeed Liu et al. (2007) have shown that the dissolved OM content of biosolids is important to check as a such OM fraction could be associated with PTEs to form organo-mineral complexes, which may lead to leaching or plant assimilation.

Biochar is globally known as an amendment that enhance the phytostabilization of various PTEs (Houben et al. 2013; Ahmad et al. 2014; Ali et al. 2017; Fresno et al. 2018, 2020; Simiele et al. 2021; Al-Lami et al. 2022). Indeed, its application increases soil pH and SOM content (mainly humified SOM), which lead to a decreased solubility of metals/metalloids and their retention by the soil organic phase. However, attention should be made when applying biochar for phytostabilization as opposite effect may be obtained on other non-targeted metals/metalloids. For example, although biochar application decreased the phytoavailability and the uptake of Cd, it increased Zn concentration in soil and its uptake (shoot and grain concentration) by wheat (*Triticum aestivum* L.) grown on a Cd-contaminated saline soil (Abbas et al. 2018). More recently, evidence of a greater mobilization and translocation of Cd, Pb and Zn into gum rockrose (*Cistus ladanifer*) was reported when biochar was added to a polluted soil, suggesting a need of particular attention for biochar use in phytostabilization perspectives (Duarte et al. 2023). In addition, biochar addition to a polluted soil planted with Chinese brake (*Pteris vittata*) increased significantly As mobility, bioaccumulation and translocation into shoot, despite a rising effect on pH (Liu et al. 2023b). This mobilization effect could be related to electrostatic repulsion between biochar negative charges and the negative charges of As as explained by Shaheen et al. (2023).

Animal wastes (horse manure, poultry manure, cow-dung manure, slurry, composts, bone meal, shell) have been tested and confirmed as phytostabilizing amendments for different PTEs (Zn, Cd, Pb, Cu) (Liu et al. 2009; Houben et al. 2012; Burges et al. 2016; Sawarkar et al. 2023). Like biochar, some animal waste amendments could have opposite effect on different PTEs. Indeed, Houben et al., (2012) showed that cow manure application lead to immobilization of Zn while having a mobilization effect of Pb in such multi-metal polluted soil. The same phytostabilizing effect toward Cd and Pb was found when rice straw and wheat staw were amended to soil polluted by Cd and Pb (Xu et al. 2016).

Although phytodegradation can allow the dissipation of considerable amount of different organic pollutants, its efficiency on the remediation of aged PHs polluted soils is relatively low due, for example, to their interaction with soil solid phase matrix (soil organic carbon "SOC", clay), particularly the for PAHs which present complex chemical structures (Hatzinger and Alexander 1995). Therefore, different soil amendments have been applied along with phytoremediation to change soil properties which can modify organic pollutants availability and enhance their dissipation in polluted soil. For example, biosolid application to a petroleum-polluted soil planted with maize (*Zea mays* L.) led to increased TPHs degradation compared to control treatment (Asadollahi et al. 2016). Similarly, application of different organic wastes (potato skin, soy cake, and tea leaf) to a fuel diesel polluted soil phytoremediated with song of India (*Dracaena reflexa*) resulted in an increased degradation rate of +40 to +65% compared to the control (Dadrastia and Pariatamby 2016). The application of an organic compost (formed by a mix of cow dung, cabbage-leaf and sawdust) during the phytoremediation of a crude-oil polluted soil

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planted with *Calendula officinalis* resulted in 73% removal rate of TPHs (Wang et al. 2021). Several studies have reported significant effect of biochar application in the phytoremediation of PHs polluted soils (Meki et al. 2022; Dike et al. 2022). Biochar coupled with wheat (*Triticum aestivum*), maize (*Zea mays*) or ryegrass (*Lolium perenne*), and compost coupled with white clover (*Trifolium repens*) and alfalfa (*Medicago sativa*) led also to a gain in removal rate of TPHs in polluted soils (Yousaf et al. 2022). Moreover, maize straw biochar application alone or in combination with phytoremediation by ryegrass improved PAHs removal (phenanthrene, pyrene, and benzo(a)pyrene) (Li et al. 2020). This reduction of PHs content following these different organic amendments were ascribed to their degradation by rhizosphere microbiota as it was accompanied by an increase in the hydrocarbon-utilizing bacterial population (Dadrasnia and Agamuthu 2013; Yousaf et al. 2022; Hussain et al. 2022). Particularly, biochar effect was related to the abundance of microbial taxa involved in PAHs degradation and thereby enhancing the soils degradation capacity toward PAHs (Kong et al. 2018; Dike et al. 2021).

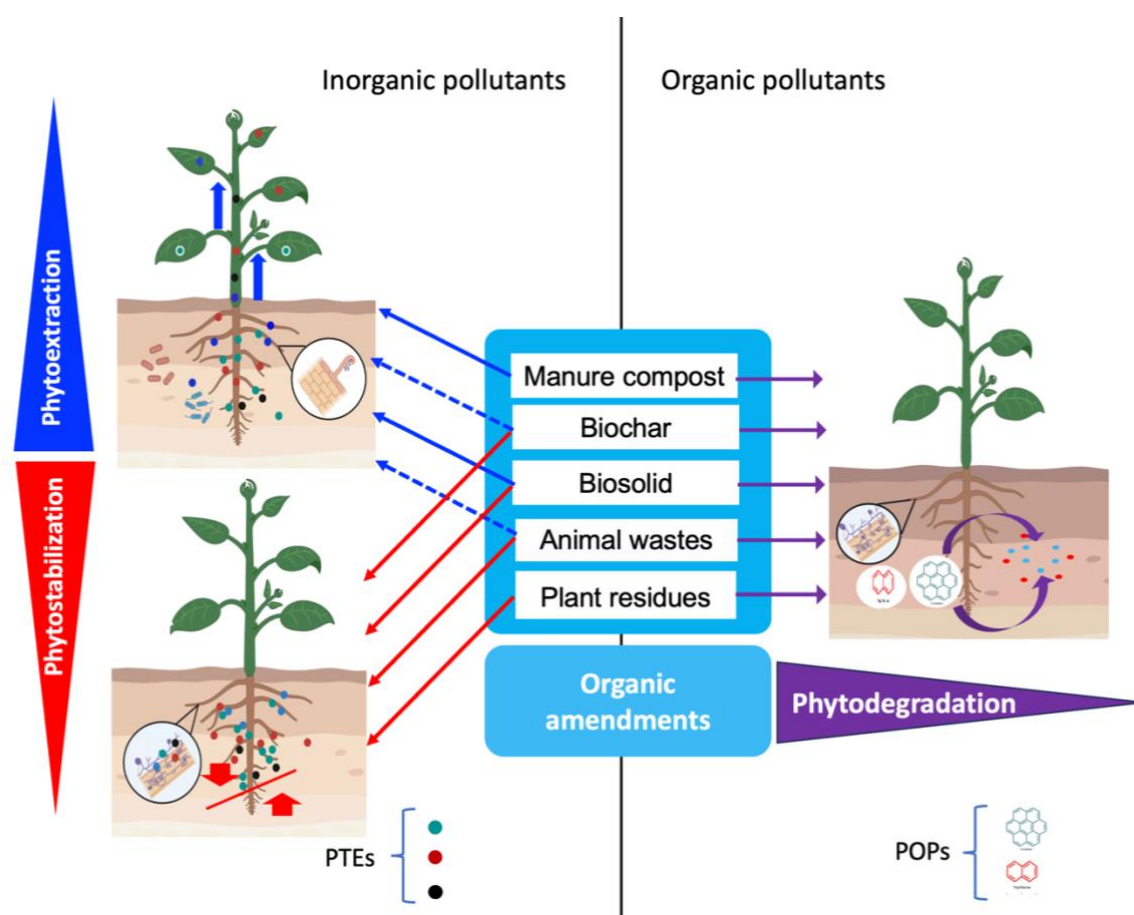


Figure 2: Effect of different types of organic amendments on the mobility and fate of inorganic (PTEs) and organic (POPs) pollutants in soil during phytoremediation. Arrows in broken lines indicated alternative (less frequent) phytoremediation pathways promoted by the application of some specific amendments.

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Table 1: Effect of organic amendments on soil ecosystem functions during the phytoremediation of soils polluted with inorganic and organic pollutants; TOC= total organic carbon, DOC= dissolved organic carbon, T-SQI= total soil quality index, SOM= soil organic matter, SOC= soil organic carbon, PCB= polychlorinated biphenyl, ROS= reactive oxygen species. Semi-field condition means an experiment carried out in big containers placed outdoors without any control apart from watering.

Ecosystem fonctions	Pollutants and phytoremediation process	Plant species	Organic amendments	Culture conditions	Observed effects	References
Microbial activities and nutrient recycling	Phytostabilization of Cd, Pb and Zn	Red fescue (<i>Festuca rubra</i>)	Cow slurry	Pot experiment	<ul style="list-style-type: none"> • Increase of β-glucosidase, urease, phosphatase, β-glucosaminidase 	(Borges et al. 2016)
	Phytostabilization of Zn, Cu, Cd	Dragontree (<i>Paulownia fortune</i>)	Biosolid and olive mill composts	Semi-field experiment	<ul style="list-style-type: none"> • Increase of TOC, available P, available K and N-Kjeldahl • Increase of enzyme activities (dehydrogenase, Aryl-sulphatase, β-glucosidase, acid phosphatase and Urease) 	(Madejón et al. 2014)
	Phytostabilization of Pb, Zn, Cd and Cu	Poplar (<i>Populus spp.</i>), willow (<i>Salix spp.</i>) or Miscanthus (<i>Miscanthus spp.</i>)	Biosolid, Biosolid+biochar Biosolid+humus substance	Pot experiment	<ul style="list-style-type: none"> • Increase of N (NH_4^+-N and NO_3^--N), TOC, • Increase of dehydrogenase activity • Increase of the treated soil quality index (T-SQI) 	(Al-Lami et al. 2022)
	Adsorption and/or phytodegradation of Chlorpyrifos	Pelargonium (<i>Peargonium graveolens L.</i>)	Biochar	Pot experiment	<ul style="list-style-type: none"> • Increased microbial biomass • Increased microbial activities (dehydrogenase, 	(Yadav et al. 2023)

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					phenoloxidase, phenol peroxidase) <ul style="list-style-type: none"> • Increased enzymatic activities (cellulase, N-acetyl glucosaminidase, alkaline phosphatase) 	
Phytodegradation of PAHs (phenanthrene, pyrene, benzo(a)pyrene	Ryegrass (<i>Lolium perenne</i>)	Biochar	Pot experiment	<ul style="list-style-type: none"> • Increased DOC, mineral N, available P, available K • Increased dehydrogenase and urease activities • Increased metabolic activity (abundance of microbial metabolites) 	(Li et al. 2020)	
Phytodegradation of PAHs	Ryegrass (<i>Lolium perenne</i>)	Biochar	Pot experiment	<ul style="list-style-type: none"> • Increased microbial activity (Urease activity, PAHs-degrader bacterial genes) • Increased bacterial richness (OTU number, Chao1) and diversity (Shannon) 	(Zhao et al. 2022)	
Phytodegradation of PAHs (phenanthrene, anthracene, pyrene, fluoranthene)	Maize (<i>Zea mays</i>)	Cow-dung manure	Pot experiment	<ul style="list-style-type: none"> • Increased enzyme activities (laccase, catechol 1,2 dioxygenase) • Increased soil protein content 	(Kumari et al., 2021)	

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	Phytodegradation of TPH (Crude oil)	Marigold (<i>Calendula officinalis</i>)	Mixed compost of cow-dung, cabbage-leaf and sawdust	Pot experiment	<ul style="list-style-type: none"> Increased soil nutrient contents (total N, Available N, Available P, SOM) 	(Wang et al., 2021)
	Phytodegradation of TPH, PCB, PTEs	Black poplar (<i>Populus nigra</i>)	Horse manure	Field experiment	<ul style="list-style-type: none"> Increased enzyme activities (dehydrogenase, phosphatase, betaglucosidase) Increased soil nutrient contents (mineral N, total N, total P, SOC) 	(Doni et al., 2012)
	Phytodegradation of TPH	Barley (<i>Hordeum vulgare</i>)	Poultry manure	Pot experiment	<ul style="list-style-type: none"> Increased soil respiration (CO₂ release) 	(Barati et al., 2018)
Phytotoxicity alleviation and plant growth	Phytostabilization of Cu, Zn and Mn	Wild spinach (<i>Chenopodium album</i>)	Olive mill manure	Pot experiment	<ul style="list-style-type: none"> Increased shoot biomass 	(Walker et al., 2004)
	Phytostabilization of Zn, Pb, Cd, Cu	Brown mustard (<i>Brassica juncea. L</i>)	Biochar (from bamboo)	Field experiment	<ul style="list-style-type: none"> Increased shoot and root biomass Increased chlorophyll content Increased carotenoid content 	(Ali et al., 2017)
	Phytostabilization of Cd	Wheat (<i>Triticum aestivum L.</i>)	Chicken manure compost	Pot experiment	<ul style="list-style-type: none"> Increased yield (dry matter) 	(Liu et al., 2009)
	Phytostabilization of Cu, Zn and Pb	Ryegrass (<i>Lolium perenne</i>)	Solid waste compost	Pot experiment	<ul style="list-style-type: none"> Increased shoot growth 	(Alvarenga et al., 2009)
	Phytoextraction of Cd, Pb, Zn	Gum rockrose (<i>Cistus ladanifer</i>)	Biochar	Pot experiment	<ul style="list-style-type: none"> Increased phytotoxicity (Plant ROS) 	(Duarte et al., 2023)

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	Phytodegradation of TPH	Ryegrass (<i>Lolium perenne</i>)	Garden waste biochar and compost	Pot experiment	<ul style="list-style-type: none"> • Increased germination rate • Increased biomass • Increased Chlorophyll content 	(Hussain et al. 2018, 2022)
	Phytodegradation of TPH	Ryegrass (<i>Lolium perenne</i>), Maize (<i>Zea mays</i>), White clover (<i>Trifolium repens</i>) and Alfalfa (<i>Medicago sativa</i>)	Biochar and biochar	Pot experiment	<ul style="list-style-type: none"> • Increased biomass 	(Yousaf et al. 2022)
Carbon sequestration	Coal-mine site restoration	Pine (<i>Pinus sylvestris</i> L.) or giant miscanthus (<i>Miscanthus x giganteus</i>)	Biosolid and compost	Field experiment	<ul style="list-style-type: none"> • Increased SOC stock • Increased SOM humification • Increased C phytosequestration 	(Placek-Lapaj et al. 2019)
	Phytostabilization of Cu, Zn, Pb and Ni	Bamboo (<i>Bambusa vulgaris</i>)	Farmyard manure and press mud	Field experiment	<ul style="list-style-type: none"> • Increased C phytosequestration (biomass production) 	(Sawarkar et al. 2023)
	Phytostabilization of multiple PTEs (Pb, As, Zn, Cu; Ni and Cr)	Bamboo (<i>Bambusa balcooa</i> , <i>Bambusa vulgaris</i>)	Farmyard manure and press mud	Field experiment	<ul style="list-style-type: none"> • Increased C phytosequestration (biomass production) 	(Shakeel et al. 2024)

2.2. How organic amendments affect microbial functioning and fertility in polluted soils?

Organic amendment-assisted phytoremediation has proven to have positive effect on microbial activities, which is indispensable for C and nutrient recycling in both PTEs and POPs polluted soils. For example, the application of cow slurry in Cd, Pb and Zn polluted soil planted with red fescue (*Festuca rubra*) has been shown to enhance enzyme activities like β -glucosidase, urease, phosphatase, β -glucosaminidase and basal soil respiration (Borges et al. 2016). In a mine tailing polluted by Zn and Pb and vegetated by a mixture of different native species (*Lygeum spartum*, *Hyparrhenia hirta*, *Cistus albidus* L, *Helichrysum stoechas*, *Lavandula dentata*, *Atriplex halimus*, *Rosmarinus officinalis* and *Phagnalon saxatile*), the application of pig slurry increased soil microbial biomass and enzyme activities along with an improvement of SOC, total N, available P, exchangeable K contents (Martínez-Martínez et al. 2019). After cow-dung manure fertilization of soil polluted by four PAHs (phenanthrene, anthracene, pyrene and fluoranthene) and planted with maize, it was observed an increase in soil enzyme activities (laccase, catechol 1,2 dioxygenase) and protein content suggesting a positive effect of such amendment on microbial activities (Kumari et al. 2021). Soil amendment with different types of manures (horse manure, poultry manure) had also positive effect on soil microbial activity during phytoremediation of TPHs polluted soils. Indeed, Barati et al. (2018) found a positive effect of poultry manure into soil respiration of TPHs polluted soils cultivated with barley while horse manure addition along with *Populus nigra* cultivation during the phytoremediation of a multi-polluted site (TPHs, PCB, and PTEs) have been reported to positively affect enzyme activities (dehydrogenase, phosphatase and beta-glucosidase) and nutrient contents (NO_3^- , NH_4^+ , total N and P, DOC) (Doni et al. 2012).

Compost addition also improved microbial activities and soil fertility in polluted soils. Indeed, Madejón et al. (2014) found that biosolid and olive mill compost application increased SOC, available P, available K, and total N-Kjeldahl contents in PTEs polluted soil vegetated with dragon tree (*Paulownia fortune*). Importantly, they found an increase in the relative surface index calculated using five different enzymatic activities (dehydrogenase, Aryl-sulphatase, β -glucosidase, acid phosphatase and urease activities) (Madejón et al. 2014). Similarly, the effect of an organic compost (formed by a mix of cow dung, cabbage-leaf and sawdust) in the phytoremediation of crude-oil polluted soil planted with *Calendula officinalis* was studied (Wang et al. 2021). The authors observed that the application of this compost resulted in a significant increase in nutrients content (SOM, total N, total P, available N, available P) compared to the control planted treatment. This compost-driven soil nutrients increase was likely related to a microbial activities boost as several enzyme activities (dehydrogenase, polyphenol oxidase, urease and alkaline phosphatase) measured in the rhizosphere of compost amended treatment were increased relatively to their control (Wang et al. 2021)

The application of biosolid alone or in combination with biochar or humus substance, improved significantly dehydrogenase activity, mineral N availability (NH_4^+ and NO_3^-), plant productivity, SOC in a tailing site contaminated with PTEs and planted with either hybrid poplar DN34 (*Populus deltoids*_*Populus nigra*), willow (*Salix pentandra*) or miscanthus (*Miscanthus x giganteus*), (Al-Lami et al. (2022) . Furthermore, biochar application increased the soil microbial biomass, enhanced several enzyme activities involved in C, N and P cycling (cellulase, N-acetyl glucosaminidase, alkaline phosphatase) and global microbial activities

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(dehydrogenase, phenol oxidase, phenol peroxidase) in the rhizosphere of *Pelargonium* (*Pelargonium graveolens* L.) planted in a soil polluted by chlorpyrifos (an organochlorine pesticide) (Yadav et al. 2023). Indeed, a such pollutant inhibits the catabolic activity of soil enzymes through chelation and reduce SOM mineralization while biochar contribute to suppress this adverse effect by facilitating energy supply and growth of soil microorganisms and catalyzing P solubilization (Tao et al. 2020). Moreover, biochar application during the phytoremediation of PAHs (phenanthrene, pyrene, and benzo(a)pyrene) polluted soil by ryegrass cultivation increased available C and nutrient contents (dissolved organic carbon (DOC), mineral N, available P, available K), dehydrogenase and urease activities, with the best responses noticed with the combination of biochar and ryegrass phytoremediation (Li et al. 2020). These results were also confirmed by Zhao et al. (2022) who showed that application of rape straw biochar to soil planted with ryegrass increased not only its bacterial alpha-diversity (OTUs numbers, Chao1 index and Channon index) but also stimulated microbial activity evidenced by the increased expression of PAHs-degrader bacterial genes (PAH-RHD α gram-negative, and gram-positive) and a higher urease activity in the rhizosphere. This biochar effect was ascribed to its adsorptive/immobilizing capacity through several mechanisms (hydrophobic partition, surface adsorption or hydrogen binding) (Liu et al. 2018; Khalid et al. 2020), and its biostimulant potential on microbial taxa involved in PAHs degradation like *Sphingomonas* spp, *Alcanivorax* spp, *Pseudomonas*, *Stenotrophomonas* etc. (Kong et al. 2018; Dike et al. 2021), resulting in a reduction of soil toxicity and an improvement of its microbial functioning. Overall, the improvement of microbial activity and nutrient cycling consecutive to organic amendments addition leads logically polluted soil to better support plant growth.

2.3. How organic amendments affect plant growth in polluted soils?

In PTEs polluted soils, organic amendments application may have positive or negative effects on plant growth according to the type of the amendments, their maturity, their effect on PTEs mobility, the soil characteristics or the plant species. Bamboo biochar application in Zn, Pb, Cd and Cu polluted soils resulted in an increased growth of brown mustard (*Brassica juncea*) (shoot and root biomass, chlorophyll and carotenoid contents) mainly due to PTEs immobilization and their reduced accumulation in plant (Ali et al. 2017). Similarly, positive effect of chicken manure compost application was noticed against wheat Cd phytotoxicity, through 70% reduction of Cd soluble/exchangeable fraction and reduced uptake resulting to an improved plant growth (dry matter yield) (Liu et al. 2009). The same result was obtained in the case of the phytostabilization of Cu, Zn and Pb by the addition of biosolid and municipal solid waste compost, which lead to an increase of ryegrass shoot growth (up to 3 times). In addition, the application of cow manure in a Cu, Zn and Mn-polluted soil reduced its phytotoxicity and increased the biomass of wild spinach (*Chenopodium album*) thanks to lower accumulation of such PTEs in plant shoots whereas olive mill compost application to the same soil has led to opposite results (Walker et al. 2004). Indeed, amendment with some organic materials may sometimes have detrimental effect on plant growth due mainly to possible metal phytotoxicity which may be consecutive to an increased availability in polluted soil. For example, application of biochar (2% w/w) has resulted in greater PTEs (Cd, Pb, Zn) uptake and translocation in the aerial part of gum rockrose (*Cistus ladanifer*), which showed signs of oxidative stress (increase of superoxide dismutase activity, increase of reactive oxygen species content, decrease of catalase activity etc.) (Duarte et al. 2023). The authors stated that this adverse effect would be related to the low organic C content of the mine soil, which lead biochar to stimulate microbial activity resulting to the degradation of SOM and the release of PTEs adsorbed in

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its labile fraction. This suggest that in PTEs polluted soil, the effect of organic amendments on plant growth could also depend on soil characteristics (SOM content, pH) and the amendment dose which may affect microbial activity and then change PTEs mobility in different ways (immobilization or solubilization/desorption).

Evidences of plant growth promotion in addition to PAHs removal following organic amendments application on polluted soils have been demonstrated in numerous studies (Hussain et al. 2018, 2022; Ali et al. 2021; Yousaf et al. 2022). For example, TPHs removal by the application of different amendments (garden waste biochar, compost, or their combination) was reported to reduce the soil phytotoxicity toward ryegrass as evidenced by the significant increase of its germination rate, biomass production and chlorophyll content in amended compared to unamended treatment (Hussain et al. 2018, 2022). Even if, each of these different amendments impacted positively plant growth in TPHs polluted soil, the best response was obtained with their combination. Similarly, biochar and compost were reported to boost phytoremediation of TPHs polluted soils planted with either *poaceae* (ryegrass, maize) or *fabaceae* (white clover and alfalfa) (Yousaf et al. 2022). In this study, the authors found specific interactions i.e., with biochar being more efficient in improving *fabaceae* plants growth while compost effect on both germination rate and biomass production were more effective with *poaceae* plants. This positive impact of organic these amendments on retrieving plant growth rate/germination potentials in PHs polluted soil could be explained by the soil fertility boost following OM supply that affect microbial activity and nutrient recycling, as discussed in the precedent sub-section.

2.4. Effect of organic amendment on carbon storage in polluted soils

Phytoremediation potential to increase C sequestration thanks to the installation of plant cover has been well documented (Rockwood et al. 2004; Kåresdotter et al. 2022; Baier et al. 2022; Bai et al. 2022). However, the influence of amendments on this ecosystem function during phytoremediation is rarely addressed. However, as organic amendments represent an important C supply for soils, they could impact C balance during the phytoremediation through several mechanisms. This includes the increase of soil C stock, the modification of SOC mineralization rate and their impact on perennial plants biomass through their positive effect on soil fertility (nutrient availability) and against the soil phytotoxicity. Indeed, while contributing to the management of polluted soils, the application of organic amendments provides soil with various quantity and quality of OM which can integrate the different soil C pools (e.g., labile, recalcitrant) and therefore can have different fates. Moreover, the improvement of polluted soils fertility following organic amendments application leads to an enhancement of plant growth/yield, increasing the removal of CO₂ from atmospheric and its fixation into the plant biomass (phytosequestration) through a higher photosynthetic activity. A such mechanism can be particularly interesting in the phytostabilization approach where the perennial plants maintained on site to immobilize PTEs can therefore contribute to reduce atmospheric CO₂ through C phytosequestration. For example, the effect of biosolid and compost applied alone or in combination with lake chalk was studied on the phytoremediation of a coal-mine site planted with either pine (*Pinus sylvestris* L.) or miscanthus (*Miscanthus x giganteus*), with a focus on carbon stock, CO₂ emission and plant biomass production (Placek-Lapaj et al. 2019). The authors found that these amendments have increased SOC stock and SOM humification compared to unamended treatment and importantly, their combination with lake chalk improved considerably their C sequestration performances. Furthermore, farmyard manure and press mud have been reported to contribute to C sequestration by increasing considerably bamboo

biomass while contributing to Cu, Zn, Pb and Ni phytostabilization after two years of cultivation (Sawarkar et al. 2023). Biochar is used to aid the phytoremediation of both PTEs and POPs polluted soils (Kong et al. 2018; Zhao et al. 2022; Dike et al. 2022; Duarte et al. 2023). Moreover, biochar has generally high content of stabilized C which may suggest its application as a mean to contribute to C sequestration and climate change mitigation as reported by several studies (Rogovska et al. 2011; Houben et al. 2013). In addition, its phyto-sequestration improvement effect relative to plant growth enhancement on polluted soils could make it relevant for climate change mitigation. Nevertheless, despite its soil C sequestration potential and its reducing effect on N₂O release, the potential of biochar to reduce greenhouse gas emission is still controversial due to its variable effects of C mineralization, which may depend on agricultural system, altitude, chemical fertilization etc., and therefore upsetting the net carbon balance (Cayuela et al. 2010; Case et al. 2014; Bamminger et al. 2018; He et al. 2019).

3. Role of mineral amendments on the ecosystem services of polluted soils

Mineral amendments combined with phytoremediation are able to modulate PTEs availabilities in the soil, their bioconcentration and translocation in plant as well as to promote rhizodegradation of organic pollutants (Qayyum et al. 2017; Vrînceanu et al. 2019; Palansooriya et al. 2020) (**Fig. 3**). Beyond such effects on pollutant mobility, they may have a role on polluted soils ecosystem functions and particularly, SOM mineralization and nutrient recycling, plant growth and biomass production and C sequestration (**Table 2**). Synthetic mineral fertilizers are largely known to positively impact plant growth as supplying plant with available forms of essential nutrient (N, P and K etc.). Here, we focused only on natural mineral amendments and by-products which are available with a great interest for circular economy in a phytoremediation context.

3.1. How mineral amendments act on soil pollutants?

PTEs phytostabilization can be enhanced by the addition of different mineral amendments including liming materials (Burgos et al. 2010; Shi et al. 2019; Hannan et al. 2021), phosphates (phosphate rock, triple superphosphate) and gypsum (Qayyum et al. 2017; Shaheen et al. 2017b), clay materials like bentonite, sepiolite, zeolite (Zhan et al. 2019; Vrînceanu et al. 2019), metal oxides such as calcium hydroxide, dolomite, red mud (Bian et al. 2016; Wang et al. 2018; Vrînceanu et al. 2019), as well as mineral industrial wastes from sugar beet and cement factory, and fly ash (Houben et al. 2012; Shaheen et al. 2017b). Globally, these mineral amendments reduce metal mobility and phytoavailability by rising the soil pH, by sorbing cationic PTEs, or by forming metal-carbonate complexes promoting therefore a phytostabilization process (**Fig. 3**).

Although mineral amendments are more frequently used in the phytoremediation of PTEs polluted soils, they can also help in the removal of PHs. For example, Liu et al. (2023) applied nano-SiO₂, nano-CeO₂ and Na-montmorillonite to soil polluted by PAHs and vegetated with the ornamental plant *showy stonecrop* (*Sedum spectabile*) which presented remarkable phytoextraction abilities toward these POPs, and observed that the mineral amendments increased the dissipation of the different PAHs from the soil by promoting their uptake by plant. Moreover, the application of different sorbents (kaolinite, zeolite, diatomite and vermiculite) to a crude oil-polluted soil has led to a significant decrease in soil residual PHs content after three seasons of red clover (*Trifolium pratense*) and bulbous oat grass (*Arrhenatherum* sp.) cultivation (Vasilyeva et al. 2022).

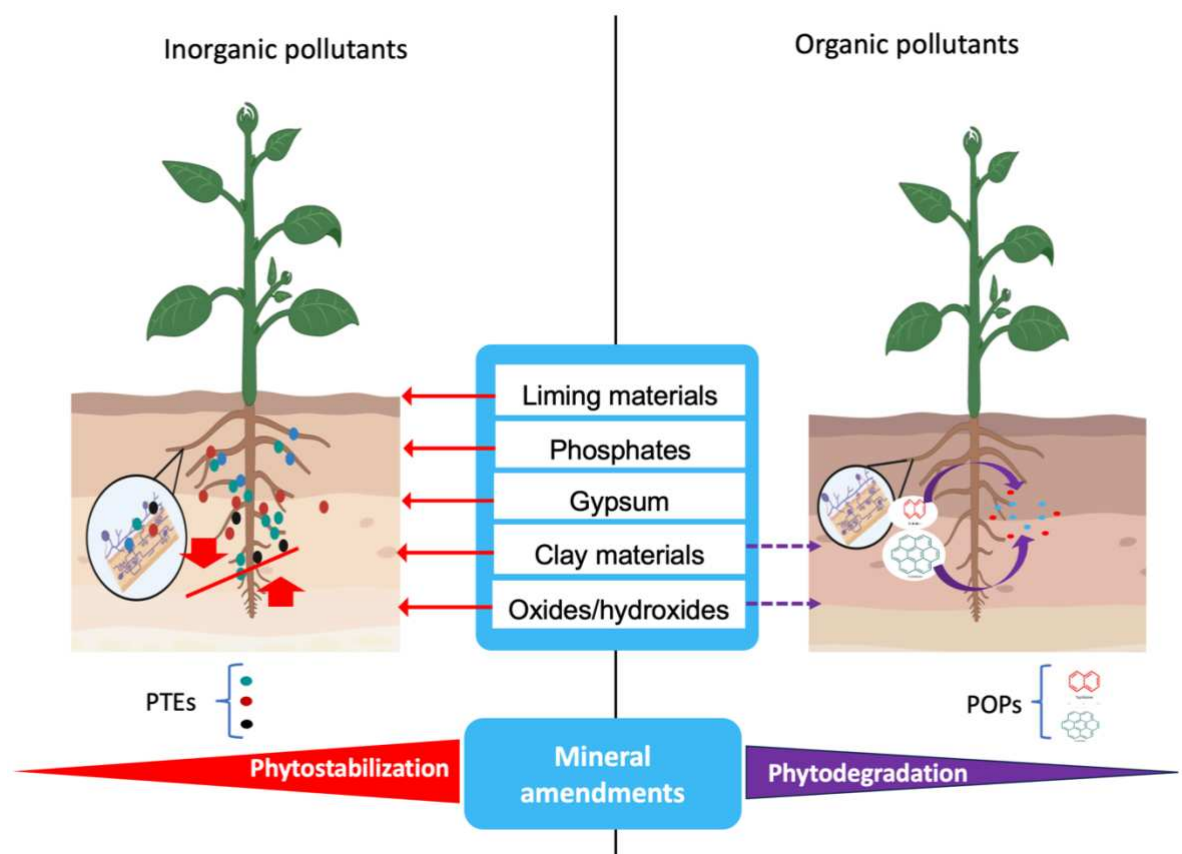


Figure 3: Effect of different types of mineral amendments on the mobility and fate of inorganic (PTEs) and organic (POPs) pollutants in soil during phytoremediation. Mineral amendments decreased the mobility of PTEs by (i) rising the soil pH, (ii) by sorbing cationic PTEs and (iii) by forming metal-carbonate complexes. Arrows in broken lines indicated alternative (less frequent) phytoremediation pathways promoted by the application of some specific amendments.

3.2. Effect of mineral amendments on soil microbial activity, nutrient recycling and fertility

Microbial activity is generally inhibited in PTEs polluted soil, mainly because of the subsequent toxicity (Kumar et al., 2022; Zamulina et al., 2021). Mineral amendments could alleviate the negative effect of these toxic metals on microbial activity allowing soil refunctionalization. For example, a liming material (constituted with 70-80% of CaCO_3), derived from sugar manufacturing process increased dehydrogenase enzymatic activity due to its positive effect on soil pH, while improving PTEs phytostabilization (Burgos et al. 2010). During the phytoremediation of a Ni-polluted soil, amendment with limestone led to the increase of several enzymatic activities (β -glucosidase, phosphatase, urease and catalase) (Hannan et al. 2021). Moreover, four year application of lime alone or lime combined with a phosphate fertilizer in Cd-polluted soil cultivated with rice led to an increase of bacterial richness and diversity suggesting potential benefic effect on microbial functioning (Shi et al. 2019). Similarly, an increase in root mycorrhizal colonization and soil AMF abundance were noticed after fly ash aided-phytostabilization of a Pb, Cd and Zn polluted soil planted with a mixed plant community, suggesting an improvement of soil nutrients (P and N) availability (Labidi et al. 2015).

3.3 How mineral amendments affect plant growth in polluted soils?

While a reduced phytotoxicity is noticed during PTEs phytostabilization, a beneficial effect of mineral amendments on plant growth remains controversial. Indeed, several studies reported that application of mineral amendments in polluted soils subject to phytoremediation resulted to an improvement of plant growth/yield whereas other ones supported no beneficial effects. Indeed, positive effect was noticed on wheat growth in Cd-polluted soil amended with phosphates and gypsum (Qayyum et al. 2017). Application of different mineral amendments (rock phosphate, phosphogypsum, triple superphosphate, sugar beet factory lime) improved also the yield of sorghum cultivated in soil derived from a multi PTEs polluted site (Shaheen et al. 2017b). Moreover, maize biomass was also increased after sepiolite (hydrous magnesium silicate clay) amendment to Cd and Pb polluted farmlands (Zhan et al. 2019). This positive effect on plant growth can be explained by several factors such as the direct effects of amendments on nutrient status, structuration and water retention of soil, but also their potential effects on microbial activity and nutrient recycling and their retrieving effects on soil phytotoxicity. Conversely, some studies reported an absence of yield improvement despite PTEs phytostabilization following mineral amendments application. For example, although the phytostabilizing effect of silicon slag and calcium hydroxide on soil available Cd, these mineral amendments did not increase rice yield (Bian et al. 2016). The rice grain yield was not changed after lime and mineral phosphate were applied on Cd-polluted soil despite the immobilization effect noticed on this PTE (Shi et al. 2019). The same results were obtained on the yield of a mixed plant community after an amendment of a mixed polluted soil (Cd, Pd and Zn) with Na-bentonite, dolomite and natural zeolite (Vrinceanu et al. 2019). The controversial results of these amendments on plant growth/yield may be explained by their composition (e.g., P-rich amendments are likely to be highly benefic for plant thanks to their potential role on P supply), the plant species and cultivar because of their specific sensibility to oxidative stresses caused by PTEs, the initial concentration of the pollutant etc.

The negative effect of POPs on plant growth can be also retrieved by mineral amendments. Indeed, nano-SiO₂, nano-CeO₂ and Na-montmorillonite were reported to have positive effect on the photosynthesis and biomass of *showy stonecrop* (*Sedum spectabile*) cultivated in PHs polluted soil (Liu et al. 2023a). Moreover, the application of different mineral sorbents (kaolinite, zeolite, diatomite and vermiculite) to a crude oil-polluted soil led to an increase of the harvested biomass, due to the reduced soil phytotoxicity and the increased of the population of hydrocarbon-degrading bacteria (Vasilyeva et al. 2022).

3.4. How mineral amendments affect C storage in polluted soils?

Mineral amendments are widely studied in the phytoremediation of soil contaminants and mainly PTEs. However, their potential effect on C sequestration is rarely investigated. This seem to be a critical as they can impact soil enzyme activities (section 3.2), which may determine the fate of SOM in polluted soils. Nevertheless, Placek et al. (2017) showed that lake chalk amendment to melter-polluted soil and post-mining soil planted with scots pine (*Pinus sylvestris*) and miscanthus (*Miscanthus giganteus*) has led to an increase in SOC concomitantly to a reduced concentration of soil available PTEs after 18 months, suggesting a positive impact on C sequestration. In addition, the reduced phytotoxicity and the improved plant growth positively contributed to C stockage though the more C phytosequestration, particularly for scots pine. Therefore, more attention should be devoted to C flow consequences of mineral amendments in future phytoremediation studies.

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- 1 **Table 2:** Effect of mineral amendments on soil ecosystems functions during phytoremediation of inorganic and organic pollutants. SOC= soil organic carbon. Semi-field
 2 experiment means an experiment carried out in big containers placed outdoors without any control apart from watering

Ecosystem functions	pollutants and phytoremediation process	Plant species	Mineral amendments	Culture conditions	Observed effects	References
Soil microbial activities and nutrients recycling	Phytostabilization of Ni	Rapeseed (<i>Brassica napus</i> L.)	Liming materials	Pot experiment	<ul style="list-style-type: none"> Increased enzymatic activities (β-glucosidase, phosphatase, urease and catalase) 	
	Phytostabilization of Cd	Rice (<i>Oriza sativa</i>)	Lime and phosphates	Field experiment	<ul style="list-style-type: none"> Increased bacterial richness and diversity 	(Shi et al. 2019)
	Phytostabilization of Pb, Cd and Zn	Mixed plant community : <u>black locust</u> (<i>Robinia pseudoacacia</i> L.), <u>black alder</u> (<i>Alnus glutinosa</i> L.), <u>sycamore maple</u> (<i>Acer pseudoplatanus</i> L.), <u>pedunculate oak</u> (<i>Quercus robur</i> L.), and <u>white willow</u> (<i>Salix alba</i> L.)	Fly ashes	Field experiment	<ul style="list-style-type: none"> Increased plant mycorrhization (root colonization, arbuscules and vesicles frequency) Increased arbuscular mycorrhizal fungi abundance of soil 	(Labidi et al. 2015)
	Phytostabilization of Cd	Wheat (<i>Triticum aestivum</i> L.)	Phosphate and Gypsum	Field experiment	<ul style="list-style-type: none"> Increased plant growth and grain yield 	(Qayyum et al. 2017)
	Phytostabilization of Al, Fe, Mn, Cr and Zn	Sorghum (<i>Sorghum bicolor</i>)		Pot experiment		(Shaheen et al. 2017b)

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Phytotoxicity alleviation and plant growth	Phytoextraction of Cd, Cu, Ni		Phosphate rock, Triple superphosphate, Sugar beet factory lime, Sulfur		<ul style="list-style-type: none"> Increased plant growth and grain yield 	
	Phytostabilization of Cd and Pb	Maize (<i>Zea mays</i>)	Sepiolite (hydrous magnesium silicate clay)	Field experiment	<ul style="list-style-type: none"> Increased biomass 	(Zhan et al. 2019)
	Phytostabilization of Cd	Rice (<i>Oryza sativa L. Japonica</i>)	Silicon slag and calcium hydroxide	Field experiment	<ul style="list-style-type: none"> No effect on yield 	(Bian et al. 2016)
	Phytostabilization of Cd	Rice (<i>Oryza sativa L</i>)	Lime and Mineral phosphate	Field experiment	<ul style="list-style-type: none"> No effect on yield 	(Shi et al. 2019)
	Phytostabilization of Cd, Pb and Zn	Mixed plant community (<i>Trifolium pratense</i> , <i>Dactylis glomerata</i> , <i>Lolium perenne</i> , <i>Agropyron repens</i>)	Na-bentonite, dolomite and natural zeolite	Field experiment	<ul style="list-style-type: none"> No effect on yield 	(Vrinceanu et al. 2019)
	Phytoextraction of PAHs	Showy stonecrop (<i>Sedum spectabile</i>)	Nano-SiO ₂ and nano-CeO ₂	Pot experiment	<ul style="list-style-type: none"> Increased biomass 	(Liu et al., 2023)
	Phytodegradation of Crude oil (TPH)	Red clover (<i>Trifolium pratense</i>) and Ryegrass (<i>Lolium perenne</i>)	Kaolinite, zeolite, diatomite and vermiculite	Semi-field experiment	<ul style="list-style-type: none"> Increased biomass 	(Vasilyeva et al. 2022)
Carbon sequestration	Phytostabilization of Cd, Pb and Zn in a Melter-polluted soil and post-mining soil	Pine (<i>Pinus sylvestris L.</i>) and Miscanthus (<i>Miscanthus x giganteus</i>)	Lake chalk	Field experiment	<ul style="list-style-type: none"> Increase in SOC Increased phytosequestration 	(Placek et al. 2017; Placek-Lapaj et al. 2019)

4. Role of microbial amendments on the ecosystem services of polluted soils

Microorganisms play key roles in the soil functioning and notably in ecosystem restoration as they contribute to C and nutrients recycling (Harris 2009). Plant inoculation with plant growth promoting bacteria (PGPR), bacterial endophytes and arbuscular mycorrhizal fungi (AMF) are recognized as a method to boost plant growth and biomass production (Glick 2012; Gao et al. 2020). To boost the phytoremediation of polluted soils by either PTEs or POPs, some of these microbial amendments including notably PGPR, bacterial endophytes and AMF are used as inoculants to enhance the bioaccumulation/immobilization of PTEs or to stimulate the degradation of organic pollutants (Ma et al. 2019; Li et al. 2021; Lebrun et al. 2021a, b; Alves et al. 2022). Here, we reviewed recent studies on the effects of different microbial amendments on pollutant fate (**Fig. 4**). We will also analyze the impact of such inoculants on nutrient recycling, plant growth/yield in different phytoremediation contexts (**Table 3**).

4.1. How bioinoculants affect soil pollutants?

Microbial inoculants have been applied and tested in phytoremediation of different polluted soils. According to their nature, they can facilitate and promote different PTEs phytoremediation pathways (**Fig. 4**). Indeed, amendment with AMF (*Funneliformis mosseae*, *Acaulospora* sp, *Gigaspora* sp, *Glomus* sp, *Claroideoglossum claroideum*) leads generally to PTEs phytostabilization in the soil (Gil-Cardesa et al. 2018; González-Chávez et al. 2019; Zhang et al. 2019; Hu et al. 2019; Pérez et al. 2021; Fontaine et al. 2022; Wang et al. 2022). In these cases, the reduced availability of PTEs is related to their immobilization by the glomalin secreted by the AMF, the wall of hyphae, or their storage in the vacuoles of resistance spores (Nayuki et al. 2014; Ferrol et al. 2016; Janeeshma and Puthur 2020).

Although some AMF inoculants have PTEs mobilizing effect and lead to their bioaccumulation in plant root, they do not ensure sufficient translocation in shoot, suggesting a potential of such AMF/plant associations to prevent food chain contamination and pollutant dispersion in the environment (González-Villalobos et al. 2021; Morais et al. 2021; Pan et al. 2023). For example, Pan et al. (2023) showed that the inoculation of *F. mosseae* in a Mn-polluted soil cultivated with nutgall tree (*Rhus chinensis*) led to an increased Mn bioaccumulation but importantly the concentration of a such PTE in plant shoot was not modified, suggesting a rhizo-immobilization.

Concerning bacterial inoculants, phytoremediation studies reporting PTEs phytostabilizing effect are very scarce (Kang et al. 2018; Ke et al. 2021a). Bacterial inoculation is therefore more likely associated with mobilization of PTEs and their phytoextraction from polluted soils. For example, inoculation with bacterial endophytes has led to Cd, Pb, Zn, Hg and As accumulation and promote their phytoextraction by various plant species (*Phytolacca acinosa*, *Sedum plumbizincicola*, *Sedum alfredii*, *Oriza sativa*, *Cynodon dactylon*, *Eleusine indica*) (Ma et al. 2015; Tang et al. 2020; Ullah et al. 2022; Ustiatik et al. 2022; Li et al. 2023a). Rhizosphere bacteria from different genera have also phytoextraction abilities when inoculated in Cu, Pb, Mn, Ni, Cd, As, Cr-polluted soils (Ma et al. 2015; Kong et al. 2015; Durand et al. 2016; Moreira et al. 2016; Funes Pinter et al. 2017; Ke et al. 2021b; Tirry et al. 2021; Chen et al. 2023). Indeed, their positive effects on PTEs bioaccumulation can be related to (i) organic acid release which enhance PTEs bioavailability, (ii) siderophore secretion which leads to PTEs chelation, (iii) metal

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biotransformation to a lesser toxic forms through oxidation/ reduction (e.g. $\text{Hg}^{2+} \rightarrow \text{Hg}$; $\text{Cr}^{6+} \rightarrow \text{Cr}^{3+}$; $\text{As}^{3+} \rightarrow \text{As}^{5+}$), methylation/demethylation, hydrogenation and (iv) plant growth promotion thanks to the increased nutrient availability (P solubilization, nitrogenase and phytohormones secretion etc.) (Diaz-Bone and Wiele 2010; Mishra et al. 2017; Alves et al. 2022).

Furthermore, some bacterial species have the potential to degrade PHs and particularly PAHs which are persistent in soils and therefore constitute a major environmental and health concern (Pandey et al. 2012; Sharma et al. 2016). This has been observed after the inoculation of a *Serratia* sp strain to *Brassica napus* L. planted a PAHs polluted soil (Guan et al. 2023). Inoculation of *Pseudomonas* sp to *Sorghum bicolor* in a pyrene contaminated soil led to an increased dissipation of such PAH (Rostami et al. 2016). The same result was observed after the inoculation of *Pseudomonas aeruginosa* to a pyrene polluted soil planted with *Medicago sativa* L (Panwar and Mathur 2023). Moreover, the potential of AMF inoculation to stimulate the degradation of various POPs (PAHs and TPHs and polychlorinated bisphenyl) during phytoremediation has been reviewed by Lenoir et al., (2016). Indeed, such fungal inoculants could improve the dissipation of POPs through several mechanisms, in particular the stimulation of the telluric activity of enzymes (dehydrogenase, peroxidase, catalase etc.) involved in the degradation and the transformation of aromatic compounds (Lenoir et al. 2016).

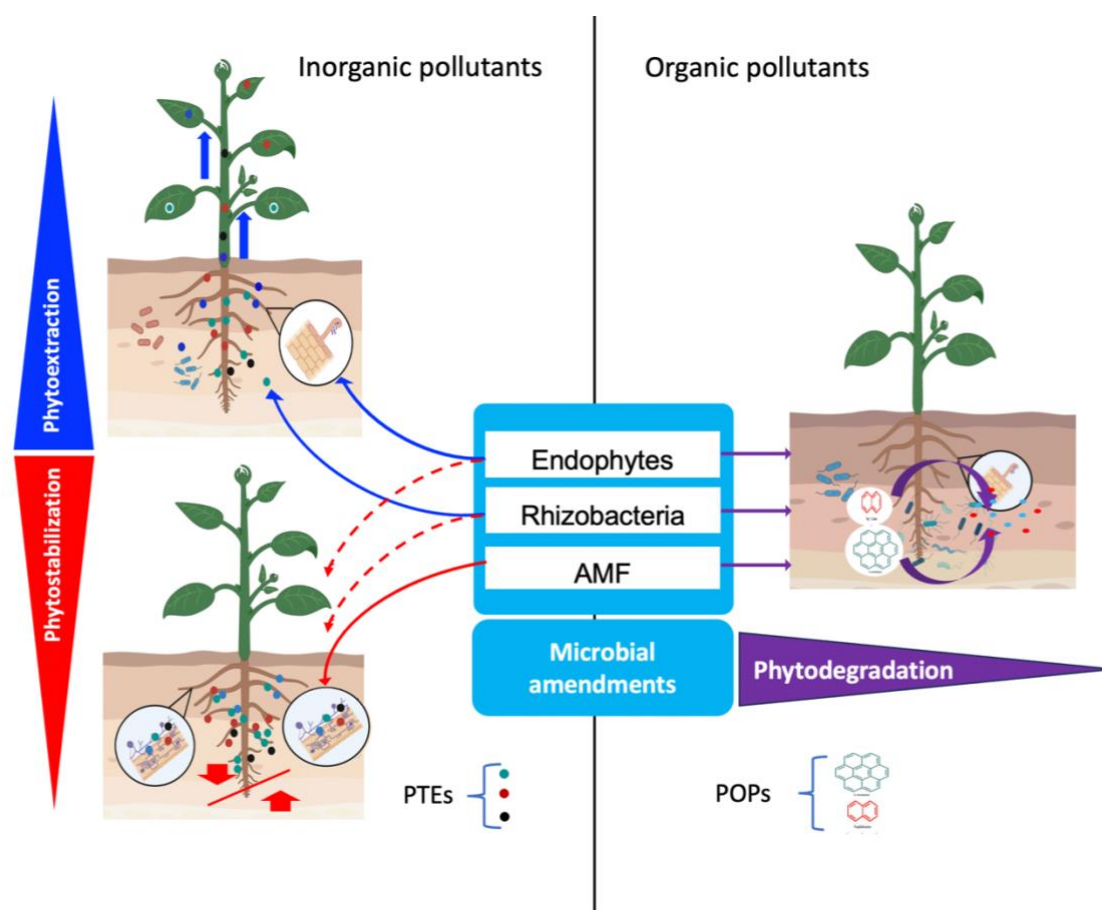


Figure 4: Effect of different types of microbial amendments on the mobility and fate of inorganic (PTEs) and organic (POPs) pollutants in soil during phytoremediation. Arrows in broken lines indicated alternative (less frequent) phytoremediation pathways promoted by the application of some specific amendments.

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Phytostabilization of PTEs by AMF is related to their immobilization by the AMF glomalin and the wall of hyphae, or their storage in the vacuoles of resistance spores. PTEs increased bioaccumulation after bacterial inoculation is related to (i) organic acids release which enhance PTEs bioavailability, (ii) siderophore secretion leading to PTEs chelation, (iii) metal biotransformation to lesser toxic forms through oxidation/reduction, methylation/demethylation, hydrogenation and (iv) improved plant growth/biomass production.

4.2. How bioinoculants affect soil microbial activity and nutrient recycling in polluted soils?

The use of microbial amendments is considered as a sustainable fertility management approach which improve plant growth through several mechanisms including the modulation of soil microbial diversity, functioning and nutrient recycling. Importantly, some microbial species applied along with soils phytoremediation have also the abilities to improve soil microbial activity/nutrient recycling. Indeed, microbial inoculants, can act by (i) improving nutrient phytoavailability and supply (e.g., N fixation, P and K solubilization etc), (ii) producing phytohormones (auxins, cytokinins, gibberellins) which regulate plant growth, alleviate metal toxicity and (iii) by producing enzymes (e.g., 1-aminocyclopropane-1-carboxylate (ACC) deaminase that regulates ethylene levels in stressed plants) (Ma et al. 2016). Therefore, these microbial amendments could contribute to improve polluted soil functioning and their nutritional status which is generally reduced because of the ecotoxicity of the polluted soils. For example, Li et al., (2023) reported that the inoculation of *Phytolacca acinosa* with a bacterial endophyte (*Bacillus paramycooides*) in a Cd-polluted soil increased the microbial metabolic functional diversity and nutrient contents of soil (available-N, P, and K). Accordingly, the inoculation of rhizobacteria strains such as *Bacillus paramycooides* and *Klebsiella huaxiensis* led to the increase of nutrient contents (organic matter, available P) and enzyme activities (urease, phosphatase and sucrase) in a multi-polluted soil (Mn, Pb, and As) cultivated with *Festuca arundinacea* (Chen et al. 2023). Importantly, these improvements in soil functioning markers were accompanied by a modification of bacterial diversity; in particular, with an increase of Firmicutes group comprising the *Bacillus* genus, known for its metal tolerance. Similarly, the inoculation with AMF have also been reported to improve the microbial activities of polluted soil in context of phytoremediation. For example, the inoculation of *Claroideoglossum etunicatum* to Cd polluted soil modified microbial communities' structure and increased the abundance of C (carbohydrate metabolism) and N cycling genes (ammonia and nitrate production: e.g., glutamate dehydrogenase[NADP⁺], glutamine synthetase, nitrate reductase and hydroxylamine dehydrogenase) (Hao et al. 2022).

Microbial amendments used to improve phytoremediation of POPs in polluted soils may also improve soil functioning. Indeed, Guan et al. (2023) inoculated Brassica napus L. cultivated on a PAHs polluted soil with a phenanthrene degrading capability Serratia sp strain and found an increased activity of enzymes related to nutrient recycling (catalase and alkaline phosphatase). Moreover, the introduction of Pseudomonas aeruginosa to a pyrene polluted soil planted with Medicago sativa L improved significantly soil enzyme activities (dehydrogenase and fluorescein diacetate hydrolysis) (Panwar and Mathur 2023). The same positive effect on soil enzyme activities (urease, polyphenol oxidase, laccase, and dehydrogenase) have been noticed when different AMF species have been combined and inoculated for the phytoremediation of PAHs (benzo(α) pyrene, pyrene and phenanthrene) polluted soil (Li et al. 2023c). Similarly, the microbial activity (dehydrogenase activity and

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fluoresceine di-acetate hydrolysis) in the rhizosphere of Alfalfa (*Medicago sativa* L.) grown in dioxin/furan polluted soil increased after inoculation with a commercial AMF mixture composed by *Funneliformis mosseae* and *Glomus sp* (Meglouli et al. 2018).

4.3. How bioinoculants affect plant growth in polluted soils?

Microbial inoculants are used to boost the phytoremediation of PTEs and POPs polluted soil through phytostabilisation, phytoextraction or phytodegradation processes. In all cases, microbial inoculation alleviated the negative effects of soil PTEs and POPs on physiology and plant growth mainly through the improvement of soil fertility (increase of nutrients phytoavailability: N, P, K) or the alleviation of organic or inorganic pollutants related phytotoxicity (Li et al. 2023c; Panwar and Mathur 2023; Guan et al. 2023). This reduction of phytotoxicity is also related an enhancement of plant antioxidant capacity following microbial amendment (Li et al. 2023c; Guan et al. 2023). For PTEs, the inoculation of Ni and Pb polluted soil planted with *Atriplex lentiformis* using *Bradyrhizobium japonicum* and/or *Pseudomonas fluorescens* improved the plant chlorophyll content, antioxidative enzyme activity and plant height and biomass (Kumar et al. 2023). Similarly, inoculation with a bacterial consortium formed by *Vibrio neocaledonicus*, *Thalassospira australica* and *Pseudarthrobacter oxydans*, increased the growth rate, the number of stem branches, photosynthetic activity and the water content of *Salicornia ramosissima* planted in a soil polluted by As, Cu, Zn and Pb (Mesa-Marín et al. 2020). In the same way, the inoculation of *Medicago sativa* planted in Cr polluted soil with a *Pseudomonas sp* strain decreased plant stress (hydrogen peroxide, and proline content) and importantly increased chlorophyll content, shoot and root dry weights (Tirry et al. 2021). Furthermore, in field inoculation of *Acaulospora sp* and *Funneliformis mosseae* in a Pb polluted soil led to its phytostabilization while improving Castor bean (*Ricinus communis*) seed weight and oil content (González-Chávez et al. 2019). Similarly, poplar (*Populus sp*) biomass production was increased in a field experiment after inoculation with a mixture of three commercial mycorrhizal inoculum (see **Table 3** for inoculum composition) (Ciadamidaro et al. 2017). Concerning POPs, *Pseudomonas sp* inoculation to a pyrene contaminated soil alleviated the phytotoxic effect on *Sorghum bicolor* and enhanced its growth (Rostami et al. 2016). Similar result was obtained by Fatima et al. (2018) who found an improvement of plant growth in *Leptochloa fusca* and *Brachiaria mutica* after their inoculation by bacterial endophytes (*Acinetobacter sp*, *Pseudomonas aeruginosa* and *Klebsiella sp*) for the phytodegradation of crude oil. In addition, AMF were also reported to have beneficial effect on the growth of plant cultivated in PAHs polluted soils in a microbial-assisted phytoremediation context (Lenoir et al. 2017).

4.4. Effect of bioinoculants on Carbon storage in the context of phytoremediation

The translocation and stabilization of atmospheric C into the soil organic matter pool is among recommended strategies for mitigating CO₂ emission (Minasny et al. 2017). Apart from its positive effect on C phytosequestration into the plant biomass, the inoculation of microbes during phytoremediation could improve soil C storage either by more C transfer into the soil or better stabilization of SOM. For example, while contributing to assist the phytoremediation of both organic and mineral soil pollutants, AMF could promote soil C sequestration through not only the development of hypha networks from the C derived from photosynthesis, but the secretion of glycoproteins such as glomalin related soil proteins which are largely known to promote soil aggregation and

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SOC stabilization (Zhang et al. 2020; Jeewani et al. 2021; Agnihotri et al. 2022). Similarly, some PGPR used to promote phytoremediation could contribute in SOC stabilization through the secretion of polymer like exopolysaccharide (EPS), which have been demonstrated to promote rhizosphere soil aggregation and SOC stabilization. Indeed, EPS production along with positive plant growth promotion have been reported after plant inoculations with several PGPR belonging to various taxonomical groups (Kaci et al. 2005; Figueiredo et al. 2008; Berge et al. 2009; Sandhya et al. 2009). In addition, the increasing aerial biomass induced by microbial amendments of marginal polluted soils could contribute to enhance soil C sequestration (e.g., after valorization of biomass and transformation of residual by-products to biochar which can be returned to soil).

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Table 3: Effect of microbial amendments on soil ecosystem functions during the phytoremediation of inorganic and organic pollutants

Ecosystem services	Pollutants and phytoremediation processes	Plant species	Microorganisms	Culture conditions	Effects observed	References
Soil microbial activities and nutrients recycling	Phytoextraction of Cd	<i>Indian pokeweed (Phytolacca acinose)</i>	<i>Bacillus paramycoides</i>	Pot experiment	<ul style="list-style-type: none"> • Increased nutrient contents (available N, P and K) • Increased microbial metabolic diversity 	(Li et al., 2023)
	Phytoextraction of Cd	Ryegrass (<i>Lolium perenne</i>)	<i>Serratia sp</i>	Pot experiment	<ul style="list-style-type: none"> • Increased enzyme activities (Urease, acid phosphatase) • Increased soil N and K 	(Li et al. 2023b)
	Phytoextraction of Cd Mn, Pb and As	<i>Tall fescue (Festuca arundinacea)</i>	<i>Bacillus paramycoides</i> and <i>Klebsiella huaxiensis</i>	Pot experiment	<ul style="list-style-type: none"> • <i>Increased nutrient content</i> (organic matter, available P) • <i>Increased enzyme activities (urease, phosphatase and sucrase)</i> 	(Chen et al. 2023)
	Phytodegradation of PAHs	Brown mustard (<i>Brassica napus L</i>)	<i>Serratia sp</i>	Pot experiment	<ul style="list-style-type: none"> • Increased enzyme activities (<u>catalase</u> and <u>alkaline phosphatase</u>) 	(Guan et al., 2023)
	Phytodegradation of Pyrene	Alfalfa (<i>Medicago sativa L</i>)	<i>Pseudomonas aeruginosa</i>	Pot experiment	<ul style="list-style-type: none"> • Increased soil enzyme activities (dehydrogenase and fluorescein diacetate hydrolysis) 	(Panwar and Mathur., 2023)

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	Phytodegradation of benzo(α) pyrene, pyrene and phenanthrene	Basket willow (<i>Salix viminalis</i> L)	AMF (<i>Funneliformis mosseae</i> , <i>Laroidoglomus etunicatum</i> , and <i>Rhizophagus intraradices</i>)	Pot experiment	<ul style="list-style-type: none"> Increased soil enzyme activities (urease, polyphenol <u>oxidase</u>, laccase, and dehydrogenase) 	(Li et al., 2023)
	Phytostabilization of Cd	Maize (<i>Zea mays</i>)	AMF (<i>Claroideoglomus etunicatum</i>)	Pot experiment	<ul style="list-style-type: none"> Increased the abundance of carbohydrate metabolism genes, ammonia and nitrate production genes, Increased of indole-3-acetic acid and 1-aminocyclopropane-1-carboxylate genes 	(Hao et al. 2022)
	Phytodegradation of dioxines/furans	Alfalfa (<i>Medicago sativa</i> L)	Commercial AMF inoculum (<i>Funneliformis mosseae</i> and <i>Glomus sp</i>)	Pot experiment	<ul style="list-style-type: none"> Increase of fluoresceine di-acetate hydrolysis and dehydrogenase activity 	(Meglouli et al. 2018)
Phytotoxicity alleviation and plant growth	Phytoextraction of Ni and Pb	Quail Bush (<i>Atriplex lentiformis</i>)	<i>Bradyrhizobium japonicum</i> and/or <i>Pseudomonas fluorescens</i>	Pot experiment	<ul style="list-style-type: none"> Increased plant chlorophyll content, Increased plant antioxidative enzyme activity Increased plant height and biomass 	(Kumar et al. 2023)
	Phytostabilization of Pb	<i>Ricinus communis</i>	<i>Acaulospora</i> sp. and <i>Funneliformis mosseae</i>	Field condition	<ul style="list-style-type: none"> Increased plant survival Not effect on plant growth (height, stem diameter) Increase of seeds weight Increase of seed oil content 	(González-Chávez et al. 2019)

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Phytoextraction of As, Cu, Zn and Pb	Twiggy Glasswort (<i>Salicornia ramosissima</i>)	Bacterial consortium (<i>Vibrio neocaledonicus</i> , <i>Thalassospira australica</i> and <i>Pseudarthrobacter oxydans</i>)	Pot experiment	<ul style="list-style-type: none"> • Increased the growth rate • Increased number of stem branches • Higher photosynthetic activity • Higher plant water content 	(Mesa-Marín et al. 2020)
Phytostabilization of Cr	Alfalfa (<i>Medicago sativa</i> L.)	<i>Pseudomonas</i> sp	Pot experiment	<ul style="list-style-type: none"> • Decreased plant stress (hydrogen peroxide, and proline content) • Increased chlorophyll content, • Increased shoot and root biomass 	(Tirry et al. 2021).
Phytostabilization of Cd, Cu, Pb and Zn	Poplar (<i>Populus</i> sp)	Fungal consortium (<i>Rhizophagus irregulare</i> , <i>Rhizophagus intraradices</i> , <i>Funneliformis mosseae</i> , <i>F. geosporum</i> , <i>Claroideoglomus claroideum</i> , <i>C. etunicatum</i> , <i>Glomus microaggregatum</i> , <i>Hebeloma mesophaeum</i> , <i>Amanita rubescens</i> , <i>Laccaria proxima</i> , <i>Paxillus involutus</i> , <i>Pisolithus arrhizus</i> and <i>Scleroderma citrinum</i>)	Field experiment	<ul style="list-style-type: none"> • <i>Increased biomass production</i> 	(Ciadamidar o et al. 2017; Phanthavong sa et al. 2017)
As, Cd, Pb and Zn	<i>Acacia saligna</i> and <i>Eucalyptus camaldulensis</i>	Consortium of fungal ectomycorrhizae (<i>Pisolithus tinctorius</i>), endomycorrhizae (<i>Entrophospora colombiana</i> , <i>Glomus clarum</i> , <i>Glomus</i>	Field condition	<ul style="list-style-type: none"> • <i>Increased growth (diameter and height)</i> • <i>Increase of glutathione S-Transferase activity</i> 	(Guarino and Sciarrillo 2017)

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			<i>etunicatum</i> , <i>Glomus intraradices</i>), and six bacteria species (<i>Bacillus licheniformis</i> , <i>Bacillus megaterium</i> , <i>Bacillus polymyxa</i> , <i>Bacillus subtilis</i> , <i>Bacillus thuringiensis</i> , <i>Paenibacillus azotofixans</i>)			
Phytodegradation of PAHs	Rapeseed (<i>Brassica napus</i> L.)	<i>Serratia sp</i>	Pot experiment	<ul style="list-style-type: none"> Increased growth (root length, stem length, biomass, leaf surface, germination rate) 	Guan et al., 2023	
Phytodegradation of Crude oil	Swamp grass (<i>Leptochloa fusca</i>) and buffalo grass (<i>Brachiaria mutica</i>)	<i>Acinetobacter sp</i> , <i>Pseudomonas aeruginosa</i> and <i>Klebsiella sp</i>	Field condition	<ul style="list-style-type: none"> Increased plant growth (plant biomass, root and shoot length) 	(Fatima et al., 2018)	
Phytodegradation of pyrene	Sorgho (<i>Sorghum bicolor</i>)	<i>Pseudomonas sp</i>	Pot experiment	<ul style="list-style-type: none"> Increased plant growth (plant biomass) 	(Rostami et al. 2016)	
Phytodegradation of pyrene	Alfalfa (<i>Medicago sativa</i> L.)	<i>Pseudomonas aeruginosa</i>	Pot experiment	<ul style="list-style-type: none"> Increased plant growth (Root and shoot length, plant biomass) 	(Panwar and Mathur., 2023)	
Phytodegradation of benzo[a]pyrene)	Barrel medic (<i>Medicago truncatula</i>)	<i>Rhizophagus irregularis</i>	Pot experiment	<ul style="list-style-type: none"> Reduction of phytotoxicity (decreased H₂O₂ accumulation) Increased root length 	(Lenoir et al. 2017)	

6. Conclusion and perspectives

Beyond their roles in the phytoremediation of PTEs and POPs polluted soils, the different types of amendments reported here are more likely prone to foster different phytoremediation processes. In additions, they may have differential impacts on other soil ecosystem functions like nutrient recycling, primary production and C sequestration.

- Globally, organic amendments lead to phytostabilization of PTEs or phytodegradation of POPs and have positive effects on soil microbial activity/nutrient recycling, as well as on plant growth and yield in phytomanaged soils. Depending on their nature and soil physico-chemical and microbiological characteristics, organic amendments could be subjected to various mineralization degree and rate, and therefore may remain in soil for variable periods of time (Diacono and Montemurro 2011; Quilliam et al. 2012). Therefore, the durability of their effects on polluted soil functions should be studied in the longer term to enable a better assessment of the sustainability of phytoremediation approaches when regular re-applications are needed. Nevertheless, a potential issue linked to organic amendments is their possibility to contain PTEs or organic pollutants, which could potentially exacerbate contamination issues if not properly screened or managed. Therefore, a continuous monitoring of their quality is required before each application throughout the phytoremediation process.
- Mineral amendments are more frequently oriented toward a phytostabilization strategies. However, only few studies reported their positive effects on polluted soil microbial functioning, whereas their impact on plant growth is controversial. This controversy could be explained by the nature of the mineral amendments (*i.e.*, phosphate rich minerals could likely improve plant P nutrition more than clay minerals), their application dose, the plant species, the soil physico-chemical parameters and the targeted PTEs.
- Several studies have highlighted positive effects of bacterial and fungal inoculants on microbial activities and nutrient status of phytomanaged soils. Globally, bacterial inoculants are more frequently efficient for PTEs mobilization and phytoextraction, whereas fungal inoculants lead generally to PTEs immobilization and phytostabilization. Both bacterial and fungal inoculants are also efficient for POPs phytodegradation.
- For the three types of amendments, little is known about their impact on C sequestration, which is crucial for atmospheric CO₂ removal and climate change mitigation. In particular, for organic and microbial amendments that may impact thoroughly microbial activities and soil organic matter degradation and dynamic, their impact on C flux/balance in the soil-plant-atmosphere system is paramount and should be investigated and integrated as a criterion when assessing phytoremediation efficiency.

The majority of assisted-phytoremediation studies were carried out in pot conditions (**Table 1, 2 &3**) and therefore, the applicability on the field remains critical. Notably, the capabilities of microbial amendments could be lost in the field due to abiotic stresses or interaction with resident soil microbial communities (Khare and Arora 2015; Kaminsky et al. 2019; O'Callaghan et al. 2022). For example, a decrease of Cd and Zn concentrations was shown in *Festuca rubra* grown in the field after its inoculation with a consortium of bacterial endophytes (*Microbacterium sp* and *Pseudomonas sp*), but no significant effect was obtained in plant biomass yield (Burgess et al. 2016). In addition, several other in field studies did not find any effect of AMF inoculation on plant growth or PTEs mobility

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(Raveau et al. 2020; Perlein et al. 2021; Langrand et al. 2023). Nevertheless, enhanced phytoremediation efficiency (phytostabilization of Zn, Cu, Pd and Cr, and an improved plant growth) has been observed after mycorrhizal inoculation of plants cultivated in polluted fields (Ciadamidaro et al. 2017; Phanthavongsa et al. 2017; González-Chávez et al. 2019) as well as a successful enhancement of PHs degradation following the inoculation of crude oil polluted soil with bacterial endophytes (*Acinetobacter sp*, *Pseudomonas aeruginosa* and *Klebsiella sp*) in a field experiment (Fatima et al. 2018). This suggests the necessity to focus more on field experiments for the assessment of amendment aided phytoremediation on soils ecosystem functions.

For agricultural polluted land or mine tailing sites, phytomanagement can allow soil reclamation, while ensuring economic and social benefits. This includes the production of feedstock in polluted sites for multiple industries (bioenergy, pulp and paper, food additives, building materials, composites and plastic alternatives, biochemical products like adhesives, detergents or essential oil etc.) (Moreira et al. 2021). For example, Raveau et al. (2021) showed that aromatic plants i.e., clary sage (*Salvia sclarea* L.) and coriander (*Coriandrum sativum*) were not adversely affected when cultivated in PTEs polluted agricultural land and the biomass valorization through essential oil (EO) production was economically viable for clary sage. However, despite a positive effect on root colonization, they found that mycorrhizal inoculation did not increase plant biomass or EO yield. Therefore, organic amendments like biochar, biosolids, manures or composts which showed positive effect on biomass production (Alvarenga et al. 2009; Liu et al. 2009; Ali et al. 2017), could represent good options not only for the immobilization of pollutants but also for improving the economic profitability by increasing the biomass and EO produced from these aromatic plants.

Sustainable nature-based solution project which captures C or reduce greenhouse gas emission can be labelled for carbon credits which can be sold to industrial/private companies aiming a net-zero C emission (Donofrio et al. 2019). Therefore, phytoremediation approaches like phytostabilization which require a long-term planting of crops/trees in polluted lands could be eligible to this funding. In such perspectives, organic amendments such as biosolid, compost and manure, which showed a real potential to enhance C sequestration in soil or in biomass (Placek-Lapaj et al. 2019; Sawarkar et al. 2023), could enhance phytostabilization attractiveness by improving the economic income thanks to this funding mechanism. Before that, more researches are needed to fully estimate the global C balance within different amendment assisted phytostabilization systems (combinations of different crop species with different amendments) in different agroclimatic conditions.

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Synthèse bibliographique

Revue 2 : Significance of Arbuscular Mycorrhizal Fungi in Mitigating Abiotic Environmental Stress in Medicinal and Aromatic Plants: A Review

La rentabilité socio-économique des phytotechnologies constitue l'un des principaux freins à leur développement. A l'heure actuelle, les enjeux majeurs conditionnant leur développement portent sur la démonstration de leurs performances (cas des multi-pollutions, efficacité des amendements, transferts et suivi des polluants dans la plante, durée de traitement, etc), l'optimisation des itinéraires techniques (du semis à la récolte) et le développement de filières de valorisation de la biomasse produite sur ces sols pollués ou fortement anthropisés. Ainsi, la valorisation de de la biomasse végétale produite sur ces terres agricoles polluées permettrait d'abaisser les coûts globaux de gestion et de requalification de ces sites, tout en répondant aux attentes environnementales et sociales. Parmi les filières présentant une rentabilité élevée, la culture de PAM a été suggérée comme une option prometteuse pour le phytomanagement des sols pollués. En effet, les PAM représentent un groupe diversifié d'espèces végétales jouissant d'un intérêt considérable en raison de leurs applications variées en médecine herboriste, pharmacologie, cosmétique et nutrition. Les plantes médicinales sont utilisées pour leurs propriétés thérapeutiques, tandis que les plantes aromatiques, riches en composés aromatiques, servent souvent d'épices, d'agents aromatisants ou sont intégrées dans des produits cosmétiques et médicinaux.

Cependant, les PAM sont souvent soumises à des stress abiotiques accrus tels que la sécheresse, la salinité et la pollution, aggravés par le changement climatique. Ces facteurs peuvent sérieusement compromettre leur croissance et, leur développement, altérant ainsi la qualité des huiles essentielles produites.

Face à ces défis, des stratégies respectueuses de l'environnement sont nécessaires pour renforcer la tolérance des PAM aux stress abiotiques. L'association des plantes avec des micro-organismes bénéfiques tels que les CMA se révèle particulièrement intéressante. En effet, les CMA établissent une symbiose avec les racines de nombreuses espèces végétales terrestres, y compris les PAM, améliorant l'absorption des nutriments essentiels et de l'eau du sol tout en augmentant la résistance des plantes aux stress environnementaux.

Cette seconde revue analyse l'impact des principaux stress abiotiques sur la croissance des PAM, ainsi que sur le rendement et la composition des huiles essentielles. Elle explore également les mécanismes

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par lesquels la mycorhization arbusculaire peut accroître la tolérance des PAM aux stress abiotiques à différents niveaux, morphologique, physiologique et moléculaire et décrit les effets synergiques des CMA et d'autres amendements (bactéries favorisant la croissance des plantes, amendements organiques ou inorganiques) sur la croissance des PAM, leur rendement et la composition de leurs huiles essentielles dans des environnements stressés.

Synthèse bibliographique

Review

Significance of Arbuscular Mycorrhizal Fungi in Mitigating Abiotic Environmental Stress in Medicinal and Aromatic Plants: A Review

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Abstract: Medicinal and aromatic plants (MAPs) have been used worldwide for thousands of years and play a critical role in traditional medicines, cosmetics, and food industries. In recent years, the cultivation of MAPs has become of great interest worldwide due to the increased demand for natural products, in particular essential oils (EOs). Climate change has exacerbated the effects of abiotic stresses on the growth, productivity, and quality of MAPs. Hence, there is a need for eco-friendly agricultural strategies to enhance plant growth and productivity. Among the adaptive strategies used by MAPs to cope with the adverse effects of abiotic stresses including water stress, salinity, pollution, etc., their association with beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) can improve MAPs' tolerance to these stresses. The current review (1) summarizes the effect of major abiotic stresses on MAPs' growth and yield, and the composition of EOs distilled from MAP species; (2) reports the mechanisms through which AMF root colonization can trigger the response of MAPs to abiotic stresses at morphological, physiological, and molecular levels; (3) discusses the contribution and synergistic effects of AMF and other amendments (e.g., plant growth-promoting bacteria, organic or inorganic amendments) on MAPs' growth and yield, and the composition of distilled EOs in stressed environments. In conclusion, several perspectives are suggested to promote future investigations.

Keywords: medicinal and aromatic plants; essential oil; abiotic stresses; arbuscular mycorrhizal fungi; amendments



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1. Introduction

Medicinal and aromatic plants (MAPs) are a diverse group of plant species that have received a great deal of interest due to their herbal medicine, pharmaceutical, cosmetic, and nutritional applications [1–3]. While medicinal plants include herbs used for therapeutic or pharmacological purposes, aromatic plants are rich in aromatic compounds and provide products that are widely used as spices or flavoring agents, and in cosmetics, or medicine [4,5]. These plant species are mainly cultivated for their various parts (roots, stems, leaves, flowers, seeds, buds, rhizomes, wood, bark, etc.) containing a wide range of secondary metabolites, including essential oils (EOs), which exert antimicrobial, antioxidant, and anti-inflammatory effects [6–8]. The cultivation of MAPs is looked upon not only as a source of effective health care products but also of income and livelihood security [9]. The World Health Organization estimates that 80% of the world's population depends on medicinal herbs for primary healthcare and wellness [10,11]. The use of MAPs has increased widely over the past three decades, and partly because of their low side effects, their demand has expanded not only in local but also international markets [12–14].

With increasing global warming, the intensity of droughts and heatwaves will increase, as along with other abiotic stress conditions such as salinity and flooding [15,16]. These various environmental stresses can severely affect the growth, development, and productivity

of MAPs [17]. Indeed, MAPs are not tolerant of the impacts of climate change [18]. It has been reported that various abiotic stresses influence the yields and the chemical composition of EOs and lead to variations in their quality [19–21]. The chemotype of EOs extracted from cumin (*Cuminum cyminum* L.) seeds changed under water stress, resulting in a change in the EO's odor [22]. Furthermore, a reduction was observed in the relative concentration of 1,3,8-*p*-menthatriene in the EO extracted from plain-leaved parsley, *Petroselinum crispum*, exposed to water stress [20]. This decrease could be detrimental to oil quality.

Due to the significance of MAPs for humans and the detrimental impacts of abiotic stress on MAP's growth and productivity, to improve plant growth and productivity there is a need to identify adequate approaches to enhance plant tolerance to abiotic stress. Amongst eco-friendly agricultural practices, the addition of microbial and/or organic or inorganic amendments could be sustainable mitigation strategies to fight against the detrimental effects of abiotic stress [23–28].

Plants rely greatly on root-associated microorganisms, particularly bacteria and fungi, to improve plant performance under abiotic stress [29,30]. Arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota are known to establish a symbiotic relationship with the roots of over 80% of terrestrial plant species [31], including MAPs [32–34]. Such symbiosis helps plants to obtain from the soil more water, and mineral nutrients including phosphorus, nitrogen, and oligo-elements. In return, the AMF takes photo-assimilates (i.e., carbohydrates) from the host plant [31,35,36]. It has been established through many studies that the inoculation of MAPs by AMF renders them more tolerant to abiotic stress conditions including water stress, salinity, and pollution [32,33,37,38], through the involvement of several mechanisms at morphological, physiological, and molecular levels [38–45].

The synergistic effect of microbial (i.e., AMF, plant growth-promoting bacteria (PGPR)) and organic or inorganic (i.e., compost, biochar, manures, NPK, silicate, and clay) soil amendments is beneficial for plant tolerance, by enhancing physicochemical properties and total organic matter content, as well as by increasing nutrient availability and the water-holding capacity of the soil [46–48]. Under abiotic stress such as water stress, salinity, or pollution, with the presence of microbial or organic or inorganic soil amendments, the improvement of plant growth and secondary metabolite production has been reported for various MAPs such as pennyroyal (*Mentha pulegium*), sage (*Salvia officinalis*), sweet basil (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), and cumin (*Cuminum cyminum*) [49–52].

The present review aims first to analyze the data of several studies reporting the impacts of various abiotic stresses (water stress, salt stress, low and high temperatures, light, and pollution) on MAPs' growth, and on the yield and composition of distilled EOs. Secondly, it deciphers the AMF-mediated mechanisms to improve MAPs' tolerance at morphological, physiological, and molecular levels. Finally, the role of PGPR with organic and inorganic amendments in alleviating abiotic stress is discussed. In conclusion, several perspectives to promote future investigations are suggested.

2. Impact of Abiotic Stresses on MAPs

2.1. Water Stress

Water stress is among the most important abiotic factors that severely affect MAPs' growth, and it can adversely affect yield and EO composition [20]. Various studies have established that water stress causes a reduction in the vegetative growth of MAPs (Table 1). For instance, in lavender species such as *Lavandula angustifolia* [19] and *Lavandula stoechas* [53], water stress reduced plant height, leaf area, and number of leaves per plant. In addition, Alishah et al. [54] showed that water stress decreased the plant height, stem diameter, number, and area of leaves for sweet basil (*Ocimum basilicum*). The restriction in shoot growth was most likely due to carbohydrate reallocation in favor of root growth, or to a decrease in photosynthesis efficiency [55]. The water stress effect can be extended to the reproductive stage by decreasing seed yield.

Table 1. Summary of water stress effects on growth and essential oil (EO) yield in various medicinal and aromatic plants (MAPs).

Family	Plant Species	Growth	EOs		Reference
			Yield	Plant Part Used for EOs Distillation	
Apiaceae	<i>Carum carvi</i>	–	+	Seeds	[56]
	<i>Coriandrum sativum</i>	–	n.d.	n.d.	[57]
	<i>Cuminum cyminum</i>	–	–	Seeds	[22]
	<i>Petroselinum crispum</i>	–	+	Roots; Leaves	[20]
	<i>Pimpinella anisum</i>	–	–	Seeds	[21]
Lamiaceae	<i>Lavandula angustifolia</i>	–	=	Leaves	[19]
	<i>Lavandula stoechas</i>	–	n.d.	n.d.	[53]
	<i>Mentha piperita</i>	–	–	Aerial parts	[58]
	<i>Ocimum basilicum</i>	–	+	Aerial parts	[59]
	<i>Ocimum americanum</i>	–	+	Aerial parts	[59]
	<i>Ocimum basilicum</i>	–	n.d.	n.d.	[54]
	<i>Rosmarinus officinalis</i>	–	–	Leaves	[60]
	<i>Salvia sclarea</i>	–	–	Aerial parts	[61]
	<i>Salvia officinalis</i>	–	+	Aerial parts	[62]
	<i>Salvia fruticosa</i>	–	+	Leaves	[19]
<i>Sureja hortensis</i>	–	+	Aerial parts	[63]	

'+' indicates increasing responses; '–' indicates decreasing responses; '=' indicates no response; 'n.d.' indicates not determined.

Numerous studies have reported that water stress has a negative effect on the number of umbels and umbellets during the reproductive stage in various Apiaceae species, such as cumin (*Cuminum cyminum* L.) [22], sesame (*Sesamum indicum* L.) [64], caraway (*Carum carvi* L.) [56], and coriander (*Coriandrum sativum*) [57]. Concomitant with this, reductions in seed yield were observed.

Several studies have shown that water stress modifies EOs yield and composition by increasing or decreasing constituent levels depending on plant species and stress duration. Water stress has been found to have a positive effect on the yield of EOs distilled from MAPs including Iranian native savory (*Satureja hortensis*) [63], Mexican oregano (*Lippia berlandieri Schauer*) [65], basil (*Ocimum* sp.), and caraway (*Carum carvi* L.) [56] (Table 1). It has been reported that the EOs of Greek sage (*Salvia fruticosa*) doubled under moderate water stress and increased by 83% under severe water stress, compared with normal conditions [19]. In another sage species, *Salvia officinalis*, Bettaieb et al. [62] reported an increase in EO yield when plants were cultivated under water stress. The effects of water deficit on the EOs of three parsley (*Petroselinum crispum*) cultivars, designated as plain-leafed, curly-leafed, and turnip-rooted, were studied by Petropoulos et al. [20]. They showed an enhancement in the EO yield for plain-leafed and curly-leafed parsley but not for turnip-rooted parsley. There is evidence that the presence of a high density of oil glands due to limited leaf area might explain such a rise in EO content [66]. Conversely, a decrease in EO yield was reported in a number of MAPs, including rosemary (*Rosmarinus officinalis* L.) [53,60], anise (*Pimpinella anisum* L.) [21], lavender (*Lavandula latifolia*) [19], and clary sage (*Salvia sclarea*) [61].

From the aforementioned studies, it is clear that water stress also influences the EO composition. Water stress was found to induce changes in the composition of EOs distilled from cumin (*Cuminum cyminum* L.) [22]. Furthermore, water stress modified the chemotype of the EO from γ -terpinene/phenyl-1,2 ethanediol in control seeds to γ -terpinene/cuminaldehyde in stressed seeds, possibly changing the EO's odor [22]. However, the EO chemotype of caraway (*Carum carvi* L.) did not change under water stress, but the content of limonene and carvone increased in the oil extracted from caraway seeds [56]. Water stress decreased certain compositions within the EO extracted from plain-leafed parsley, particularly 1,3,8-*p*-menthatriene [20]. As a result, this decrease could be detrimental to oil quality [67], although an increase in myristicin, which is another essential aromatic

constituent, was observed [20]. Under water stress, a decrease in total monoterpenes and an increase in oxygenated monoterpenes were observed in lavender (*Lavandula angustifolia*) and Greek sage (*Salvia fruticose*) [19]. This variation in EO yield and composition might be due to enzyme activity and metabolism enhancements [22,68,69].

2.2. Salt Stress

Salt stress, generally resulting from poor quality irrigated water, is considered a major environmental factor limiting plant growth and productivity [70]. There are several common features between salt and water stress, as in both cases, the primary effect is a lower water potential in the soil around the roots [71].

Many researchers have studied the effect of soil and water salinity on the growth of MAPs, as well as on the yields and composition of their distilled EOs. A decrease in shoot and root dry weight, as well as seed yield in response to salinity, was reported in sweet fennel (*Foeniculum vulgare*) [72] and ajwain (*Trachyspermum ammi* L.) [73]. Many other plant growth parameters including plant height, root length, number of leaves per plant, branches per plant, and flowers per plant decreased in a number of MAPs under salt stress (Table 2).

Table 2. Summary of salt stress effects on growth and EO yield in various MAPs.

Family	Plant Species	Growth	EO		Reference
			Yield	Plant Part Used for EO Distillation	
Apiaceae	<i>Coriandrum sativum</i>	n.d.	–	Leaves	[74]
	<i>Coriandrum sativum</i>	–	+	Fruits	[75]
	<i>Coriandrum sativum</i>	–	+	Roots	[76]
	<i>Foeniculum vulgare</i>	–	+	Seeds	[72]
	<i>Trachyspermum ammi</i>	–	=	Seeds	[73]
Asteraceae	<i>Matricaria chamomila</i>	–	–	Flowers	[77]
Lamiaceae	<i>Calendula officinalis</i>	–	+	Flowers	[78]
	<i>Melissa officinalis</i>	–	–	Aerial parts	[79]
	<i>Mentha x piperita</i>	–	–	Aerial parts	[80]
	<i>Menhta piperita</i>	n.d.	–	Aerial parts	[81]
	<i>Mentha suaveolens</i>	–	–	Aerial parts	[80]
	<i>Ocimum basilicum</i>	–	+	Aerial parts	[81]
	<i>Origanum majorana</i>	–	–	Shoots	[82]
	<i>Saliva officinalis</i>	–	–	Leaves; Fruits	[83,84]
	<i>Satureja hortensis</i>	–	=	Aerial parts	[85]
	<i>Thymus daenensis</i>	–	n.d.	n.d.	[86]
	<i>Thymus maroccanus</i>	–	=	Aerial parts	[87]
	<i>Thymus vulgaris</i>	–	n.d.	n.d.	[86]
	<i>Thymus vulgaris</i>	–	+	Aerial parts	[88]
Poaceae	<i>Cymbopogon schoenanthus</i>	–	+	Aerial parts	[89]

‘+’ indicates increasing responses; ‘–’ indicates decreasing responses; ‘=’ indicates no response; ‘n.d.’ indicates not determined.

From a wide array of experiments, it has been demonstrated that EO yield and composition can vary with plant species, cultivation conditions, and salt concentrations in the soil. An increase in EO yield was observed for English marigold (*Calendula officinalis*) [78], thyme (*Thymus vulgaris*) [88], and sweet basil (*Ocimum basilicum* L.) [90]. The increase in EO yield was attributed to a higher oil gland density [66]. In contrast, other studies have revealed that high salinization of soil and water had a negative effect on oil yield for several MAPs including sage (*Saliva officinalis*) [84], chamomile (*Matricaria chamomile*) [77], mint (*Mentha* sp.) [80], and marjoram (*Origanum majorana*) [82] (Table 2). In sage (*Saliva officinalis*) leaves and fruits, the EO yield increased under moderate salt stress (50 and 75 mM), but

when the NaCl concentration was increased to 100 mM, the oil yield decreased [83,84]. The application of salt stress induced marked changes in the major EO compounds of sage (*Salvia officinalis*) fruits [84], in particular viridiflorol, which acts as an antifungal potential, and manool, which is a precursor for aromatic products and has an ambergris odor [91]. In control plants and at a low concentration of NaCl (25 mM), viridiflorol was the main compound in EOs, whereas at a high salt concentration (100 mM) manool was the dominating compound. These variations might be due to the induction of enzyme activities involved in the biosynthesis of the latter compounds [84]. In coriander (*Coriandrum sativum* L.) leaves, the EO yield was increased at 25 and 50 mM NaCl concentrations while it decreased at a high NaCl concentration (75 mM) [74]. On the other hand, in coriander fruits and roots, the EO yield increased with increasing NaCl concentration (0, 25, and 50 mM) [75,76]. The compositions of EOs from coriander leaves and roots were affected differently by salt treatment. Coriander fruit contains linalool and camphor as major constituents, and their amounts increased along with the NaCl concentrations.

2.3. Low and High Temperatures

Temperature is one of the most important abiotic factors limiting plant growth and productivity [92]. Heat stress (high temperature) and cold stress (low temperature) can cause a series of changes at physiological, biochemical, and molecular levels [93]. Heat stress induces leaf senescence, cell membrane damage, degradation of chlorophyll content, and denaturation of various proteins [94]. Several studies are available on the effect of temperature stress on MAPs' growth [95,96] as well as on EOs' quantity and quality [97,98]. The effect of high temperature on sweet basil (*Ocimum basilicum*) was studied by Al-Huqail et al. [95]. The authors observed inhibition in plant growth under all high temperature treatments (35, 45, and 55 °C) compared with the control treatment (25 °C), which might be due to the effects of high temperature on plant metabolism [95]. Similarly, plant growth parameters (flower yield and plant height) for chamomile (*Matricaria chamomilla*) decreased at high temperatures (15, 20, and 25 °C) while these parameters increased at a low temperature (12 °C) [97]. In contrast, the fresh and dry weight of fennel (*Foeniculum vulgare*) were reduced under cold stress (2 °C) [96]. Another study has shown that increased temperature can cause early onset of senescence in American ginseng (*Panax quinquefolius*) and, subsequently, decreased photosynthetic rate. Furthermore, a decline in total biomass was observed [99]. The decrease in plant growth parameters might be because stomata become closed or partially closed under temperature stress, which causes a reduction in the CO₂ availability, and consequently the efficiency of photosynthesis decreases [97,100]. In addition, the reduction in leaf area and stomatal conductance affects the plant's capacity to intercept light and capture CO₂ and, consequently, its ability to produce biomass [97,101].

Temperature stress can also affect the production of secondary metabolites including EOs [93,97]. For example, at low temperatures the EO yield of chamomile (*Matricaria chamomilla*) increased, while it decreased at a high temperature [97]. In another study, the EO yield of chamomile (*Matricaria chamomilla*) increased at 10 and 20 °C and decreased under cold stress (5 °C) [102]. Some studies also showed that EO composition can be affected by low temperature. For example, Nguyen et al. [98] indicated an increase in some compounds such as eugenol, methyl eugenol, and β -caryophyllen in the EO of holy basil (*Ocimum tenuiflorum*), cultivated at low temperatures. Similarly, Rastogi et al. [103] reported that eugenol and methyl eugenol content in holy basil (*Ocimum tenuiflorum*) EOs decreased under cold stress.

2.4. Light

As well as being the primary source of energy for photosynthesis, light also acts as a signal and can regulate plant development [104,105]. Different parameters such as light quality (wavelengths), quantity (fluence rate), and duration strongly influence plant growth and development, as well as plant productivity [106–108]. Although light provides energy for photosynthesis and regulates plant development, it may also function as a

stress factor [108]. Many experiments have been conducted to assess how light quality, quantity, and duration affect the growth and EO yield of MAPs. For instance, the fresh weight of mint (*Mentha* spp.) was highest under combined red and blue LED light (70/30%) compared to normal growing conditions [109]. Likewise, the combination of red and blue LED light increased the fresh and dry weights of the shoots and the leaf numbers of lemon balm (*Melissa officinalis*) compared with red light alone, blue light alone, or white light [110]. Another study showed that the leaf and shoot numbers of coriander (*Coriandrum sativum*), as well as its fresh and dry mass, increased under different ratios of red: blue compared with red light alone [111]. In contrast, the total fresh lateral-shoot weight of sweet basil (*Ocimum basilicum*) was highest under blue light compared with other lights (red, green, blue-green, or white light) [112]. The combination of blue and white light increased the leaf area of peppermint (*Mentha piperita*) [113]. These studies demonstrate that combined red and blue light is more effective than monochromatic red light for plant growth, and their conclusions explain that the red light wavelength (650–665 nm) matches the absorption peak of chlorophyll a/b found in the chloroplast [114], while blue light has a complementary effect [115]. Furthermore, blue light has been shown to affect the opening of stomata [116], allowing more CO₂ entrance for photosynthesis, which is reflected by an increase in dry matter [117]. The effect of increasing light intensity (approximately 4, 7, 11, or 20 mol m⁻² d⁻¹) on the growth of African basil (*Ocimum gratissimum*) was conducted by Fernandes et al. [118]. Results from that experiment indicated that the number of leaves, leaf area, and plant height increased up to 10 mol m⁻² d⁻¹, but decreased after this value. In the presence of green LED light, the fresh weight and leaf area of sweet basil (*Ocimum basilicum* L.) were higher than under other tested lights [112].

Ultraviolet (UV) light is stressful for plants, causing dwarfing and loss in photosynthesis, but appropriately using UV light can increase plant yield in some species [119]. Supplementary UV-B light for two hours each day for seven days had a greater effect on plant height, leaf area, fresh weight, and dry weight of sweet basil than one hour supplemental UV-B light [120]. In peppermint (*Mentha piperita*), combining white and UV-A or UV-B light increased the leaf area and leaf area index. In contrast, Johnson et al. [121] reported that the leaf area of sweet basil (*Ocimum basilicum*) was reduced by UV-B light. Another study reported that the combination of UV-B and white light affected neither the number of leaves nor the leaf area of Japanese mint (*Mentha arvensis*) [122].

It has been established that red, blue, and UV light can improve EO yield in various MAPs compared to white light. For instance, the effect of various LED lights (red, blue, red + blue (70% + 30%), or white) on some species of mint (*Mentha piperita*, *Mentha spicata*, and *Mentha longifolia*) was investigated by Sabzalian et al. [109]. The results showed that the EO content of all mint species was higher under red or combined red and blue light than under white light. In sweet basil (*Ocimum basilicum* L.), the EO content under blue light was 1.2–4.4 times higher than in plants cultivated under white light, and was lowest under red light, in experiments conducted at 50 μmol. m⁻².s⁻¹ PPFD for 70 days [112]. Changes in the EO composition of basil depended on light treatments. The second and third major compounds, respectively, were myrcene and linalool under blue light, and α-pinene and β-pinene under green and red light; under white light, these produced an intermediate response [112]. Even though UV light has a generally negative effect on plant growth, it might improve EO yield in some MAPs. For instance, Karousou et al. [123] studied the effect of supplementary UV-B radiation on the EO yield of two distinct chemotypes of spearmint (*Mentha spicata*) growing under field conditions. A significant increase in EO production was observed in chemotype II, while in chemotype I, the increase was insignificant. An increase in EO yield in Japanese mint (*Mentha arvensis*) was observed under UV-B or combined UV-A and UV-B light with white light [122]. Under the same conditions, increases in *l*-menthol and limonene concentrations were observed. Similarly, the combination of UV-A or UV-B with white light increased the EO yield in peppermint (*Mentha piperita*) [113,124]. There is limited information on the enhancement of EOs in MAPs under light [109]. However, the accumulation of EOs in MAPs may be due to

the effects of light on the metabolic pathways of MAPs leading to an increase in EO yield [109,112]. Therefore, further investigations are needed to study the mechanisms that affect EO accumulation in MAPs under light, including UV light.

2.5. Pollution

Soil pollution is becoming a major environmental problem, due to rapid urbanization and industrialization [125]. Trace elements (TEs) are the major pollutants found in polluted soils. Soil pollution by TEs may occur naturally (i.e., erosion, geochemical background, volcanic eruption, etc.), but it mainly originates from anthropogenic activities (i.e., industrial and agricultural activities, traffic, mining, etc.) [126]. The most commonly found TEs in contaminated soil include arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn), nickel (Ni), and mercury (Hg) [127]. TEs cause significant risks to human health and the environment due to their non-biodegradability, bioaccumulation, and extreme toxicity [128,129]. Concerns have been raised about the accumulation of TEs in the soil, and their ability to penetrate and become incorporated into plants. These accumulated TEs adversely affect plant growth and productivity, as well as possibly contaminating the human food chain [129]. TEs can adversely affect MAPs' development [130–132] as well as the quantity and composition of their secondary metabolites such as EOs [130,133].

Several studies have indicated that the response of MAPs to TEs is highly variable. This is probably due to the different experimental conditions (plant species, concentration, duration of treatment, composition of growth medium, culture conditions, etc.). The effect of TEs on plant growth has been observed in various Lamiaceae species. For instance, the uptake of As by the *Ocimum* spp. (*Ocimum tenuiflorum*, *O. basilicum*, and *O. gratissimum*) significantly decreased plant growth (plant height and dry weight) [134]. However, Cd, Pb, and Cu did not affect the growth of sweet basil (*Ocimum. basilicum*) [135]. Moreover, another study has shown that Cd, Pb, and Zn reduced root and shoot dry biomass by 15 and 10%, respectively, in garden sage (*Salvia officinalis*) [130]. Likewise, Raveau et al. [136] showed that the plant height and dry weight of clary sage (*Salvia officinalis*) were decreased by polluted soil (Pb, Zn, and Cd) compared with unpolluted soil. Similarly, a decrease in the dry biomass of shoot and root in chamomile (*Matricaria recutita*) was observed when grown in soil contaminated with Cd and Pb [137]. Despite being members of the same genus, menthol mint (*Mentha arvensis*), peppermint (*M. piperita*), and bergamot mint (*M. citrata*) responded differently to soil contaminated by Cr and Pb. *M. arvensis*'s shoot and root yields were not significantly affected by the application of Cr and Pb in the growth medium, whereas the shoot and root yields of *M. citrata* decreased, and those of *M. piperita* increased [131]. In addition, Sá et al. [138] reported that the application of Pb in soil did not affect the growth of garden mint (*Mentha crispata*). Another study reported that the application of Ni up to 30 ppm increased the shoot yield of *Tagetes minuta*. However, a further increase in the level of Ni decreased the shoot yield of that species [132]. Plant height and the number of branches per plant increased at low concentrations of Ni, while these parameters decreased with increasing Ni concentrations [132].

It has been hypothesized that the reduction in plant growth on TE-polluted soils is due to poor nutrient uptake because TEs compete with other plant nutrients [139]. Arsenate (AsV) accumulates in plants via the phosphate transporter (PHT) as an analog of phosphate (Pi), resulting in an inhibition of phosphorus (P) uptake [140–142]. Cd can reduce the uptake and translocation of essential nutrients including calcium (Ca), Cu, iron (Fe), Zn, and manganese (Mn) [143]. Cd is a divalent cation and may compete with plant nutrients for the same transporters [144]. Cd uptake by plants is mediated through cation transport systems, which are generally involved in the uptake of essential nutrients such as zinc–iron permease (ZIP) (ZRT–IRT-like proteins), natural resistant associated macrophage protein (NRAMP) transporters, or Ca channels and transporters [145]. In leaves, Cd penetrates via the Ca channel, disrupting the plant–water relationship [145], causing stomatal closure and leading to inhibition of photosynthetic activity, which subsequently reduces plant

growth [143]. Furthermore, excessive Pb concentrations in soil inhibit plants' uptake of minerals including Ca, Fe, Mg, Mn, P, and Zn, resulting in reduced plant growth [146].

The effects of TEs on the EO yields of many MAPs have been studied, including basil spp. (*Ocimum* spp.), mint spp. (*Mentha* spp.), lemon balm (*Melissa officinalis*), and sage (*Salvia officinalis*) [131,136,147,148]. Previous studies carried out on *Ocimum* species reported an increase in EO yield with the application of As [134,149]. Similarly, an increase in EO yield following Cd and Pb treatments was observed in sweet basil (*Ocimum basilicum* L.) [148]. Furthermore, differences in EO yield appeared between plant species within the same genus. The application of Cr and Pb in the medium growth, for example, decreased EO yields in menthol mint (*Mentha. arvensis*) and bergamot mint (*M. citrata*) while increasing the EO yield in peppermint (*M. piperita*) [131]. In contrast, the EO yield of *M. arvensis* was not affected by the application of Pb and Cd in soil [150], while in another mint species, garden mint (*Mentha crispa*), the EO yield increased when the concentration of Pb increased [138]. Moreover, the application of Pb in the soil decreased the EO yield of lemon balm (*Melissa officinalis*) [147]. In contrast, an increase in the EO yield of sage (*Salvia officinalis*) was observed in the presence of Cd, Pb, and Zn [130,136]. Differences in EOs' chemical compositions were also observed. For example, menthol content in *M. arvensis* and *M. piperita* was not affected in response to Cr and Pb treatments, but minor constituents such as α -pinene and β -pinene were reduced in *M. arvensis* [131]. The reason for the changes in the EO yield of plants after the application of TEs is not known, but it might be attributable to the effect of TEs on enzymatic activity and carbon metabolism [68].

Phytoremediation is a green approach that relies on plants, including MAPs, to clean pollutants from contaminated soil [133]. It has been demonstrated through many studies that MAPs can be used in the phytoremediation of TE-polluted soils. For instance, vetiver (*Vetiveria zizanioides*) and rose (*Pelargonium roseum*) can act as phytoextractants for Pb. A study conducted by Chen et al. [151] showed that the formation of Pb-EDTA complexes (i.e., a chelating agent) in vetiver increased the accumulation of Pb in the roots and its translocation from roots to shoots. In addition, rose geranium (*Pelargonium roseum*) possesses a hyperaccumulator phenotype due to the accumulation of a high concentration of Pb in shoots relative to roots (8644 and 5550 mg Pb kg⁻¹ DW, respectively) [152]. Other MAPs, including basil (*Ocimum* spp.), lavender (*Lavandula angustifolia* L.), sage (*Salvia sclarea*), and rosemary (*Rosmarinus officinalis* L.), act as multi-hyperaccumulators or excluders of multiple TEs including Cd, Zn, and Ni [52,153–157]. On the other hand, several MAPs have been shown to act as phytostabilizers for TEs. For example, palmarosa (*Cymbopogon martini*) can accumulate TEs (Cr, Ni, Pb, and Cd) with less translocation of TEs from roots to its aerial parts [158]. Furthermore, rosemary (*Rosmarinus officinalis* L.), clary sage (*Salvia officinalis*), and vetiver (*Vetiveria zizanioides*) can be useful for the phytostabilization of TEs such as Cu, As, Cd, Zn, and Pb [136,159,160].

In plants, the uptake and translocation of TEs occur through specific transporters such as ZIP (ZRT-IRT-like proteins), cation diffusion facilitators, heavy metal transport ATPases, metal transporter proteins, natural resistant associated macrophage proteins, and ATP-binding cassette transporters, which are localized at the plasma membrane and on the vacuole membrane of cells. These transporters facilitate TE uptake into plants and participate in their sequestration into vacuoles or cell walls [127,161,162]. In fact, plants have an antioxidant defense system which is triggered as a consequence of the increased level of reactive oxygen species (ROS). Glutathione (GSH) shows a high affinity for toxic metals, and plays an important role in metal sequestration and tolerance in plants [163–165].

3. Arbuscular Mycorrhizal Fungi Help MAPs to Cope with Abiotic Stress

It is evident from the studies cited above that water stress, salinity, temperature, light, and pollution are among the major abiotic stresses that can affect plant growth and consequently reduce biomass production and EO yield in some MAP species. In addition, abiotic stresses induced changes in the composition of distilled EOs. Since plants cannot move, they have evolved numerous strategies to cope with abiotic stress, among which

AMF are known to promote plant growth and to confer better tolerance to abiotic stresses in host plants [31], including MAPs [32,33]. The following sections of the current review focus on the mechanisms used by AMF to alleviate the deleterious effects of abiotic stress in MAPs (Figure 1).

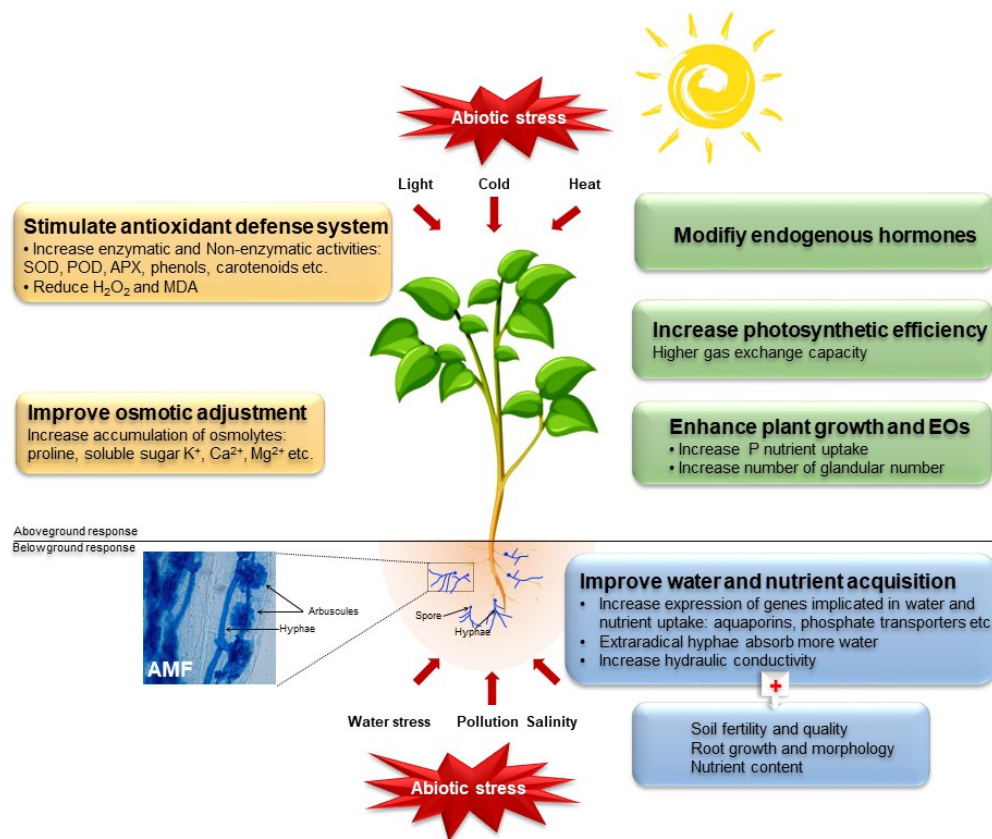


Figure 1. Mechanisms used by arbuscular mycorrhizal fungi (AMF) to improve stress tolerance and enhance the growth of medicinal and aromatic plants. AMF triggers the response of plants at morphological, physiological, and molecular levels to cope with the detrimental effects of abiotic stress. Arbuscular mycorrhizal symbiosis improves water and nutrient acquisition, enhances plant growth and abiotic stress tolerance. “+” indicates a positive effect. EOs, essential oils; SOD, superoxide dismutase; POD, peroxidase; APX, ascorbate peroxidase; H_2O_2 , hydrogen peroxide; MDA, malondialdehyde; P, phosphorus.

3.1. AMF Improves MAPs Growth and EOs Yield

Many studies have shown that AMF inoculation improves plant growth and biomass in addition to EO yield under abiotic stresses; see Table 3. Under conditions of water stress, Indian coral tree (*Erythrina variegata*) inoculated with *Funneliformis mosseae* presented a significant increase in plant growth parameters (shoot and root length, stem diameter, and leaf area) compared to non-inoculated plants [166]. The increase in dry biomass was explained by an improvement in water uptake by AMF extraradical hyphae [166]. Furthermore, sesbania (*Sesbania sesban*) colonized by different AMF species (*F. mosseae*, *Rhizophagus irregularis*, and *Claroideoglomus etunicatum*) showed an increase in fresh weight and lengths of shoot and root compared to non-inoculated plants [43]. The enhancement of MAPs' growth by AMF could be attributed to AMF improving nutrient acquisition, especially P nutrition [167].

The accumulation of EOs in MAPs is generally attributed to glandular trichomes, which are specialized external secretory structures that secrete secondary metabolites including EOs [168,169]. Hence, there is a strong correlation between the density of glandular trichomes and EO yield. Interestingly, several studies revealed an enhancement in EO yield

in many MAPs colonized with AMF, and these improvements were attributed to increases in the number of glandular trichomes in mycorrhizal plants [170–172]. It has been shown that the inoculation of oregano (*Origanum vulgare* L.) with *Glomus viscosum* increased the glandular density on the upper leaf epidermis [170]. Furthermore, sweet basil (*Ocimum basilicum* L.) inoculated with a mixture of AMF species, viz., *F. mosseae*, *Gigaspora margarita*, and *Gigaspora rosea*, showed an increase in EO yield [171]. This increase was associated with an abundance of glandular trichomes on the basal and central leaf zones [171]. Moreover, it has been reported that the inoculation of rose geranium (*Pelargonium graveolens*) with *F. mosseae* and *R. irregularis* increased the EO yield under conditions of water stress [37,173]. Similarly, basil (*Ocimum gratissimum* L.) plants inoculated with *R. irregularis* showed an increase in plant height and EO yield when experiencing water stress [44]. Likewise, sweet basil (*Ocimum basilicum* L.) inoculated with *R. irregularis* showed an increase in plant biomass and EO yield with a high level of metals in the soil [174]. The AMF can protect plants against TE toxicity by binding the metals in their hyphae, which limits the translocation of TEs from roots to shoots [174,175]. In addition, AMF hyphae produce glomalin, a glycoprotein that plays a role in the immobilization of TEs by generating protein–metal complexes, resulting in a reduction of TE content in the soil [176].

However, in addition to the advantages described above, some studies have reported that AMF can promote the content of active compounds in MAPs. Such benefits may be related to changes in the gene expressions involved in the biosynthesis of these compounds [177]. For example, Lazzara et al. [178] showed that *Hypericum perforatum* inoculated with a mixture of nine different AMF species increased the concentration of bioactive secondary metabolites such as hypericin and pseudohypericin in flowers, compared with non-inoculated plants under conditions of low P availability [178]. This enhancement could be attributed to the involvement of methyl jasmonate or salicylic acid in the mycorrhizal symbiosis, which can positively affect hypericin content [178,179].

Table 3. Effect of mycorrhizal inoculation on MAPs growth and EOs yield under abiotic stress.

Stress Type	Plant Species	AMF Species	Growth	EO Yield	Reference
Water stress	<i>Ephedra foliata</i>	<i>Claroideoglomus etunicatum</i> , <i>Funneliformis mosseae</i> , <i>Rhizophagus irregularis</i>	+	n.d.	[33]
	<i>Erythrina variegata</i>	<i>Funneliformis mosseae</i>	+	n.d.	[166]
	<i>Foeniculum vulgare</i>	<i>Funneliformis mosseae</i> , <i>Rhizophagus irregularis</i>	+	n.d.	[180]
	<i>Glycyrrhiza uralensis</i>	<i>Rhizophagus irregularis</i>	+	n.d.	[40]
	<i>Lavandula spica</i>	<i>Funneliformis mosseae</i> , <i>Rhizophagus intraradices</i>	+	n.d.	[32]
	<i>Matricaria chamomilla</i>	<i>Funneliformis mosseae</i>	+	n.d.	[38]
	<i>Ocimum gratissimum</i>	<i>Rhizophagus irregularis</i>	+	+	[44]
	<i>Pelargonium graveolens</i>	<i>Funneliformis mosseae</i> , <i>Rhizophagus irregularis</i>	+	+	[37,173]
	<i>Ricinus communis</i>	<i>Funneliformis mosseae</i> , <i>Rhizophagus intraradices</i>	+	n.d.	[181]
	<i>Tagetes erecta</i>	<i>Glomus constrictum</i>	+	n.d.	[182]

Table 3. Cont.

Stress Type	Plant Species	AMF Species	Growth	EO Yield	Reference
Salinity	<i>Acacia nilotica</i>	<i>Glomus fasciculatum</i>	+	n.d.	[183]
	<i>Chrysanthemum morifolium</i>	<i>Diversispora versiformis</i> <i>Funneliformis mosseae</i> <i>Claroideoglomus etunicatum</i> ,	+	n.d.	[184]
	<i>Ephedra aphylla</i>	<i>Funneliformis mosseae</i> , <i>Rhizophagus irregularis</i>	+	n.d.	[185]
	<i>Ricinus communis</i>	<i>Funneliformis mosseae</i> , <i>Rhizophagus intraradices</i> <i>Claroideoglomus etunicatum</i> ,	+	n.d.	[181]
	<i>Sesbania sesban</i>	<i>Funneliformis mosseae</i> , <i>Rhizophagus irregularis</i>	+	n.d.	[43]
	<i>Valeriana officinalis</i>	<i>Funneliformis mosseae</i> , <i>Rhizophagus irregularis</i>	+	n.d.	[41]
High temperature	<i>Cyclamen persicum</i>	<i>Glomus fasciculatum</i>	+	n.d.	[186]
Trace elements	<i>Ocimum basilicum</i>	<i>Rhizophagus intraradices</i> <i>Acaulospora laevis</i> ,	+	+	[174]
	<i>Trigonella foenum-graecum</i>	<i>Gigaspora nigra</i> <i>Glomus monosporum</i> , <i>Glomus clarum</i>	+	+	[187]

'+' indicates increasing responses; '-' indicates decreasing responses; '=' indicates no response; 'n.d.' indicates not determined.

Similarly, an enhancement in the concentration of artemisinin was observed in leaves of *Artemisia annua* inoculated with *R. intraradices*, and such an increase was attributed to the induced expression of the artemisinin biosynthesis genes including amorpha-4; 11-diene synthase, cytochrome P450, double bond reductase 2, and aldehyde dehydrogenase 1 [188]. Recently, Li et al. [189] reported that the inoculation of *Paris polyphylla* var. *yunnanensis* with mixtures of AMF species induced increased content of polyphyllin, which was associated with the expression of *PpSE*. A study carried out by Duc et al. [190] showed that a mixture of AMF species (*F. mosseae*, *Septoglomus deserticola*, and *Acaulospora lacunose*) promoted the accumulation of polyphenol content in *Eclipta prostrata* under salt stress [190]. This change in the polyphenol profile could be explained by the changes in the expression of genes involved in the polyphenol biosynthesis pathway [190]. Moreover, the AMF *R. irregularis* promoted glycyrrhizin and liquiritin in *Glycyrrhiza uralensis* plants experiencing water stress, and this enhancement was associated with an increase in the expression of glycyrrhizin biosynthesis genes (squalene synthase, β -amyrin synthase), P450 monooxygenase, and cytochrome P450 monooxygenase 72A154) [40].

3.2. AMF Improves Mineral Nutrient Uptake

It has been shown that AMF can boost the uptake of relatively immobile mineral nutrients in soils, including P, Zn, and Cu. The extraradical hyphae of AMF can extend beyond the depletion zone of the rhizosphere to absorb and transfer nutrient elements to the host plant [167]. Several studies have shown the impact of AMF inoculation on nutrient uptake in MAPs. For instance, two AMF species (*F. mosseae* and *R. irregularis*) significantly increased the leaf P concentration in fennel (*Foeniculum vulgare* Mill.) plants under different levels of water stress [180]. Under conditions of water stress, colonization of the roots by two AMF, *R. intraradices* and *F. mosseae*, alone or in combination, resulted in a significant increase in the concentrations of N, P, Fe, and Zn in rose geranium (*Pelargonium graveolens* L.) compared with non-inoculated plants [173]. It has been observed that water stress reduces P absorption, but this reduction rate was lower in mycorrhizal marigolds (*Tagetes erecta*) than in non-mycorrhizal plants [182]. Many studies have further shown that AMF inoculation enhanced the mineral nutrition of MAPs cultivated under abiotic stress. For example, it was shown that chamomile plants inoculated with *F. mosseae* demonstrated an increase in the

shoot and root P and K concentrations under osmotic stress [38]. Similarly, enhancement of P, K, and Mg levels was reported in valerian (*Valeriana officinalis* L.) inoculated with *F. mosseae* and *R. irregularis* under salt stress [41]. In addition, hangbaiju (*Chrysanthemum morifolium*) inoculated with *F. mosseae* or *Diversispora versiformis* showed an increase in root concentration of N under salt stress [185]. Autochthonous *F. mosseae* (i.e., a drought-tolerant AMF strain) was found to be better than allochthonous *F. mosseae* (i.e., a drought-sensitive AMF strain) in terms of drought tolerance, with higher contents of N and K established in lavender (*Lavandula spica*) [32].

The main mechanism behind the higher nutrient concentration of mycorrhizal plants, especially in terms of P nutrients, is the increase in surface area for P uptake. The uptake of phosphate (Pi) from soil and AMF is mediated by the PHT1 gene family [191]. AMF induced Pi transporter genes during plant–AMF symbiosis [192]. AMF up-regulated the expression of phosphate transporter (PT) genes (*LePT4* and *LePT5*) in tomato plants to enhance plant tolerance under conditions of water stress [193]. Furthermore, AMF induced the expression of ammonium transporter protein and potassium (K⁺) transporter genes under conditions of water stress, which is crucial for N and K uptake by host plants in arid regions [194]. The secretion of acid phosphates by extraradical hyphae has also been proposed [195].

3.3. AMF Improves Plant Water Status

It has been demonstrated that AMF can significantly improve plants' water uptake to alleviate abiotic stresses such as water stress, salinity, and light stress [196,197]. This increase in water uptake could be attributed to an increase in the hydraulic conductivity of the roots, which results from a change in root morphology [198,199]. The formation of external hyphae by AMF can also improve access to larger areas in soil, resulting in increased uptake and transport of water from soil to roots [200,201]. Mycorrhizal plants have improved access to soil water compared with non-mycorrhizal plants because the extraradical hyphae involved in water transport have a diameter of 2–5 µm, allowing them to easily penetrate small soil pores that are not accessible to root hairs [200,202]. It is noteworthy that there has been no research using MAPs to confirm water uptake by extraradical hyphae under abiotic stress.

Due to negative water potential, meaning low water availability in the soil, plants can be faced with the problem of acquiring a sufficient amount of water [203], a process that necessitates the involvement of aquaporins [204–206]. Aquaporins, also called water channel proteins, belong to the family of membrane channel proteins that facilitate the transport of water following an osmotic gradient [205–207]. Numerous studies on various plant species have indicated that AMF can modify the expression of genes' coding for plant aquaporins under different environmental stresses [203,208,209]. Under salt stress conditions, common bean (*Phaseolus vulgaris*) roots inoculated with *R. irregularis* showed an up-regulation of three plasma membrane intrinsic protein (PIP) genes, *PvPIP1;1*, *PvPIP1;3*, and *PvPIP2;1*, compared with non-inoculated plants [208]. Under conditions of water deficit, soybean (*Glycine max*) and lettuce (*Lactuca sativa*) plants inoculated with *F. mosseae* and *R. irregularis* showed rapid decreases in the expression of certain PIP genes in comparison with non-inoculated plants [210]. A study carried out on liquorice (*Glycyrrhiza uralensis*), which is an important medicinal plant, found that inoculating plants with *R. irregularis* increased the expression of aquaporin genes when compared with non-inoculated plants, implying that AMF has a direct role in improving plant water status during water stress [40]. These studies clearly showed that the expression of aquaporin genes in host plants may respond differently according to levels of AMF colonization and stress imposed. Taken together, the up-regulation of aquaporin genes might be linked to an increase in the water transport capacity of plants [211], especially under abiotic stresses [208–210]. Further studies are required to analyze the expression of plant aquaporins in mycorrhizal MAPs exposed to different abiotic stresses, and to investigate the roles of aquaporins in facilitating plants' water uptake.

Some studies have reported that mycorrhizal MAPs such as liquorice (*Glycyrrhiza uralensis*) [40], pangola-grass *Digitaria eriantha* [39], and *Polygonum cuspidatum* [212] showed an increase in stomatal conductance, thereby increasing their photosynthetic rate. Indeed, it has been well demonstrated that water stress prompts stomatal closure, causing a reduction in CO₂ availability, which reduces photosynthesis in plants [100]. In this way, AMF could enhance photosynthesis by improving the plant's water status, because the increase of stomatal conductance could result in an increase in gas exchange [213].

Under water stress, rose geranium (*Pelargonium graveolens* L.) inoculated with AMF species (*R. irregularis* or *F. mosseae*) showed higher water use efficiency (WUE) than non-inoculated plants [173]. Similarly, *R. irregularis* had a positive effect on WUE in liquorice (*Glycyrrhiza uralensis*) subjected to water stress [40]. The mechanisms behind the enhancement of WUE can be attributed to higher water uptake by extraradical hyphae [196,214], an increase of stomatal conductance and transpiration rate [196,215], and adjustment of osmotic potential [216].

3.4. AMF Modifies Endogenous Hormones

Abscisic acid (ABA) is a phytohormone that regulates plant development, including seed dormancy, inhibition of seed germination, growth regulation, and stomatal closure [217–219]. Its role in stress tolerance has received significant attention [220]. Indeed, ABA is considered a stress hormone due to its fundamental role in the responses of plants to abiotic stresses [221]. Under abiotic stress conditions, ABA levels increase, maintaining plant water status, regulating stomatal closure, and inducing changes in the expression of stress-inducible genes [217,221–224].

Several studies have documented that ABA levels were higher in mycorrhizal MAPs having experienced water stress [33,225]. For example, *Ephedra foliata* inoculated with *Claroideoglossum etunicatum*, *R. irregularis*, and *F. mosseae* showed an increase in ABA concentration under conditions of water stress compared with control plants [33]. Under dehydration, the leaves of mycorrhizal chile ancho pepper (*Capsicum annuum*) plantlets had higher ABA concentrations than non-mycorrhizal plantlets [225]. Thus, in stressed-inoculated plants, the modulation of ABA levels in guard cells induces the closure of the stomata in order to prevent water loss [226]. In contrast, AMF can also reduce the ABA levels in MAPs under conditions of water and salt stress [40,43]. For example, sesbania (*Sesbania sesban*) colonized by *F. mosseae*, *R. irregularis*, and *Claroideoglossum etunicatum* exhibited lower ABA levels than non-mycorrhizal plants under salt stress [43]. In addition, lower ABA concentrations were observed in the roots of liquorice (*Glycyrrhiza uralensis*) inoculated with *R. irregularis* compared with non-mycorrhizal plants under drought stress [40]. It has been reported that AMF could improve plants' drought tolerance by decreasing root ABA concentration, which in turn up-regulated the expression of plasma membrane intrinsic proteins (PIPs) and antioxidant SOD genes [227]. Based on the above studies, it seems that the regulation of ABA levels depends on the AMF and the host plant species.

3.5. AMF Mediates Osmotic Adjustments

Osmotic adjustment is another important mechanism that allows plants to tolerate abiotic stresses including water stress, salinity, and osmotic stress. Osmotic adjustment allows the plant to maintain its turgor pressure and physiological activity by accumulating organic osmolytes including proline, soluble sugars, glycine betaine, and polyamines, as well as inorganic osmolytes such as K⁺, Ca²⁺, and Mg²⁺ [228,229]. Several studies have shown that AMF inoculation enhanced the stress tolerance of MAPs by increasing osmolyte accumulation [38,41,180]. Accumulation of soluble sugars in mycorrhizal plants leads to an adjustment of osmotic potential, constituting an important defense mechanism against abiotic stress [230]. Studies conducted on valerian (*Valeriana officinalis* L.) colonized with *R. irregularis* and *F. mosseae* indicated a higher accumulation of total soluble sugars in shoots and roots under salinity stress compared with non-mycorrhizal plants [41]. Compared with non-mycorrhizal plants, German chamomile (*Matricaria chamomilla* L.) plants

inoculated with *F. mosseae* presented significantly higher levels of soluble sugars under osmotic stress [38]. An increase in soluble sugars was also reported in fennel (*Foeniculum vulgare* Mill.) plants inoculated with *F. mosseae* and *R. irregularis* during water stress [180]. Inoculation of *Ephedra foliate* with a mixture of AMF species, viz., *Claroideoglossum etunicatum*, *R. irregularis*, and *F. mosseae*, prompted a higher accumulation of glucose than in non-inoculated plants [33]. Recently, a study by Sun et al. [231] reported an increase in sucrose content in the roots of *Polygonum cuspidatum* inoculated with *F. mosseae*.

Sugar accumulation in mycorrhizal plants is due to an increase in photosynthesis activity and also due to the hydrolysis of starch [232]. Furthermore, an increase in the expression of sugar transporter encoding genes may also lead to sugar accumulation in mycorrhizal plants [213,233]. It has been reported that, during AMF symbiosis, the carbon allocation from source leaves to the roots increases, and that this process requires an increase in the expression of genes encoding for sugar transporters [234]. In the roots of tomato (*Solanum lycopersicon*) plants colonized by *F. mosseae*, a higher accumulation of sucrose and fructose content was observed. These results suggest that sucrose synthesized in source organs is loaded into the phloem. Therefore, the expression of the three genes encoding the sucrose transporters SUT1, SUT2, and SUT4 was increased [213]. However, as far as we know, the role of sugar transporter genes in the osmotic adjustment of mycorrhizal MAPs under abiotic stresses has not been reported, and further investigations are needed to elucidate this subject.

Proline is one of the most important osmolytes, and its high accumulation in response to abiotic stresses maintains the cell's osmotic balance. Several studies reported an accumulation of proline in MAPs inoculated with AMF under abiotic stresses, such as chamomile (*Matricaria chamomilla*) [38], castor bean (*Ricinus communis*) [181], basil (*Ocimum gratissimum* L.) [44], *Ephedra alata* [184], *Ephedra foliate* [33], and valerian (*Valeriana officinalis* L.) [41]. However, some plants do not show this increase in osmolytes. For instance, the Indian coral tree (*Erythrina variegata* L.) inoculated with *F. mosseae* showed tolerance to water deficit by accumulating chlorophyll and carotenoids but not proline or soluble sugars [166].

3.6. AMF Stimulates Plants' Antioxidant Defense Systems

Exposure of plants to abiotic stresses induces an accumulation of ROS including hydroxyl radicals ($\cdot\text{OH}$), hydrogen peroxide (H_2O_2), singlet oxygen (O_1^-), and superoxide anion radical (O_2^-). An excess accumulation of ROS might be harmful to plants as a result of oxidative damage to proteins, lipids, and nucleic acids [235,236]. Malondialdehyde (MDA) is the end product of lipid peroxidation and is widely used as a marker of oxidative lipid damage [237,238]. The detoxification of excess cellular ROS requires an antioxidant machinery composed of enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants comprise superoxide dismutase (SOD), peroxidases (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and glutathione reductase (GR), while the non-enzymatic system includes reduced GSH, ascorbic acid (AA), phenols, tocopherols, and carotenoids [235,236,239]. Several studies have shown that AMF colonization reduces oxidative damage by enhancing the antioxidant system in plants (including MAPs) under conditions of water stress, salinity, and heat stress. For instance, rose geranium (*Pelargonium graveolens* L.) inoculated with *F. mosseae* or *R. irregularis* presented lower accumulations of H_2O_2 and MDA in their leaves. These findings suggested that mycorrhizal plants had higher antioxidant enzyme activity (CAT, APX, GPX, and SOD) than non-mycorrhizal plants [37]. AMF-treated *Ephedra foliate* plants showed an increase in enzymatic (CAT, APX, GPX, SOD, and GR) and non-enzymatic antioxidants (GSH and AA), allowing them to maintain ROS levels that better prevent cell damage under conditions of water stress compared with non-mycorrhizal plants [240]. The activation by AMF of antioxidant activities enhanced tolerance to water stress. Autochthonous *R. irregularis* conferred greater drought tolerance to lavender (*Lavandula spica*) plants than allochthonous *F. mosseae* did, because higher antioxidant concentrations and greater development of intraradical and extraradical mycelium and arbuscular formation were produced in lavender inoculated

with *R. irregularis* [32]. *Sesbania* (*Sesbania sesban* L.) [43] and *Ephedra aphylla* [184] colonized with a mixture of AMF species, viz., *Claroideoglomus etunicatum*, *R. irregularis*, and *F. mosseae*, showed an increase of different enzymatic antioxidants under salt stress. An enhancement in non-enzymatic antioxidants was also observed in *Sesbania* (*Sesbania sesban* L.) [43]. A low level of MDA was observed in AMF-treated *Ephedra aphylla* plants compared with control plants [184]. Roots of cyclamen (*Cyclamen persicum* Mill.) plants colonized by *Glomus fasciculatum* subjected to heat stress presented an increase in the production of SOD and APX, as well as non-enzymatic antioxidants (AA), compared with non-mycorrhizal stressed plants [186].

AMF can also stimulate the production of non-enzymatic antioxidants other than GSH and AA, such as carotenoids, phenolic, and flavonoid compounds [37,182,184,241]. For example, rose geranium (*Pelargonium graveolens*) plants colonized by different *Glomus* species had higher phenol and flavonoid contents than non-colonized plants subjected to water stress [37]. Furthermore, *Ephedra aphylla* inoculated with a mixture of AMF, viz., *Claroideoglomus etunicatum*, *R. irregularis*, and *F. mosseae*, showed an increase in phenolic compounds under salt stress [241]. In addition, an enhancement in carotenoid content was documented in marigold (*Tagetes erecta*) plants colonized with *Glomus constrictum* [182]. These studies suggest that mycorrhizal MAPs can withstand abiotic stresses by maintaining ROS homeostasis through the accumulation of enzymatic and non-enzymatic antioxidants. Overall, the improvement in antioxidant defense systems caused by AMF could be attributed to the fact that AMF can accumulate ROS and also that AMF possesses SOD genes [242,243].

4. Synergistic Effects of AMF and Other Amendments on MAPs

4.1. Microbial Amendments

PGPR are soil bacteria living around or on the root surface, and in addition to AMF can also be used to improve MAPs' growth under stress conditions. Indeed, PGPR are directly or indirectly involved in promoting plant growth and development via secretion into the rhizosphere soil of various regulatory chemicals (phytohormones, aminocyclopropane-1-carboxylic acid deaminase, and volatile growth stimulants) [244,245]. They also perform important biological functions, protecting plants against many pests or helping them to mitigate abiotic stresses such as drought, salt, or pollution [246,247].

Many studies have reported the beneficial effects of PGPR on the growth and yield of MAPs under drought stress. For example, Asghari et al. [248] showed that PGPR conferred drought tolerance and stimulated the biosynthesis of secondary metabolites in pennyroyal (*Mentha pulegium* L.) under water shortage conditions. The authors demonstrated high ABA, salicylic acid (SA), soluble sugars, phenolics, flavonoids, and oxygenated monoterpene contents, as well as radical scavenging activity in PGPR-inoculated plants under severe drought stress. The contribution of PGPR has also been shown to promote seed germination and root elongation of sage (*Salvia officinalis* L.) as well as growth of *Hyoscyamus niger* under water deficit conditions [51]. The improvement in seed germination rate could be due to an increase in gibberellin (GA) synthesis, which triggers the activity of α -amylase enzymes that promote early germination by improving starch assimilation [51,249]. This mechanism has been reported in hopbush (*Dodonaea viscosa* L.) under saline stress [250]. Likewise, plant growth promotion by PGPR involves the production of plant growth regulators such as indole acetic acid, GA, cytokinins, and ethylene [251–254]. In addition, through the production of biofilms, PGPR improve the availability of water for the plant, helping to manage abiotic stresses. Several works have reported modifications of MAPs' metabolisms and increases in EO production using PGPR inoculants [255]. Indeed, PGPR are known to improve plant biomass and trichome production and to stimulate terpene biosynthesis, which improves EO production [49,51,256]. For example, increases of *cis*-thujone in sage (*Salvia officinalis* L.), α -terpineol in sweet basil (*Ocimum basilicum*) and marjoram (*Origanum majorana* L.), trans-sabinene in marjoram (*O. majorana* L.) and hybrid marjoram (*Origanum*

x majoricum), and EO yield in fennel (*Foeniculum vulgare* Mill.) and geranium (*Pelargonium graveolens*) have variously been observed [49,51,257,258].

Further studies have demonstrated the beneficial and synergistic effects of dual PGPR and AMF inoculation on MAPs under metal, water, and salt-stressed conditions [259–263]. It has been demonstrated under conditions of water stress that the combined inoculation of myrtle (*Myrtus communis* L.) with AMF (*R. irregularis*) and PGPR (*Pseudomonas fluorescens*, *P. putida*) species improved hydromineral supply and EO production as well as enhancing plants' oxidative defense [262]. Furthermore, it was reported that combined inoculation of PGPR and AMF improved plant growth, metabolite content, and root length under drought stress in various MAPs [264,265]. Similarly, co-inoculation with plant-growth-promoting fungi (like *Trichoderma* spp. and AMF) and PGPR has been reported to increase the production of secondary metabolites and hairy roots of Chinese salvia (*Salvia miltiorrhiza*) under conditions of drought or salinity stress [261].

4.2. Organic Amendments

Organic amendments including biochar, composts, manures, and mulch are known to limit biotic and abiotic stresses on plants by influencing the physico-chemical properties, nutrient availability, retention capacity, or microbial activity of the soil [48]. In addition, organic amendments are rich in humic acids, which decrease the availability of metals through adsorption and the formation of stable organometallic complexes [266], which could contribute to reducing TE stress for MAPs. If well-chosen, all these organic amendments could (depending on the MAP species and soil type) significantly improve soil fertility and nutrient cycling, nitrogen fixation, organic matter amount, plant growth, and consequently plant establishment under conditions of water and temperature stress [267,268]. The decomposition of organic amendments has also been reported to provide useful weed control for MAPs, and as a solution to avoid water runoff while maintaining soil moisture [269]. Thus, compost application increased the biomass and EO yields of black cumin (*Nigella sativa* L.), cumin (*Cuminum cyminum*), and sweet basil (*Ocimum basilicum* L.) under drought stress conditions, and of *Chenopodium album* L. in TE-polluted soil. These improvements in plant biomass could be due to an increase in NPK availability, the physical, chemical, and biological properties of soils, and increased soil cation exchange capacity and water retention, resulting in better plant growth [270,271]. The addition of biochar allows the immobilization of TEs such as Pb and Cd through various mechanisms including surface complexation, surface precipitation, electrostatic attraction, physical adsorption, and ion exchange, improving soil quality by increasing water retention and nutrient mineralization, and reducing soil bulk density [272]. Biochar-based amendments can improve plant biomass, stimulate production of pharmaceutically active compounds, including phenolic and flavonoids, consequently increasing antioxidant activities, and could reduce the risk of metal accumulation in the plant [272]. Indeed, biochar has been shown to improve the quality of bioactive compounds and the biomass yield of various MAPs, including kalmegh (*Andrographis paniculata*) cultivated under conditions of water stress, and brahmi (*Bacopa monnieri* L.), kalmegh, and ashwagandha (*Withania somnifera* L.) cultivated on TE-polluted soil [272,273].

Synergistic effects of combined organic amendment and AMF have been reported in many studies [274–277]. For example, the combination of AMF inoculation with organic amendment improved the biomass and active principle yields of lemon balm (*Melissa officinalis* L.) [274]. Similarly, Kaleji et al. [275] reported that the co-application of AMF and compost enhanced the growth and EO content of water mint (*Mentha aquatica*) [275].

4.3. Mineral Amendments

Mineral fertilizers such as NPK, silicate, or clay are known to affect soil porosity, pore distribution, water retention, and water availability in the soil [52,278,279]. Under conditions of water stress, the EO and biomass yields of sage (*Salvia officinalis* L.) have been reported to be significantly higher when fertilized with a conventional NPK mineral

fertilizer [280]. Indeed, by changing soil pH, silicates reduced the phytoavailability of TEs, which are precipitated or chelated on soil aggregates [52]. Furthermore, silicate has been reported to be useful against various stresses, e.g., salinity, temperature, and flooding [281]. Generally, stress tolerance mechanisms can be attributed to physiological improvements in plants, activation of antioxidant systems, elicitation of secondary metabolites including ABA implicated in the osmotic stress response, or enzymatic activity (SOD, GPX, and CAT) [281]. For example, under stress conditions, silicate has been known to improve plant enzymatic activities such as SOD, CAT, GPX, and APX, as well as those of non-enzymatic antioxidants such as glutathione and proline, or plant transporters [281]. Likewise, clay amendment was reported to promote root elongation and drought tolerance in Pakchoi (*Brassica chinensis* L.) and hybrid sage varieties (*Salvia officinalis* × *S. pomifera*, *S. officinalis* × *S. tomentosa*, *S. officinalis* × *S. ringens* and *S. fruticosa* × *S. ringens*) under conditions of water stress [278,282], and silicate fertilizers reduced Cd transfer and accumulation in basil (*Ocimum basilicum*) shoots under metal stress [52].

Very few studies have demonstrated the synergistic effects of AMF inoculation and mineral amendments on MAPs under stress conditions. Indeed, AMF are often presented as an alternative to the application of chemical fertilizer in the production of MAPs. Recently, it has been shown that the application of a mixture of mycorrhizal species (*R. intraradices*, *F. mosseae*, *Glomus hoi*) and phosphorus fertilizer could improve the EO yield and physiological characteristics of peppermint (*Mentha piperita*) cultivated under different water stress conditions [283]. On the other hand, several studies have reported beneficial effects after the co-application of mineral and organic fertilizers [284,285]. For example, synergistic effects on fruit quality improvement have been described in the case of co-application of mineral and organic amendments under water deficit in pomegranate [286].

4.4. Biostimulants

Biostimulants are categorized as biological amendments and contain a wide range of molecules which may include chitosan, amino acids, humic substances, or plant extracts [46]. Their functional richness and potential interest in relation to MAPs grown under abiotic stress conditions have been highlighted in numerous studies [46,287–290]. It was shown that moderate water stress combined with SA application (300 ppm) increased EO yield [291] in lemon verbena (*Lippia citriodora* L.). The foliar application of SA and chitosan increased the EO content of thymbra (*Thymbra spicata* L.) and, particularly, the amount of thymol produced under reduced irrigation conditions [289]. Likewise, palm pollen grain extracts increased EO yield and the level of osmoprotectants (proline, amino acids, and soluble sugars) in basil (*Ocimum basilicum* L.) cultivated under drought conditions [290]. It has also been reported that the use of microalgae extracts caused a change in the composition of basil (*Ocimum basilicum* L.) and parsley (*Petroselinum crispum* L.) EOs, and increased the content of certain compounds [292]. Foliar spray of pluramin (a powdery compound of amino acids) was reported to reduce water stress damage in lemon balm (*Melissa officinalis* L.) [293].

To the best of our knowledge, no data have been reported on the synergistic effects of biostimulants and AMF inoculation on MAPs. On the other hand, some studies have demonstrated the beneficial and synergistic effects of combinations of various biostimulants, including those with organic amendments, on MAPs' biomass production or EO yield [289,294]. For example, it was reported that co-application of biostimulants (such as *Azotobacter chroococcum* and *Azospirillum lipoferum*) and vermicompost improved the fresh and dry weights of coriander (*Coriandrum sativum* L.) [294].

5. Conclusions and Future Perspectives

With the increasing demand for natural products originating from MAPs, the cultivation of these plant species has become of great interest throughout the world. Unfortunately, MAPs face various environmental stresses due to anthropic activities, intensified by climate change, which have triggered harmful effects on their growth as well as the productivity

and quality of their EOs. Therefore, much effort has gone into developing eco-friendly strategies that can tackle the problems related to ongoing climate change and improve plant tolerance. Hence, the actual challenge is to increase plant growth and biomass production while maintaining or improving the quality and yield of herbal materials such as EOs.

Microbial and organic or inorganic amendments can confer greater tolerance against abiotic stress in MAPs. AMF not only enhanced water and nutrient acquisition but also rapidly triggered the MAPs' morphological, physiological, and molecular responses, which increased their ability to overcome the adverse effects of abiotic stress (Figure 1). It has been shown in the current review that AMF can improve plant growth as well as the quantity and quality of EOs. Many studies have demonstrated the synergetic effect of AMF with other soil amendments to improve MAPs' growth and, consequently, EO yield and quality under abiotic stress. However, insights into the mechanisms behind the interaction of microbial soil amendments and MAPs under abiotic stress are very complex, and there remains a need for further investigation. Based on the data outlined in the current review, there is a need in the future for fundamental and applied investigations into different aspects:

1. Most of the previously cited studies were conducted in the laboratory or under greenhouse conditions (i.e., pot experiments). However, further field experiments are required as many factors, including climate and microbial rhizosphere biodiversity, may influence the results compared with those obtained in controlled conditions.
2. In studies where different strains were tested, the extent of AMF response on plant growth and root colonization varied with AMF species and also with the type and level of stress. Therefore, choosing the appropriate host plant and AMF species is important for using plant-AMF symbionts successfully. In future research, it will be important to screen indigenous and stress-tolerant AMF isolates to improve the effectiveness of arbuscular mycorrhizal symbiosis.
3. More research should focus on the use of AMF in combination with PGPR or with organic or inorganic amendments to obtain more advantages in enhancing MAP growth and productivity. In addition, there is a considerable lack of data underlying the molecular mechanisms involved in the synergistic effects observed, and more generally in the modification of secondary metabolite pathway biosynthesis.
4. Finally, various advanced techniques (proteomics, genomics, and metabolomics) could provide new insight into the mechanisms exerted by arbuscular mycorrhizal symbiosis, which confer stronger productivity and enhanced resistance to MAPs under abiotic stresses.

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II. Apport de l'étude du microbiote du sol dans la phytoremédiation

Pour répondre aux attentes sociétales en termes de requalification écologique des sols, des techniques de remédiation biologique utilisant des micro-organismes (bioremédiation) et/ou des espèces végétales (phytoremédiation), seules ou en combinaison, ont été développées (Quintella et al., 2019). Ces approches exploitent le potentiel métabolique naturel des micro-organismes du sol et des plantes pour réduire la toxicité environnementale des contaminants du sol (Alegbeleye et al., 2017; Burges et al., 2018; Verma et al., 2017).

Cependant, malgré son potentiel prometteur, le phytomanagement des sites pollués rencontre encore des succès variables et reste loin d'une utilisation généralisée à grande échelle, principalement en raison de la complexité des interactions biologiques entre polluants, plantes, sols et micro-organismes, ainsi que des conditions climatiques et édaphiques spécifiques à chaque site (Bell et al., 2014; Teng et Chen, 2019).

Néanmoins, les récentes avancées dans les technologies "omiques" (métagénomique, métranscriptomique, métabolomique) et les outils bioinformatiques ouvrent de nouvelles perspectives pour l'étude des communautés microbiennes du sol (Bell et al., 2014; Li et al., 2018; Zhang, 2019). En particulier, les données de séquençage d'ADN environnemental (eADN) sont de plus en plus utilisées pour développer de nouveaux outils de surveillance environnementale et de phytomanagement des sols pollués.

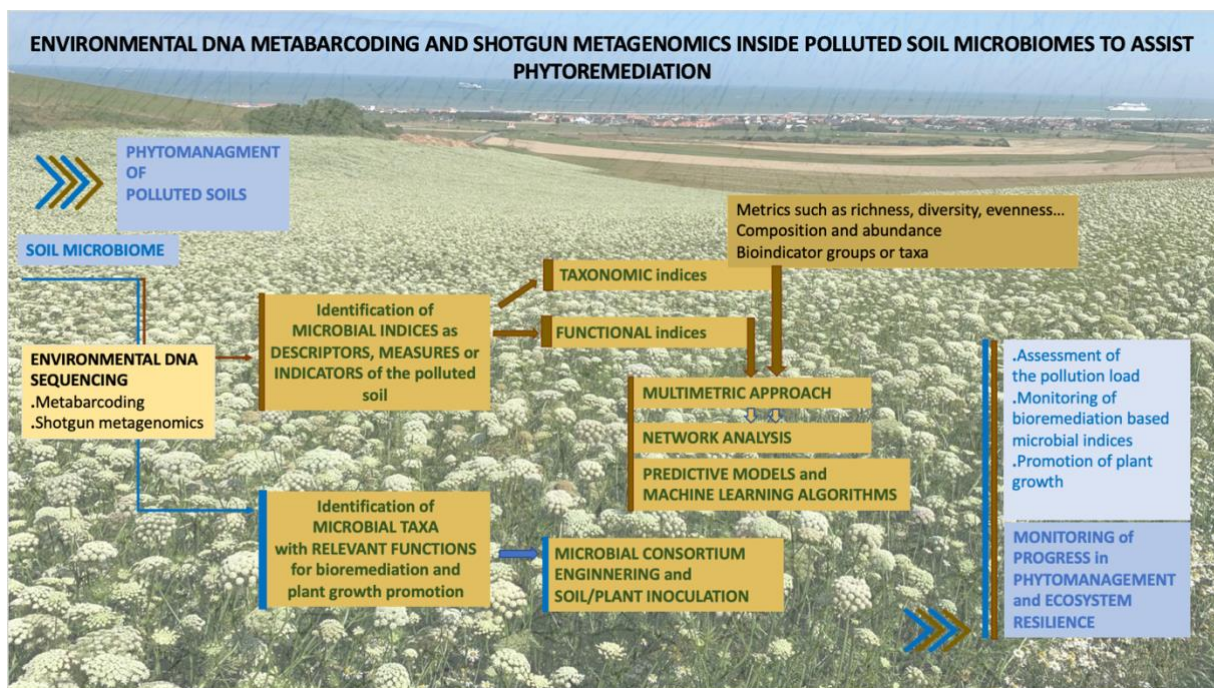
C'est pourquoi, dans cette troisième revue, nous mettons l'accent sur l'utilisation des données de métabarcoding et de métagénomique obtenues à partir de l'ADN environnemental pour évaluer l'état de santé des sols et faciliter les projets de phytomanagement. Nous décrivons comment ces données sont utilisées pour identifier des indices taxonomiques et fonctionnels microbiens afin de diagnostiquer la pollution du sol et de suivre sa remédiation. De plus, nous discutons comment ces données peuvent conduire à l'identification de souches microbiennes potentiellement utilisables pour l'immobilisation, la dégradation des polluants et la promotion de la croissance des plantes. Enfin, nous abordons les tendances futures et les développements pour exploiter pleinement le potentiel de ces données dans les projets de phytomanagement.

Revue 3 : Environmental DNA metabarcoding and shotgun metagenomics inside polluted soil microbiomes to assist phytomanagement: potentialities, limitations and prospects. A review

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Graphical abstract



Summary

Soils fulfil several crucial ecological functions. However, with anthropogenic activities, pollution has reached alarming rates and threatens soil health. Thus, several rehabilitation methods have been developed to restore the polluted soils. Notably, phytomanagement approaches, combining a sustainable reduction of soil pollutant toxicity while generating valuable biomass for the (bio)economy and essential ecosystem services, has gained an increased attention and acceptance, as a relevant cost-effective green alternative for phytoremediation of polluted soils. However, many limitations for its broad and large-scale commercial use persist and new directions for research are required. Recent developments in high-throughput sequencing, omic tools and bioinformatic approaches are proposed for developing a new generation of tools for environmental monitoring and phytomanagement of polluted soils. In this review, we focus on contributions of metabarcoding and shotgun metagenomics from environmental DNA (eDNA). We highlight and discuss some studies that propose to translate high-throughput sequencing data of microbial eDNA into effective and reliable environmental tools assisting, potentially, phytomanagement of polluted soils. Thus, first, we investigated how the identification of microbial taxonomic and functional data are proposed as microbial indices to be used for the diagnosis of soil pollution and the monitoring of the soil remediation. Then, we highlight how eDNA-sequencing data open new perspectives for the discovery of strains with potential in both pollutant immobilization and/or degradation and plant growth promotion. These microbial strains can subsequently compose microbial inoculants to enhance biomass production and phytoremediation efficiency. Finally, we discuss some future developments to fully exploit the potential of microbial eDNA data in a phytomanagement project.

Keywords: Phytomanagement, Microbiome, Soil Pollution, Metabarcoding, Shotgun Metagenomics, Microbial Indices.

I. From phytoremediation to phytomanagement: sustainable options for polluted soil rehabilitation

Soils fulfill crucial ecological functions, such as plant production, nutrient cycling, water maintenance and carbon storage (Hatfield et al., 2017). However, anthropogenic activity (mining activities, use of fossil fuels, metallurgical activities, industry, waste management, road traffic, intensive agriculture) and the rapid expansion of urbanization linked to the demographic explosion in the world, have led to an increase in the pollutant load on soils (Dhaliwal et al., 2020). Therefore, a large range of potentially toxic molecules, including persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), or volatile organic compounds (VOCs) or inorganic compounds like metal(-oid)s (also referred to as trace elements (TE), , radionuclides, inorganic salts (Garrido-Sanz et al., 2019; Geissen et al., 2021; Quintella et al., 2019; Shah and Daverey, 2020; Verma et al., 2017) is recorded in abandoned industrial sites or natural and agricultural areas. These pollutants threaten animal and human health due to their toxic, mutagenic or carcinogenic effects. In addition, they modify the physical properties of the soil (permeability or water content), as well as its chemical properties (pH or nutrient availability), impact plant growth, or lead to changes in microbial communities, in the functionality of soil and consequently in ecosystem services. Growing awareness of the need to remediate these soils has led to a plethora of approaches using cutting-edge scientific technologies to control and mitigate this difficult global problem (Dhaliwal et al., 2020; Raza et al., 2020; Verma et al., 2017).

Today, the most used technologies are based on physical (surface washing, soil covering or replacement, isolation, encapsulation), chemical (chemical leaching, precipitation, ion exchange, fixation, adsorption), or thermal protocols (heat incineration) (Khan Ag, 2022; Rajendran et al., 2022; Shah and Daverey, 2020). However, despite their performances, these physico-chemical methods are expensive and consequently not suitable for large surfaces (Ahmed et al., 2021). In addition, they sometimes generate by-products that are more harmful to the environment (Gaur et al., 2014) and lead to the loss of soil functions, which becomes a waste product. In response to these drawbacks and to meet society's expectations in terms of ecological sustainability, biological remedial techniques using microorganisms (bioremediation) and/or plant species (phytoremediation), alone or in combination, have been developed (Quintella et al., 2019). They aim to exploit the natural metabolic potential of soil microorganisms and plants to reduce the environmental toxicity of the soil contaminants (Alegbeleye et al., 2017; Burges et al., 2018; Verma et al., 2017). In particular, soil microorganisms, which are also major drivers of litter decomposition, nutrient cycling, soil fertility

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maintenance and carbon sequestration (Delgado-Baquerizo et al., 2016; Fierer, 2017; Mendes et al., 2013), have become dominant tools in bioremediation processes. Indeed, microbial communities, through their responses to pollutant exposure represent both sensitive bioindicators for revealing the ecological quality of the environment and promising actors for the remediation of polluted soils. So, numerous soil microorganisms were identified for their capabilities to sequester, transform or break down different environmental pollutants (Wang et al., 2018; Xu et al., 2019).

Today, the importance is more particularly attached to bacteria and fungi in bioremediation, due to efficient mechanisms (horizontal gene transfer, mutation, rearrangement or differential regulation of genes, plasmid exchange) involved for their adaptation and survival in unfavorable polluted environments (Borowik et al., 2017; Dhaliwal et al., 2020; Heuer and Smalla, 2012; Quintella et al., 2019; Sentchilo et al., 2013). Furthermore, they constitute the largest genetic reservoir of enzymatic activities involved in degradation of toxic compounds. (Borowik et al., 2017; Teng and Chen, 2019).

Besides bioremediation mediated by microorganisms, many plants species can naturally contribute to bioremediation through several ways such as pollutant immobilization, transformation or mineralization into lesser toxic forms which lead to remove, reduce, degrade or immobilize the various pollutants. Phytoremediation includes different strategies such as phytoextraction and phytostabilization (extensively used for remediation of TE-polluted soils), rhizodegradation or phytovolatilization, for organic pollutants (Ashraf et al., 2019; Ma et al., 2011; Raza et al., 2020). In addition to limit the impact of pollution on the environment and human health and to improve ecosystem services provided by soils, phytoremediation of polluted soils could make polluted sites economically attractive again by using plant biomass in non-food sectors (Sugawara and Nikaido, 2014). Indeed, the economic valorization of biomass through obtention of added-value by-products for bioeconomy is an option referred to as phytomanagement (Burgess et al., 2018; Raveau et al., 2021a). Here, the production of plant biomass (as renewable bioenergy, fodder or organic manure compost, high-market-value essential oils, ecomaterials, added-value subproducts for biosourced-chemistry) is essential for the economic viability of phytomanagement. However, increasing attention is being paid to the capacity of phytomanagement to provide a wide variety of ecosystem services, such as primary production and carbon sequestration, water runoff/drainage management and control of soil erosion or aesthetic value (Burgess et al., 2018; Evangelou et al., 2015; Garbisu et al., 2020). Therefore, phytomanagement is gaining increasing acceptance as a green approach and sustainable ways for soil reclamation as compared to conventional soil remediation techniques (Mahar et al., 2016). Hence, it is estimated to be around 60% to 80% less expensive than conventional physico-chemical techniques (Ashraf et al., 2019).

The selection of suitable plant species is another critical aspect of successful plant management. Indeed, plant species must be able to grow under polluted soils and develop plant cover in a relatively short time while producing large amounts of biomass. Consequently, different plant species have been tested, including annuals, perennials or woody species, food or animal fodder crop species, as well as aromatic plant species (Burgess et al., 2018; Evangelou et al., 2015; Fontaine et al., 2022; Langrand et al., 2023; Moreira et al., 2021; Raveau et al., 2021a). Research in conventional plant breeding or construction for new genome and phenotype (transgenic plants, Crispr technology), are all factors enhancing progress in plant management (Agarwal and Rani, 2022; Gogolev et al., 2021; Rakkammal et al., 2022). In addition, for helping plants to cope with pollution, some culture conditions or cropping patterns (*e.g.* application of organic amendments, tillage practices, intercropping, winter cropping) can be used to improve the phytomanagement efficiency (Brereton et al., 2020; Moreira et al., 2021). In addition to the above methods, soil inoculation with microorganisms actively facilitating the bioremediation process (Andreolli et al., 2015; Dar and Kaushik, 2023). Moreover, microbe-assisted phytosanitary management, as well as the formulation of microbial consortiums as inoculants have been developed and sometimes successfully used (Ashraf et al., 2017; Sharma et al., 2023; Thijs et al., 2016).

In practice, despite great promise, phytomanagement of polluted sites using plants and microorganisms with relevant capabilities, even using ad hoc culture systems, still encounters varying degrees of success and is still far from achieving widespread, large-scale use, mainly due to the uncertainty of complete and rapid site remediation (Bell et al., 2014). Here, it is generally accepted that current interventions do not always deliver the desired results in the field due to an incomplete understanding of the multicomponent biological interactions between pollutants, plants, soil and microorganisms, preventing any major improvements in phytosanitary management (Teng and Chen, 2019). In addition, soil and climatic conditions are site-specific, so bioremediation models need to be contextualized according to where remediation will take place.

Nevertheless, recent advances in "omics" technologies (metagenomic, metatranscriptomic, metaproteomic and metabolomic methods) from environmental samples and the computational tools for the analyses of massive amounts of datasets provide new avenues for studying soil prokaryotic and eukaryotic communities (Bell et al., 2014). While metatranscriptomic, metaproteomic and metabolomic methods are also gradually implemented for studying microbiomes of soils, environmental DNA (eDNA) is arguably the most extensively used technical advances in providing accurate description and quantification of biodiversity and its functions (Li et al., 2018; Zhang, 2019).

In this review, we focus on how DNA-metabarcoding and shotgun metagenomic datasets may become key components to assess health status of soils and assist phytomanagement project. First, we begin with a brief overview about the major characteristics of both approaches, with merits and limitations of each one. Second, we investigate how they are proposed in the literature for identification/calculation of taxonomic and functional microbial indices aiming a generation of microbial indicators, based on eDNA data, to diagnose ecological disturbance, pollution risk and soil quality recovery. Third, we highlight how metagenome data can lead to the bioprospecting of unknown or unusual microorganisms with bioremediation capabilities, and provide some guidelines for monitoring the impact of synthetic microbial inoculum with catabolic capabilities towards pollutants, but also with plant growth promoting traits. Finally, we conclude with some trends and future developments to fully exploit the potential of environmental microbial DNA-sequencing data.

II. Environmental DNA metabarcoding and shotgun metagenomic to characterize soil microbiome biodiversity

Adverse outcomes caused by pollutants to soils are determined not only by ecotoxicological assays but also by the characterization of the richness, biodiversity and the composition of the indigenous microbial communities. Classically, the bioassessment of ecosystems may be based on morphological identification of various selected taxonomic groups, used to define the ecological quality status, as well as the vulnerability of structures and functions of ecosystems in response to toxic substances (Pawlowski et al., 2018). These conventional indices have some limitations. They are based on the direct observation of the microorganisms, which have been proven to be time consuming and resource-intensive. In addition, they are often related to difficulties in morphological identification of bioindicator taxa. Indeed, to date, only a tiny part of soil microorganisms is made up of cultivable microorganisms and the traditional morphology-based species identification approach is limited (Li et al., 2018; Pham and Kim, 2012).

The high-throughput sequencing-based strategies and biocomputing are now, arguably, the main tools for environmental studies of the microbial black box (cultivable and uncultivated microorganisms). They include a variety of procedures to holistically study any biological system and to investigate the organism community inhabiting a given environment (Bell et al., 2014). These approaches (metagenomics, metatranscriptomics, metaproteomics, and metabolomics) are referred to as “omics”, a neologism that designs various methods of detection, characterization and quantification of multiple microbial communities, in one sequencing run, with an unprecedented resolution. Among them, is

metagenomics (Daniel, 2005; Escobar-Zepeda et al., 2015) with two central next-generation sequencing-based strategies developed for microbial genome analysis from eDNA sampling: i) the amplicon-targeting of specific genes and sequencing (*i.e.* eDNA amplicon-based metabarcoding) and ii) the whole metagenome shotgun sequencing (shotgun metagenomics) herein defined as the study of the entire DNA material recovered from environmental samples (Fierer et al., 2007; Quince et al., 2017; Ranjan et al., 2016). There is now a growing body of literature summarizing the potential of eDNA metagenomics for biological monitoring and highlighting its importance for environmental management. In such technology, a taxonomically informative genomic marker that is common to virtually all organisms of interest is targeted and amplified by PCR. For the identification of bacteria, amplicon sequencing studies usually target the 16S rRN1A gene, which is both a taxonomically and phylogenetically informative marker. As well, amplification of the internal transcribed spacer (ITS) regions of the rDNA has been adopted as the universal fungal barcode sequence (Anderson and Cairney, 2004; Schoch et al., 2012). The amplicons are then sequenced and the amplicon sequences are clustered into, for instance, operational taxonomic units (OTU), exact sequence variants (ESV) or amplicon sequence variants (ASV). After clustering, the SILVA, RDP, Greengenes, and NCBI databases are frequently used for bacterial sequence analysis, while UNITE and Warcup are generally used for fungal sequences. In such conditions, the DNA metabarcoding data determine which microorganisms are present in the sample. It also provides i) a proxy for the relative abundances of microbial species from variations in the copy number of the same amplicon sequences, ii) variations in DNA copy number within and between species, at different taxonomic levels, and information regarding the phylogenetic diversity of microorganisms. However, while powerful, metabarcoding is not without limitation (*i.e.* biases associated with PCR). So, the shotgun metagenomic appears as an alternative approach that avoids some limitations (Sharpton, 2014). Notably, this approach does not target a specific genomic locus from PCR amplification that avoids primer bias. In addition, total DNA is extracted from all cells of soil samples (Quince et al., 2017; Sharpton, 2014). In practice, DNA is subsequently sheared into tiny fragments that are independently sequenced. This results in DNA sequences (*i.e.* reads) that align to various genomic locations for the myriad genomes present in the sample. Some of these reads will be sampled from taxonomically informative genomic loci (*e.g.* 16S, ITS or 18S), and others will be sampled from coding sequences that provide insights into the biological functions encoded in the genome (with gene prediction and gene annotation). Advances in metagenome-assembled genomes technology, such as long-read sequencing and single-cell metagenomics, can improve the quality of shotgun metagenomic sequencing data. Despite such benefits, shotgun metagenomics meet several challenges which still limit their application. It requires a considerable increase in sequencing power and extensive bioinformatics analysis of the data, which are more costly than the metabarcoding approach (Douglas et al., 2020; Ranjan et al., 2016). Consequently, to date, this technology is increasingly used for the

characterization of the metagenome in diverse ecosystems and research fields, because of its advantages in terms of operational costs, with lower computational hardware and data management requirements (Ruppert et al., 2019).

Nevertheless, together, both approaches have been used to uncover the structure and composition of microbial communities, their taxonomic, genetic and functional diversity, phylogenetic lineages, to assemble novel genomes, to identify new taxa and genes present in the microbial community, and to determine which metabolic pathways are encoded in the community, in line with environmental parameters.

III. Diagnosis of soil pollution and phytomanagement efficacy using high-throughput sequencing and metagenome

Taxonomic indices

Analysis of microbial community profiles from metagenome data have been used to uncover the structure and composition of microbial communities in response to disturbances (Bahram et al., 2018; Delgado-Baquerizo et al., 2020, 2016; Wagg et al., 2019). In particular, ecological studies in polluted habitats have shown that soil pollutants and their toxicity induce drastic changes in the profiles of microbial communities (Borowik et al., 2017; Song et al., 2018; Tang et al., 2019). Thus, by comparing polluted soils with those derived from reference conditions, over time or across geographic regions, some dissimilarities recorded between two or more microbial communities have been identified as bioindicators of soil ecological disturbance, toxicity or quality (*i.e.* the ability of soil to perform its function). Consequently, some recent studies have been proposed to monitor the remediation of polluted soils, the restoration of soil functioning and also the effectiveness of phytomanagement (Hellal et al., 2023; Tang et al., 2019; Wang et al., 2021).

Taxonomic biodiversity analysis is the dominant approach to track ecological changes in communities. It can be then quantified through a multitude of indices, in particular, metrics such as the taxonomic alpha, beta and gamma diversities (Legendre and Legendre, 2012). Also, diverse α -diversity metrics (such as Margalef's index for richness, the Shannon-Weaver's index for diversity and the Simpson's index for dominance) can be computed. Besides, richness (how many taxa are there), evenness (relative abundances resulting in rare and dominant species), composition (in terms of taxonomic groups), phylogenetic relatedness can be analyzed. All these metrics are diversely used to diagnose soil pollution and quality. For instance, it has been observed that the pollutant can adversely

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affect both the α - and β -diversities of the soil microbial communities. For example, TE pollution has been shown to reduce Shannon bacterial diversity (Feng et al., 2018; Singh et al., 2014; Yang et al., 2018). On the contrary, when evaluating the phytosanitary management of polluted soil for essential oil production, some authors report that the Chao microbial richness parameter and the Shannon microbial diversity index are not systematically different between samples from polluted and unpolluted sites (Langrand et al., 2023; Raveau et al., 2021b, 2020; Saran et al., 2022).

Concomitantly, the **community structure** (*i.e.* the taxonomic compositions as well as absolute and relative abundance measures) is a major descriptor of microbial community diversity, and thus, provide putative bioindicators of shifts in communities in response to pollution in ecosystems of interest. The shifts can be then analyzed at high taxonomic ranks (phylum, class, order, family) or at lower and finest taxonomic resolution (genus, species). Examining microbial responses at low taxonomic level, the presence/dominance of some taxa can be correlated with variations in pollution. For example, from a 16S rRNA gene metabarcoding in bacterial communities, Saran et al. (2022) pointed out the predominance of the genus *Serratia* in the rhizosphere of *Helianthus petiolaris* cultivated for essential oil production in lead-polluted soils. In addition, plants colonized by *Serratia* developed higher biomass compared with plants grown on non-polluted control sites. The authors suggest that the bioaugmentation and distribution of *Serratia* taxa may improve the survival of plants in Pb-polluted soils. In line, Yang et al. (2018) identified a significant shift in prokaryotic community composition via substitution of dominant species across a broad range of taxa groups, in response to increased copper concentrations. Raveau et al. (2022) observed that the bacterial genera *Pseudarthrobacter*, *Streptomyces*, and *Actinoplanes* were highly abundant in the rhizosphere of clary sage cultivated on the aged TE-polluted soils and suggested their relevance in increasing phytomanagement efficiency.

As well, **indicator species** analysis (Legendre and Legendre, 2012) could provide putative microbial indicators and can be proposed to diagnose pollutant loads and guide the choice of phytomanagement options (Wang et al., 2020). For instance, Li et al., (2018) using an eDNA metabarcoding, concluded that the relative abundance of indicative OTUs was significantly correlated with pollution levels leading to their use to predict the pollution status up to 79 % accuracy on testing data sets.

Interest has also focused on **key microbial taxa** identified by analysis of different properties (nodes, centrality, modularity, stability) inside the microbial co-occurrence network (Berry and Widder, 2014; Fuhrman, 2009). Some taxa are highly connected groups that individually or in a guild exert a

considerable influence on microbiome structure and functioning regardless of their abundance across time and space (Banerjee et al., 2018). Thus, it has been proved that keystone taxa could show significant correlations with soil physicochemical conditions (Zhao et al., 2016). Some authors have begun to propose them as sensitive species for indicating pollution level and for their use in diagnosis/prediction of contamination. For instance, Wang et al. (2020) have highlighted keystone taxa of bacterial communities that could be used to successfully predict (with 88.9 % accuracy) contamination levels in wetlands, and so guide ecosystem management. Also, bacterial keystone taxa were identified in soil with oil contamination (Liang et al., 2016). Interestingly, using metabarcoding analysis Zhang et al., (2018) also underlined that some unassigned taxa are potentially good bioindicators and predictors of ecological status. Besides, they showed that the alterations of phylogenetic biodiversity of some communities were sensitive indicators of sediment contamination by copper. Also, Xu et al., (2023) revealed fungal community changes were mostly driven by total amounts TE contamination, and their data illustrated that the fungal community might serve as indicator for TE-polluted status. Also, they suggested specific TE-responsive fungal species can be considered relevant bioindicators due to their less relative abundance in polluted areas.

Functional indices

The restoration of soil functionality and ecosystem services is a major concern in soil remediation and phytomanagement projects. To guide environmental management decision-making in phytomanagement, data about soil functionality are essential. There is growing evidence that a loss in microbial diversity will inevitably lead to a loss of multifunctionality (Delgado-Baquerizo et al., 2020, 2016). However, although powerful and relevant, diversity and structure indices computed alone, limit the diagnosing/assessing of soil pollution and consequently, the monitoring of phytomanagement efficiency. Directly measuring key ecosystem functions, when possible, can assess the toxic effects of pollutants on the microbiome (Morin and Artigas, 2023). Thus, microbial respiration, nitrification and denitrification have been widely used to account for chemical disturbances on ecosystem functioning (Lyautey et al., 2021; Simonin et al., 2019; Wakelin et al., 2013) as well as diverse enzyme activities (Lyautey et al., 2021). However, this approach is limited by the small number of functions that can be analyzed *in vitro* or directly in soil. Recently, predictive computational approaches have provided new ways to connect genes to functions from metagenome and taxonomy-based investigations. Thus, several software packages and databases suited for inferring functional profiles from metagenome dataset have been developed, such as Tax4Fun (Aßhauer et al., 2015; Wemheuer et al., 2020), PICRUSt2 (Douglas et al., 2020), BactoTraits (Cébron et al., 2021), FunGuild (Nguyen et al., 2016), FungalTraits (Pöhlme et al., 2020), Piphillin (Iwai et al., 2016), Vikodak (Nagpal et al., 2016), or FAPROTAX (Louca et al., 2016) (Table 1).

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Table 1: Overview of functional assignment tools used in metagenomics

Data base	Number of entry	Traits information	Organism	Reference
Tax4Fun2	12 377 genomes	Habitat specific functional profiles	Bacteria or archaea	(Wemheuer et al., 2020)
PICRSt2	41 926 bacterial and archaeal genomes from the IMG database (JGI)	20 000 genomes	Bacteria or archaea	(Douglas et al., 2020)
BactoTraits	19 455 strains from BacDive	19 455 (At least one trait described per strain)	Bacteria	(Cébron et al., 2021)
FunGuild	9 476 entries	12 ecologic guilds	Fungi	(Nguyen et al., 2016)
FungalTraits	10 210 genera	30 guilds in the "primary_lifestyle"	Fungi	(Pölme et al., 2020)
Vikodak	25 060 genomes from IMG database (JGI)	3 275 enzymes	Bacteria	(Nagpal et al., 2016)
FAPROTAX	4 700 taxa	> 80 functions	Bacteria	(Louca et al., 2016)

As well, from shotgun metagenomic data, functional annotation of metagenomic sequences can be operated via gene prediction and gene annotation (Quince et al., 2017). This usually involves identifying metagenomic reads that contain protein coding sequences and comparing the coding sequence to a database of genes, proteins, protein families, or metabolic pathways for which some functional information is known. Thus, it provides insight into the collective functions that are encoded in the genomes of the whole community, and the functional diversity of a community can be quantified from annotations. Doing this for all metagenomic sequences produces a profile that describes the number of distinct types of functions and their relative abundance in the metagenome. Thus, it may reveal the presence of novel genes (Morgan et al., 2012; Nacke et al., 2012) or provide insight into the ecological conditions associated with those genes for which the function is currently unknown (Buttigieg et al., 2013).

Of course, the functional diversity encoded in the DNA metagenome is a proxy for the community functional activity since the presence of a gene does not mean that it is expressed at the time of sampling. Besides, diverse strategies for taxonomic and functional classification, relying on a variety of reference databases can bias the results depending on the approach taken. Despite these limitations, helping to predict functions or traits based on metagenomic data and taxonomic identities, the analytical power of barcoding or shotgun metagenomic data is explicitly increased. Notably, it provides

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new insights for our understanding of the impacts of environmental contaminants on the functional richness/diversity of environments, with the opportunity to evaluate more accurately, ecosystem functionality, and area previously neglected in ecological risk assessment. Consequently, it can be ascertained how various pollutants influence the functional potential of the community (Looft et al., 2012) or it can give access to the identification of new putative functional biomarkers for environmental diagnosis, associated potentially with unknown metabolites in microbial communities.

For instance, He et al., (2018) indicated that the overall functional richness/diversity of groundwater microbiomes decreased with increased uranium concentrations. But some specific functional genes/populations were stimulated under high concentrations of uranium and could be used to successfully predict uranium contamination and, potentially, ecosystem functioning. Besides, Mukherjee et al., (2017) interestingly performed a general characterization of bacterial community composition in petroleum hydrocarbon polluted environments, using 61 previous publications dealing with metagenomes predicted from 16S rRNA gene sequences through PICRUSt. So, they showed that significant taxonomic, phylogenetic and functional differences exist between geographically isolated oil polluted sites. In the process, they inferred potential taxonomic and functional biomarkers along with competitive and cooperative interactions in bacterial correlation networks at diverse oil polluted sites, which may be used as biomarkers for successful distinction of different oil polluted habitats as well as for monitoring of bioremediation efforts in the same.

Interestingly, using a DNA barcoding approach, Martos et al., (2021) detected substantial variations (related to diversity, composition, network) in the microbial communities of taxonomically related plants that differ in the hyperaccumulator phenotype and cohabit in non-metalliferous soils. Surprisingly, enriched levels of bacteria previously described as metal-tolerant or formerly associated with hyperaccumulators in metal-rich soils were observed in these non-metalliferous soils. In addition, through a predictive functional analysis of the hyperaccumulator bacteria that were differentially abundant, performed using FAPROTAX software, they also found that a distinctive functional trait of the bacterial communities is their role in the nitrogen cycle. Although this function is not a direct mechanism of metal acquisition or tolerance, they underlined that increased nitrogen availability can be advantageous for plants growing in TE-polluted soils.

Besides, computational methods have also been developed to approximately predict the metabolomic activities or features of microbial communities from metagenomes, based on their taxonomic and functional profiles (*i.e.* gene family abundances) obtained from metabarcoding or shotgun metagenomic sequencing data (Mallick et al., 2019).

Consequently, various studies have shown the potential power of taxonomic and functional indices obtained from eDNA sequence-based data as indicators of exposure to pollutants, due to their sensitivity, speed of response, ecological relevance and ability to provide information that integrates numerous environmental factors (Epelde et al., 2014; Touceda-González et al., 2017). They have been translated into usable approaches in medical and clinical research or in water environments (rivers or sea). In this line, Beale et al., (2022) give an overview of the science of eco-monitoring, an emerging field of environmental management and policy, where multiple levels of omics-based technologies have been beneficially applied in different systems to capture the biological response to disturbance. Implementation of taxonomic and functional indices extracted from metabarcoding and metagenomics should be considered more systematically and widely as a prerequisite for any phytomanagement project for polluted sites, in order to monitor and assess the full recovery of soil quality (*i.e.* the capacity of soil to perform all its functions and ecological services) over time.

IV. Production of inoculants with bioremediation capacity based on taxonomy and functions derived from metagenomic data

Some soil microorganisms are significantly key players in improving the transformation, degradation or immobilization of polluting compounds. Besides, some soil microorganisms, referred to as plant growth promoting microorganisms (PGPM), are able to promote plant growth and development, which is particularly relevant in phytomanagement practices where high biomass production is sought. Indeed, plants can be viewed as a superorganism (referred to as the plant holobiont), hosting in the rhizosphere or in the endophytic compartment, a complex microbial community, often referred to as a host's extended genome. This microbiome therefore provides a huge range of additional functional capacities to plants. Some contribute to plant establishment, survival, growth, fitness and performances (Fierer et al., 2013; Mendes et al., 2013; Santoyo et al., 2016; Thompson et al., 2017; Turner et al., 2013), even leading to biomass productions similar to those reported for unpolluted soils (Gupta et al., 2018; Moreira et al., 2021). Various processes are involved here, such as nutrient acquisition (nitrogen fixation, phosphorus solubilization), phytohormone synthesis or production of various anti-pathogenic metabolites (Ma et al., 2011; Shah and Daverey, 2020). But, in addition to plant growth-promoting activities, PGPM have also attracted attention due to their pollutant-degrading abilities, making them less toxic or/and available for plants. Their beneficial effect on plant growth in polluted soils have been investigated extensively while numerous studies have demonstrated that they play an extensive role for the enhanced decontamination of polluted soils (Feng et al., 2018; Santoyo et al., 2016). So, it has been shown that inoculation of PGPM

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can greatly benefit phytomanagement (Gupta et al., 2018; Moreira et al., 2021). For instance, the efficiency of Cd phytostabilisation was improved by using plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) (Raza et al., 2020). As well, some endophytes (Feng et al., 2018; Franco-Franklin et al., 2021; Ma et al., 2011) or Actinobacteria (Alvarez et al., 2017; Behera and Das, 2023) have received special attention to be active in phytoremediation. Notably, Baoune et al., (2019) successfully investigated plant-Actinobacteria partnerships to clean up crude petroleum and PAH polluted soils.

In addition, numerous data from literature highlight a joint effort of microorganisms, not acting as individuals but rather as a dynamically microbial community, engaged in networks based on various processes (*i.e.* quorum sensing, biofilms, competition and cooperation) (Festa et al., 2016; Teng and Chen, 2019). Thus, it has been highlighted that consortiums of microorganisms showed better effects than a single strain in the process of bioremediation through increasing competitiveness, persistence, and activity of inoculants once introduced into polluted soils (Kang et al., 2016). Gurav et al., (2017) constructed a consortium of four strains with higher efficiency of petroleum hydrocarbons and PAHs degradation than any single strain among the four selected. In line, a relevant review of applications of microbial consortiums in bioremediation of soil polluted by PAH, crude oils, TE or plastics is provided by Teng and Chen (2019).

Thus, the development of microbial-based inoculants with potential in pollutant remediation and growth promotion of plants exposed to different environmental stresses has deserved special attention in recent years (Basiru and Hijri, 2022; Hijri, 2023; Mokarram-Kashtiban et al., 2019). Interestingly, focusing on a complex pollutant composed of a mixture of aromatic and aliphatic hydrocarbons, Garrido-Sanz et al., (2019), reported the isolation and characterization of an indigenous soil bacterial consortium that can grow aerobically with diesel oil and other aliphatic and aromatic constituents of diesel as sole carbon and energy source. Through successive enrichment cultivation, by metagenomic analysis of the diesel-degrading microbiota, they identified a consortium of 76 ASV of the 16S rRNA gene. They successfully determine the bacterial genera composition of the consortium, by taxonomic assignment of DNA sequences; representatives of *Pseudomonas*, *Aquabacterium*, *Chryseobacterium* and *Spingomonadaceae* were prevalent inside the consortium. In addition, after a shotgun metagenome sequence analysis of the consortium, different key genes encoding for enzymes involved in oxidation of alkanes or in catalytic degradation of aromatic and polyaromatic hydrocarbon degradation were identified. Using the consortium in microcosms, further rhizoremediation of polluted soils with petroleum hydrocarbons was obtained. In conclusion, the authors advantageously proposed the use of indigenous bacteria isolated from polluted soils rather than using allochthonous strains. They introduced in the consortium several specialized bacteria with

different abilities adapted to the polluted sites. In the same manner, Eze et al., (2022), through successive enrichment of microorganisms from a petroleum-polluted soil using diesel fuel as the sole carbon and energy source, successfully isolated a bacterial consortium that can degrade diesel fuel hydrocarbons. Metagenome analysis revealed the specific roles of different microbial populations involved in the degradation of benzene, toluene, ethylbenzene and xylene, and the metabolic pathways involved in these reactions. The inoculation of diesel fuel polluted soils with the consortium resulted in approximately 70 % hydrocarbon biodegradation, indicating the potential of the consortium for environmental remediation of petroleum hydrocarbons.

V. Future trends and outlooks

Progress in identification of microbiological indices from eDNA metabarcoding to provide accurate description of soil microbiome shifts due to pollutant exposure are increasing. However, for their successful and routinely integration in phytomanagement, some issues, limitations and challenges still required consideration.

1. First, the microbial indicators vary not only according to the type of pollutants and their concentrations in soil, but also by many other factors such as soil abiotic and/or biological factors (physicochemical properties, the complex network of biological interactions, seasons, agronomic practices). Therefore, their use remains complicated and it is now crucial to better understand the processes driving microbial indicators by developing interdisciplinary approaches including soil chemistry, plant physiology and plant-microbe interaction (Diaz-Vanegas et al., 2022; Laroche et al., 2021; Shah and Daverey, 2020).
2. The bioinformatic analysis of predictive soil functionalities inferred from metagenomes have usually focused on relatively few processes, such as denitrification, primary production, or organic carbon degradation. Yet, it is essential to have large-scale data sets of functional indicators that could provide information on soil functioning in order to properly assess the effectiveness of phytoremediation and improvement of the soil quality (Jung et al., 2016). Independent studies have occasionally reported contrasting results due to the high heterogeneity of pollutants, soils from different localities, soil properties, and variable experimental conditions, which result in a lack of a consensus concerning microbiota responses to pollutants (Ben-Moshe et al., 2013; Fan et al., 2017; van der Heyde et al., 2022; Zhang et al., 2018). Therefore, the lack of functionally characterized reference microorganisms of known genomic sequences, as well as the large

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proportion of genes with unknown functions in sequence databases, still limits the use of inferred functionalities for robust prediction of pollution status from taxonomic diversity. Besides, statistical multivariate analyses, classically applied to a group of variables to measure structural and functional diversity, have many technical limitations (Aylagas et al., 2017; Sims et al., 2013).

3. Last but not least, is the choice of the most sensitive and precise taxonomic indicators to help diagnose pollution, monitor progress in phytomanagement and assess ecosystem resilience. This remains a challenge to manage and analyze soil remediation efficiency. Indeed, an in depth-understanding of the merits and limitations of each index is required for each pollutant/soil of interest. To cope with such difficulties, incorporating multiple taxonomic microbial indices for the assessment of pollution status has been proposed (Aylagas et al., 2017; Simonin et al., 2019; Sims et al., 2013).

Despite the multiple challenges described above, some solutions are in progress and can improve phytomanagement process. Amongst theme, we can cite :

4. The data compilation of the diverse meta-omics sources could provide valuable information to improve the phytomanagement of polluted soils and the appropriate use of telluric microbial communities. Multi-omics analysis may shape a new area in phytomanagement research by providing complementary information into the genomic structure and metabolic potential as well as activity of microbiomes.
5. Also, there is an increasing interest in implementation of artificial intelligence (AI) algorithms, including machine learning (ML), for studies in environmental microbiology. With DNA-sequencing costs being relatively cheap, AI should in future become a relevant tool for microbiome-based diagnostic, capable of refining the choice of microbial indices to be deployed within a phytomanagement project (Ahneman et al., 2018; Ban et al., 2020; Oh et al., 2016; Wright et al., 2021; Yuan et al., 2020). Of particular interest would be the ability to predict shifting microbial activities overtime to optimize phytomanagement activity as, for instance, contaminant composition changes and culture growth. Therefore, some huge efforts of the future implementation of DNA metagenomics in biological assessment of polluted soils and routine biomonitoring programs should be extensively recommended in the future in the phytomanagement projects. Thus, these powerful approaches have helped to monitor changes in the composition of the microbiome in response to environmental stresses (Hernández Medina et al., 2022). In particular, ML has shown promising in tracing of pollutants and predictions of soil

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health metrics environmental quality (Sperlea et al., 2022; Techtmann and Hazen, 2016; Wheeler, 2019; Wilhelm et al., 2022). Also, some were used for identifying ecologically meaningful indicator taxa or microbial biomarkers in the metabarcoding or shotgun datasets from microbiomes (Ghannam and Techtmann, 2021; Hallin, 2023; McElhinney et al., 2022). For instance, Wang et al. (2020) provided guidance for the use of keystone taxa to predict ecosystem contamination after assessment by RF-ML algorithm. Also, (Ke et al., 2022) identified how pesticides affect microbial stability and keystone species using modularity and network cohesion. In line, (Andrade et al., 2021) suggest the development of a soil quality index based on soil microbiome data, crop productivity and a range of abiotic environmental factors to improve crop production systems using AI.

Conclusion

The progress in eDNA metabarcoding or shotgun metagenomics technologies in providing accurate description about soil microbiome, emerge as promising method and of much value in the identification of microbiological indices for biological assessment/monitoring and rehabilitation of polluted soils and ecosystem restoration. However, significant progress in terms of efficiency is still needed to achieve higher levels of environmental and economic sustainability and for routine use of the methods in various sites. However, recently, As well, taxonomy-based investigations and inferred-functions from metagenomes open new perspectives to enhance phytomanagement efficiency through for the production of adapted autochthonous microbial inoculums thanks to the discovery of relevant microorganisms with potential in bioremediation and/or plant growth promotion for biomass production.

Adoption of microbial metabarcoding and shotgun metagenomics data in soil phytomanagement projects with the identification of well-validated microbial indices or isolates is therefore an important field of research to invest in the future.

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Problématique et objectifs

Problématique et objectifs

Au travers de cette analyse bibliographique, il apparait que parmi les méthodes émergentes de gestion des SSP, la phytostabilisation aidée permet d'une part, de limiter les flux de polluants dans l'écosystème sol-plante et d'autre part, de rétablir la dynamique et la fonctionnalité des sols pollués en favorisant leur résilience. Cependant, la rentabilité socio-économique de cette phytotechnologie constitue l'un des principaux freins à son développement. C'est pourquoi la valorisation de la biomasse végétale produite sur ces terres agricoles polluées est envisagée. En effet, elle permettrait d'abaisser les coûts globaux de gestion et de requalification de ces sites, tout en répondant aux attentes environnementales et sociales. Parmi les filières non-alimentaires présentant une rentabilité élevée, la filière PPAM-HE, qui allie la phytostabilisation aidée des ET, et la production de molécules à haute valeur ajoutée, les HE, semble être une option prometteuse (Raveau et al., 2021a). Afin d'optimiser les performances de la phytostabilisation, diverses stratégies, dont l'utilisation d'amendements organiques, inorganiques et biologiques, ont été développées ces dernières années (Sarwar et al., 2017). Outre la réduction de la mobilité et de la biodisponibilité des polluants, ces amendements ont pour effet de favoriser l'installation et le développement du couvert végétal (Shah and Daverey, 2020) et parfois d'améliorer la qualité biotique du sol.

En effet, la qualité biologique des sols, qui fait référence à l'abondance, à la diversité et à l'activité des organismes vivants, est généralement affectée dans les sols agricoles et de surcroît pollués par les ET (Chen et al., 2016; Ding et al., 2017; Wang et al., 2018). Nous savons que le microbiote du sol constitue un élément capitale de la vie et du fonctionnement des sols (Delgado-Baquerizo et al., 2020; Saccá et al., 2017). Les communautés microbiennes du sol sont aussi influencées par la présence d'un couvert végétal (Dagher et al., 2019; Singh et al., 2009). Par conséquent, la végétalisation du sol par la culture de PPAM pourrait contribuer au maintien ou à la requalification écologique de ces sols. Cependant, les effets de la végétalisation dans le cas de cultures de PPAM ont été très peu étudiés et restent donc méconnus, en particulier dans un contexte de pollution des sols par les ET.

L'objectif général de mon projet de thèse, consiste donc développer et optimiser la filière PAM-HE initiée dans le cadre d'un précédent projet (PhytEO) en (i) poursuivant les essais *in situ* de mise en culture de l'angélique, (ii) améliorant les rendements de biomasse et du processus de phytostabilisation en testant différents amendements minéraux, issus de l'économie circulaire (utilisation de sous-produits issus de certaines activités locales comme les laitiers sidérurgiques) et biologiques à base d'inoculum mycorhiziens, (iii) en étudiant les propriétés biologiques des HE issues de la biomasse végétale produite sur un sol pollué afin de proposer des voies de valorisation dans le domaine de la protection des cultures. Enfin, il s'agira également (iv) d'étudier la résilience écologique du sol pollué grâce à l'installation de ce couvert végétal.

Ce travail s'articule donc autour de trois questions :

1. *L'angélique constitue-t-elle un bon candidat pour le phytomanagement des sols pollués par les ET ?*

La présence d'ET dans le sol affecte-elle le développement, les rendements et la qualité des HE produites ?

L'introduction d'inoculum mycorhizien dans le sol influence-t-elle la croissance de l'angélique, sa production de biomasse, les rendements ou la qualité de ses HE ?

La filière PPAM-HE sur sols pollués par les ET est-elle économiquement viable ?

2. *Quelle est la qualité des HE d'angélique produites sur les sols pollués par les ET, sont-elles valorisables dans des domaines non alimentaires telles que la protection des cultures ou la santé humaine ?*

La composition ou la qualité des HE est-elle modifiée par la présence d'ET ou l'inoculation mycorhizienne ?

Quelles sont les propriétés biologiques des HE d'angélique, sont-elles applicables dans le domaine de la protection des cultures (antifongique, herbicide) et/ou de la santé humaine ou animale (anti-inflammatoire, antioxydante) ?

3. *Quel est l'apport de la culture de PPAM (coriandre ou angélique) et de l'introduction d'amendements minéraux (laitiers sidérurgiques), ou biologique (inoculum mycorhizien) sur la requalification écologique des sols pollués par les ET ?*

Quelle est la composition et la structure des communautés microbiennes rhizosphériques de sol agricole pollué par les ET ?

Qu'apportent la végétalisation du sol par les PPAM ou l'introduction des amendements sur la biomasse microbienne, le potentiel métabolique du sol ou la structure des communautés microbiennes du sol ?

Qu'en est-il des co-occurrences microbiennes après végétalisation du sol et introduction d'un inoculum mycorhizien dans le sol ?

Partie 2 : Site d'étude et choix des espèces végétales

Site d'étude

Ce travail repose sur une parcelle expérimentale, située à Evin Malmaison (Coordonnées GPS : 3°02'25.5", 50°25'55.5") dans le périmètre de l'ancienne fonderie Metaleurop (Figure 1), caractérisée par des teneurs en Cd (7 ppm), Pb (376 ppm) et Zn (429 ppm), dépassant respectivement de 17, 11 et 6 fois le fond géochimique régional (Sterckeman et al., 2007).

Dispositifs expérimentaux *in situ* utilisés dans le cadre de ma thèse :

Une parcelle d'angélique de 7 100 m²

Cette parcelle a été divisée en deux parties (Figure 4) : une parcelle de 2 800 m² inoculée par un amendement mycorhizien (Agtiv[®] cultures spécialisées, Premier tech, *Rhizophagus irregularis*) apporté sous forme de poudre (125 g/ha) et appliqué au moment du semis par enrobage des graines, ainsi qu'une parcelle non inoculée de 4 300 m². Ces parcelles sont séparées de 2 m afin d'éviter la propagation du champignon mycorhizien arbusculaire dans la parcelle non inoculée.



Figure 2 : Parcelles expérimentales d'Evin Malmaison, dispositif angélique. NI : cadre jaune, I : cadre rouge.

Un dispositif en microparcelles (coriandre) «amendements minéraux et biologique»

Un dispositif en microparcelles, reposant sur l'utilisation d'amendements minéraux et biologiques a été utilisés dans le cadre de cette thèse.

Ce dispositif de microparcelles a été mis en place en 2019. Il comporte 20 zones de 9 m² (3 m x 3 m) (Figures 3 & 4) avec différentes conditions croisées : végétalisées ou non avec la coriandre, deux types de laitiers sidérurgiques (laitiers de poche et de four ; composition cf. Annexe 1), deux concentrations testées (1,5 t/ha - NF U 44-001, contexte agricole, et 45 t/ha - contexte de

Site d'étude

phytostabilisation), inoculées ou non par un amendement biologique composé de champignons mycorhiziens utilisé à la dose de 10 kg/ha (Symbivit® - INOCULUM plus, Bretenière, France) et contenant six espèces de CMA : *Rhizophagus irregularis*, *Funneliformis mosseae*, *Glomus microaggregatum*, *Glomus claroideum*, *Claroideoglomus etunicatum* et *Funneliformis geosporum*.

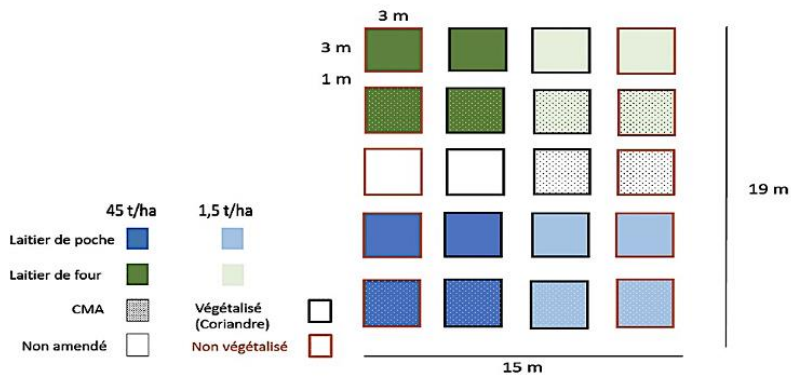


Figure 3 : Dispositif en microparcelles « amendements minéraux et biologique »



Figure 4 : Mise en place des microparcelles amendements minéraux

I. Les plantes aromatiques (PAM) utilisées dans le cadre de cette thèse

Au sein du règne végétal, les PAM comprennent une grande variété d'espèces cultivées ou sauvages. En raison de leurs propriétés intéressantes, les PAM ont de nombreuses utilisations possibles, dans divers domaines tels que la parfumerie, les produits cosmétiques et la pharmaceutique. Le secteur des PAM se divise ainsi en trois branches complémentaires, en fonction de leurs usages : la filière des plantes à parfum, celle des plantes aromatiques et condimentaires, et enfin, la filière des plantes médicinales. Dans le cadre de ce travail, deux PAM ont été testées :

L'angélique

L'angélique (*Angelica archangelica* L.) est une PAM originaire du nord de l'Europe, appartenant à la famille des Apiacées (Chauhan et al., 2016). Elle est utilisée dans les pays nordiques comme plante médicinale depuis l'Antiquité et a voyagé vers le sud avec les moines lorsque la Scandinavie a été christianisée (Karlsson Strese 2008) entre 829-830 et 1100 après J.-C. (Gräslund 1994). L'angélique est un membre de la famille des Apiaceae, qui contient de nombreuses plantes potagères aromatiques connues. Outre les caractéristiques aromatiques, la principale caractéristique des Apiacées est la structure de l'inflorescence en ombelle (Jacobsen & Jensen 1999b) et sa taille qui peut atteindre jusqu'à trois mètres de haut (Hegi & Beger 1926, Starý & Jirásek 1999, Lindholm 2003, Mossberg & Stenberg 2003). L'angélique apprécie un sol humide et nutritif (Kylin, 2012), bien qu'elle soit capable de se développer dans des sols très pauvres (Chauhan et al., 2016). Les graines possèdent un pouvoir germinatif relativement faible, mais germent dès que les conditions sont favorables, permettant de cultiver l'angélique, par re-semis, sur des cycles relativement longs, pouvant atteindre jusqu'à 10 ans (Rautio et al., 2016). Ses racines sont épaisses et présentent une coloration intérieure blanche voire jaune clair (Bhat et al., 2011). À l'extrémité de la tige, de couleur rougeâtre, l'inflorescence est une ombelle composée, portant 20 à 40 fleurs de petite taille et de couleur blanche ou jaune verdâtre (Figure 2) (Ghedira and Goetz, 2014; Joshi, 2016). La période de floraison s'étend de juillet à août mais peut varier en fonction de la localisation géographique (Pop et al., 2011; Teixidor-Toneu et al., 2020). Pendant son cycle de développement, l'angélique émet un parfum caractéristique agréable, émanant aussi bien des parties aériennes que souterraines. La plante est principalement cultivée pour sa racine aromatique, valorisée pour les traitements de divers soucis de santé (migraines, bronchites, fatigue chronique, etc.), en alimentaire et dans la fabrication de liqueurs (Chalcha and Garry, 1997; Chauhan et al., 2016). Elle est également recherchée pour son HE, produite dans l'ensemble des organes de la plante (Roslon et al., 2016). Toutefois, les HE provenant des racines et des graines sont celles affichant

Plantes aromatiques étudiées

le plus de valeur. Il est à noter également que les graines contiennent en proportion davantage d'HE, de l'ordre de 0,6 à 1,5 %, en comparaison des 0,5 à 1 % affichés pour les racines (Sowndhararajan et al., 2017; Zitterl-Eglseer et al., 2015). Les graines peuvent être récoltées à partir de la deuxième année, avec un rendement affiché pouvant varier de 1 à 2,5 tonnes par hectare, sur des sols agricoles non pollués (Sowndhararajan et al., 2017).

(A.)



(B.)



Figure 5 : Photographies de l'ombelle composée en fleurs (A.) et à la maturité des graines (B.). Photographes : Robin Raveau et Anissa Lounès-Hadj Sahraoui.

La coriandre

Coriandrum sativum L., plus communément connue sous le nom de coriandre, est une plante annuelle herbacée appartenant à la famille des Apiacées (Bhuiyan et al., 2009; Freires et al., 2014). Toutes les parties de la plante sont comestibles, mais ce sont les feuilles fraîches et les graines séchées qui sont le plus souvent utilisées (Mandal and Mandal, 2015). Son feuillage vert, qui contient des protéines, des vitamines et des minéraux (comme le calcium, le phosphore et le fer), des fibres et des hydrates de carbone, est utilisé comme condiment dans les salades. La coriandre a été utilisée par Hippocrate (460-377 av. J.-C.) dans les médicaments grecs traditionnels. La décoction et la teinture de graines de *C. sativum* réduites en poudre, seules ou en association avec d'autres plantes, sont recommandées pour

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les troubles dyspeptiques, la perte d'appétit, les convulsions, l'insomnie et l'anxiété (Mandal and Mandal, 2015). De petite taille, atteignant communément 30 à 40 cm de hauteur, elle possède un cycle de développement rapide, fleurissant 45 à 60 jours après semis et atteignant la maturité au bout de 65 à 120 jours (Mandal and Mandal, 2015; Sharma and Sharma, 2012). Les feuilles les plus élevées sont fines et délicatement divisées, tandis que les feuilles plus basses sont plus larges, non divisées et à trois lobes (Figure 3A.). Chaque tige se termine en ombelle, portant chacune 10 à 50 fleurs, souvent de couleur blanche (Figure 3B.) (Sharma and Sharma, 2012).

(A.)



(B.)



Figure 6 : Photographies de la coriandre lors de la croissance des parties aériennes (A.) puis à la floraison (B.). Photographes : Robin Raveau et Anissa Lounès-Hadj Saharaoui.

La coriandre est également connue pour son HE, traditionnellement extraite par hydrodistillation à partir des graines de coriandre, avec des rendements de l'ordre de 0,4 à 0,6 % (Bandoni et al., 1998; Bhuiyan et al., 2009; Sharma and Sharma, 2012). Toutefois, l'HE peut également être distillée à partir des parties aériennes de la coriandre, avec des rendements plus faibles variant entre 0,1 et 0,3 %, et possédant une composition chimique ainsi que des propriétés biologiques distinctes (Bhuiyan et al., 2009; Freires et al., 2014).

Historiquement utilisée en tant qu'arôme en agro-alimentaire, l'HE issues des graines de coriandre est également utilisée en aromathérapie pour ses propriétés médicinales (analgésique, digestive, antispasmodique et anti-rhumatique) et est reconnue pour ses propriétés antimicrobiennes et antioxydantes (Ghobadi and Ghobadi, 2010; Mandal and Mandal, 2015; Shahwar et al., 2012). La

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coriandre a également été évaluée, parmi d'autres plantes aromatiques telles que l'aneth, le basilic ou la camomille, pour sa capacité à se développer sur un milieu pollué par des ET (Zheljazkov et al., 2008).

Partie 3 : Résultats et discussions

Publication 1

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Relevance of Angelica use for essential oil production in a phytomanagement strategy: a three-year field experiment on an aged TE-contaminated agricultural soil

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Présentation de la publication 1

Parmi les nombreux polluants détectés dans les sites et sols pollués, les ET font l'objet d'une attention particulière en raison de leurs effets néfastes sur l'environnement mais également sur la santé humaine (Jing et al., 2023). La requalification des sites contaminés par les ET représente donc un enjeu majeur à l'échelle mondiale.

Actuellement, la dépollution des sols pollués fait appel principalement à l'excavation et/ou aux méthodes physico-chimiques conventionnelles. Celles-ci sont coûteuses, entraînent la perte des fonctions du sol (Kumpiene et al., 2019) et ne sont pas adaptées à de grandes superficies telles que les parcelles agricoles (Raveau et al., 2021a). Ainsi, parmi les solutions de réhabilitation douces et vertes qui sont actuellement en cours de développement, figure le phytomanagement. Ce dernier consiste en une gestion intégrée des sites et sols pollués, reposant sur la mise en place *in situ* d'un couvert végétal visant à : (i) réduire le risque de toxicité des sols pollués en immobilisant les éléments traces (ET) dans la rhizosphère (phytostabilisation), (ii) permettre la réhabilitation écologique des sols pollués en augmentant la biodiversité et l'activité microbienne du sol qui est à l'origine de nombreux services écosystémiques et, (iii) redonner un bénéfice socio-économique aux sites pollués en valorisant la biomasse dans des secteurs non alimentaires, via le développement de filières éco-responsables (Burgess et al., 2018; Cundy et al., 2016; Mench et al., 2018; Shah and Daverey, 2020).

Parmi ces phytotechnologies, la phytostabilisation est bien adaptée aux sols pollués par les ET (Barbosa and Fernando, 2018). Bien que cette méthode n'élimine pas les ET, elle peut les inactiver ou les immobiliser en réduisant leur biodisponibilité et leur dispersion dans les différents compartiments de l'environnement (Kidd et al., 2015; Shah and Daverey, 2020). (Epelde et al., 2014).

En outre, l'efficacité de la phytostabilisation pourrait être améliorée par l'utilisation d'amendements appropriés afin d'améliorer l'immobilisation des ET, et/ou de faciliter l'établissement et la croissance des plantes (Barbosa and Fernando, 2018). À cet égard, l'utilisation d'amendements biologiques, tels que les champignons mycorhiziens à arbuscules (CMA), s'est avérée efficace pour améliorer la croissance des plantes et leur tolérance aux polluants (Israel et al., 2022; Vilela, 2020).

L'efficacité du phytomanagement dépend également de l'espèce végétale utilisée. Dans ce contexte, la culture de plantes aromatiques et médicinales, destinée à la production d'huiles essentielles (HE) a été récemment présentée comme une alternative innovante et économiquement viable pour le phytomanagement des sols pollués (Gupta et al., 2013; Raveau et al., 2021, 2022).

Présentation de la publication 1

Ainsi, dans le cadre de cette première publication, nous avons étudié le potentiel de l'angélique, à produire des HE à partir des graines lorsqu'elle est cultivée sur un sol pollué par les ET sous deux conditions, inoculée (I) ou non (NI) avec *Rhizophagus irregularis* (AGTIV[®], cultures spécialisées, Premier Tech, 125g/ha). L'angélique est une espèce bisannuelle pouvant être cultivée pendant une dizaine d'années (par re-semis lors de la récolte) sur la même parcelle (Aćimović et al., 2022). Cette étude a été réalisée sur une parcelle agricole de 7 100 m² située à Evin-Malmaison, à environ 500 m de l'ancienne fonderie de plomb et de zinc de Metaleurop. La culture y a été suivie pendant quatre années successives afin de dresser un bilan technico-socio- économique de la filière angélique-HE. Le semis a été réalisé en 2018 (état initial) et les premières récoltes ont eu lieu en juin 2020, 2021 et 2022.

Cette étude avait pour objectif de répondre à plusieurs questions : (1) L'angélique est-elle capable de se développer sur un sol fortement pollué par les ET et de produire des quantités suffisantes de biomasse et d'HE ? (2) L'inoculation mycorhizienne améliore-t-elle les rendements en biomasse et/ou en HE ? (3) Quelle est la qualité de l'HE produite (contaminations métalliques et de résidus pesticides) ? (4) La culture de l'angélique et l'inoculation mycorhizienne influencent-elles la biomasse et l'activité bactérienne du sol pollué ? (5) Quel est l'impact de quatre années de culture d'angélique sur l'écotoxicité du sol ? (6) La filière de production d'HE d'angélique sur un sol agricole fortement pollué par la ET est-elle économiquement viable ? (7) Enfin, qu'en est-il de l'acceptabilité sociale pour le consommateur final de ces HE produites sur des sols pollués ?

Les caractéristiques pédo-agronomiques de ce sol ayant déjà été décrites par Raveau *et al.* (2021), nous avons commencé ce travail en évaluant les paramètres de croissance de l'angélique. Nos résultats montrent que l'angélique est capable de se développer sur des sols fortement pollués par les ET (7 ppm de Cd, 394 ppm de Pb et 443 ppm de Zn) dépassant largement (respectivement 17, 11 et 6 fois) le fond géochimique régional, ce qui démontre la tolérance de l'angélique aux ET. En effet, l'angélique atteint des hauteurs de 158 à 187 cm et des biomasses sèches de 483 à 517 g/plante, comparables à celles observées chez des plantes cultivées sur des sols non pollués (Kylin, 2010).

Bien qu'une mycorhization spontanée de l'angélique ait été mise en évidence, le taux de mycorhization de l'angélique était significativement supérieur dans la parcelle I par comparaison à la parcelle NI après quatre années de culture. L'inoculation mycorhizienne a eu un effet positif sur la biomasse aérienne de l'angélique, mais aucun gain n'a été observé sur les rendements en graines et en HE. Les rendements en HE issues des graines d'angélique, compris entre 1,08 et 1,93 kg/ha sont inférieurs à ceux décrits dans la littérature, et sont sensiblement comparables dans la modalité inoculée ou non.

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Par ailleurs, la qualité des HE obtenues a été vérifiée en y recherchant la présence éventuelle d'ET et de résidus de pesticides. Nos résultats démontrent que les HE ne contiennent que des traces ou de faibles teneurs inférieures aux limites de quantifications, que ce soit pour les résidus de pesticides ou les ET. De plus, les teneurs en ET et résidus de pesticides des HE d'angélique produites sur un sol pollué ne sont pas différentes d'une HE d'angélique commerciale produite sur un sol non pollué. Ces teneurs ne semblent donc pas être limitantes pour une valorisation de ces HE dans des filières non alimentaires telles que la protection des cultures ou la santé humaine.

Une augmentation significative de la biomasse bactérienne et de l'activité métabolique globale bactérienne, corrélées au temps de culture d'angélique ont été mise en évidence. Enfin, nous avons montré que l'écotoxicité du sol diminuait après quatre années de culture de l'angélique.

L'étude de viabilité économique a révélé un bilan positif, avec le dégagement d'une marge brute d'environ 814 €.ha⁻¹. Cependant ce rendement pourrait être amélioré et atteindre un maximum de 4,550 €.ha⁻¹ en optimisant les pratiques culturales et/ou la récolte des graines. Enfin, l'étude d'acceptabilité sociale indique clairement qu'il n'y a pas de refus massif d'utiliser des substances produites sur des sols pollués, et ce d'autant plus pour des usages non alimentaires et qu'une minorité de personnes montrent une importante défiance à l'égard de cette nouvelle filière.

Dans le cadre de cette étude, je me suis chargé du suivi des cultures, de l'évaluation des paramètres de croissance de l'angélique et des taux de mycorhization racinaire, des calculs de rendements et du dosage de la biomasse bactérienne. J'ai également réalisé l'ensemble des analyses statistiques. L'analyse des ET et des résidus de pesticides dans les HE a été sous-traité dans des laboratoires d'analyses. Les tests d'écotoxicité ont été réalisés en collaboration avec A. Verdin (IR, équipe TCEA-UCEIV). Les semis, ont été réalisés avant mon arrivée en thèse à l'UCEIV. La distillation des HE a été effectuée par P. Ferrant (Ferrant PHE). L'analyse technico-économique a été réalisée par moi-même, en collaboration avec P. Ferrant. L'analyse d'acceptabilité sociale a été réalisée par H. Flanquart (TVES – ULCO). J'ai également rédigé cette publication avec l'aide des co-auteurs.

Publication 1 : *Relevance of Angelica use for essential oil production in a phytomanagement strategy: a three-year field experiment on an aged TE-contaminated agricultural soil*

Abstract

Phytomanagement combine the selection of optimal phytotechnologies to mitigate risks associated with soil toxicity and a strategy for valorizing biomass produced on the polluted soil. This three-year study aims at establish a high value-added chain by cultivating angelica (*Angelica archangelica* L.), an aromatic plant, *in situ* on an aged trace elements (TEs) polluted soil (7 ppm Cd, 394 ppm Pb, and 443 ppm Zn) in order to produce essential oils (EO). We assessed the impact of soil vegetation with angelica on bacterial biomass, metabolic potential, and soil toxicity over 3 years of cultivation, compared with the initial state (pre-cultivation), and investigated the influence of mycorrhizal inoculation on plant biomass and EO yield. Additionally, we examined the economic viability and social acceptability of this high-value-added chain. Our results demonstrated that angelica thrived on heavily TE-polluted soil, yielding up to 1.9 kg/ha of EO. Analyses of TE and pesticide residue revealed that EO distilled from angelica seeds contained negligible pollutants, below or close to the detection limits, comparable to commercial EO. The vegetation of the soil with angelica for 3 years significantly increased total bacterial biomass of the polluted soil, and also significantly increased the bacterial metabolic potential compared with the initial state. While mycorrhizal inoculation did not improve angelica biomass yields, it significantly increased the mycorrhizal rate of angelica, and contributed to reducing soil ecotoxicity after three years of cultivation. Moreover, the social acceptability study indicated that the production of EO from angelica seeds on the heavily TE-polluted soil garnered favorable reception from the majority of the public. The positive economic balance of this biomass sector underscores additional arguments for its great potential and for its development within phytomanagement contexts.

Introduction

Originating from anthropic activities, trace elements (TEs) are among the most prevalent pollutants in soil (Mench et al., 2018). Due to their non-biodegradability and toxicity, these pollutants are harmful to both animal and human health (Wang et al., 2021). Indeed, TEs interact with cellular components such as DNA and nuclear proteins, leading to cell cycle modulation, carcinogenesis, or apoptosis (Beyersmann and Hartwig, 2008; Leonard et al., 2004; Wang and Shi, 2001). Currently, conventional physico-chemical soil remediation methods are frequently employed to manage such polluted soils. However, these methods are expensive, lead to the loss of soil functions (Kumpiene et al., 2019; Verma et al., 2017), and may not be suitable for large areas such as agricultural lands (Raveau et al., 2021). Among gentle and green remediation solutions currently in development, phytomanagement, a combination of phytotechnology with sustainable and cost-effective land and biomass use, is gaining traction (Burges et al., 2018; Cundy et al., 2016; Mench et al., 2018; Shah and Daverey, 2020). Within phytotechnologies, phytostabilization is becoming increasingly widespread (Barbosa and Fernando, 2018). Although this method does not eliminate TEs, it can inactivate or immobilize them by reducing their bioavailability or leaching and soil erosion (Kidd et al., 2015; Shah and Daverey, 2020). Additionally, TE-polluted soils could regain economic interest through plant biomass valorization in non-food sectors (Sugawara and Nikaido, 2014) while improving ecosystem services provided by soils and limiting the impact of pollution on the environment and human health (Burges et al., 2018; Cundy et al., 2016; Mench et al., 2018). Nowadays, the aim of any soil metal management method is no longer simply to remove metals from the soil or limit their harmful effects, but also to restore soil quality (Epelde et al., 2014). From this perspective, soil microbial parameters such as the microbial biomass or the metabolic potential of soil microbial communities are used as biological indicators to estimate phytomanagement effectiveness on the biotic quality of the phytomanaged soils (Fontaine et al., 2022). Furthermore, phytostabilization efficiency could be improved by using suitable amendments to enhance TE immobilization and/or facilitate plant establishment and growth (Barbosa and Fernando, 2018; Langrand et al., 2023). In this regard, the use of biological amendments, based on beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) inoculum, has been demonstrated to be effective in enhancing plant growth and their pollutant tolerance (Israel et al., 2022; Vilela, 2020). These fungi form beneficial symbiotic relationships with more than 80% of plant species, resulting in improved water and mineral nutrition, particularly phosphorus and nitrogen uptake (Smith and Read, 2008). In addition, AMF produce various compounds such as metallothioneins, siderophores, glomalin, and organic acids (*e.g.* oxalic acid, malic acid, and succinic acid) that improve soil structure and reduce metal bioavailability (Cicatelli et al., 2014; Ferrol et al., 2016; González-Chávez et al., 2004; Vilela, 2020).

Moreover, the success of the phytotechnology relies on the careful selection of appropriate plant species (Burgess et al., 2018). These selected plant species must thrive in TE-polluted conditions and produce sufficient biomass for valorization in non-food value chains (Ansari et al., 2015), such as bioenergy, eco-materials, or high value-added products (Ansari et al., 2015; Raveau et al., 2021). Hence, medicinal and aromatic plants (MAP) capable of adapting to various soil types with minimal chemical inputs can be a viable option for TE-polluted soil phytomanagement (Raveau et al., 2021; Israel et al., 2022). Indeed, certain MAP species offer the potential to produce essential oils (EO) with high added value, finding applications across perfumery, biocontrol, cosmetics and medicine (Lydaki-Simantiris et al., 2016; Raveau et al., 2022). Moreover, the global demand for EO is steadily increasing and is projected to reach \$24.7 billion by 2030 (Samriddhi and Roshan, 2022). Previous studies have demonstrated the technical feasibility and relevance of cultivating MAP species such as coriander and clary sage on TE-polluted soil, notably highlighting the profitability of sage (Raveau et al., 2021). However, the effects of TE pollution on the growth, biomass production, and EO quality of the aromatic plant angelica (*Angelica archangelica* L.) have been poorly studied, with limited information on its use in TE-polluted soil phytomanagement. Angelica, a biennial plant belonging to the Apiaceae family, is native to northern Europe and Asia (Chauhan et al., 2016; Kylin, 2012). While historically valued for its medicinal properties since the Middle Ages, angelica is now also used for flavoring and perfumery purposes (Kylin, 2012; Rautio et al., 2016). In addition, angelica boasts several advantages, including high biomass production, soil coverage capabilities, and a crop lifespan of approximately ten years without the need for resowing. This minimizes agricultural intervention costs and enhances the potential profitability of the crop.

Therefore, the aim of the current study was to evaluate the relevance and the socio-economic feasibility of the EO production chain, derived from angelica seeds cultivated on a TE-polluted agricultural soil *in situ* over three consecutive years. This investigation seeks to address the following questions: (1) Can angelica thrive on TE-contaminated soil and yield sufficient biomass and EO? (2) Does AMF inoculation improve biomass and/or EO yields? (3) Are the EO quality standards met (regarding metallic and pesticide contaminations)? (4) Does angelica cultivation and mycorrhizal inoculation reduce soil ecotoxicity and improve bacterial activity in the polluted soil? (5) Is the EO production chain of angelica economically viable on heavily TE-polluted agricultural soil? (6) Finally, what is the social acceptability of EO produced on polluted soils among consumers?

Materials and methods

1. Experimental site

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This study was conducted on a 7,100 m² aged TE-polluted site (50°25'55.5" N, 3°02'25.5" E; elevation 23 m) in the northern France, situated approximately 500 meters away from a former Pb and Zn smelter (Metaleurop Nord). The smelter's operations produced large volumes of TE-rich dust, leading to TE-pollution of the topsoil (0–30 cm) (Sterckeman et al., 2002). High amounts of Cd, Pb and Zn (7, 394, and 443 ppm, respectively) (Raveau et al., 2021) were detected, with concentrations approximately 17-, 11-, and 6-fold higher than regional background levels for agricultural soils (Sterckeman et al., 2007). The soil, classified as a silt loam, exhibits slightly alkaline pH (7.86) and high agronomic value with a C/N ratio of 10.5, organic matter content of 29 g/kg, and nitrogen (N) content of 1.62 g/kg (Raveau et al., 2021).

2. Experimental Setup

Angelica (*Angelica archangelica* L.), a perennial aromatic plant species, was sown in September 2018 at a seeding rate of 300,000 seeds per hectare and cultivated for 3 years on the polluted site. The seeds were purchased from the French research institute ITEIPMAI, ensuring their certification. The experimental setup spanned an area of 7,100 m² and included two conditions: non-inoculated (NI) and inoculated (I) with the mycorrhizal inoculum AGTIV[®] (Specialty Crops, PremierTech) containing the AMF *Rhizophagus irregularis* at a concentration of 12,000 viable spores per gram of product. The mycorrhizal inoculum was introduced during sowing, directly mixed with the seeds, and applied at a rate of 125 g/ha.

3. Determination of angelica emergence rates

The emergence of the angelica seedlings was assessed *in situ* for each condition (NI and I) six weeks after sowing. Seedling emergence was visually counted within a one square meter quadrat at three randomly selected positions for each condition (Gunaratne et al., 2010). The positions of the seedlings were georeferenced (TDC100[®], Trimble) for accurate spatial mapping.

4. Weed control

Annually, to manage the spontaneous growth of both gramineous and dicotyledonous species, two herbicide applications were conducted with a three-week interval, utilizing half doses of the chemicals as per ITEIPMAI guidelines (Table S1). No fungicidal or insecticidal treatments were applied.

5. Soil and plant sample collections

At the beginning of the experiment in 2018, 6 soil samples representing the initial conditions (3 NI and 3 I) were collected and stored at - 20°C until analysis. Since angelica follows a biennial development cycle, the first seed harvest took occurred in 2020. Each year thereafter (2020, 2021, and 2022), 12 soil samples were collected, 6 from the NI condition and 6 from the I condition. To cover completely the experimental conditions, all soil samples were obtained using a stratified sampling method, with an

auger, from the 0 – 20 cm soil horizon. Each sample was geo-referenced (TDC100[®], Trimble) and stored at - 20°C until analysis. Additionally, at the location of each soil sample, a whole plant was harvested (aerial and root parts). The height stem top diameter of the aerial parts were measured. Subsequently, the plants were dried and weighed, while the fresh roots were washed and stored at 4°C until stained for determining the root mycorrhizal rate.

6. Determination of root mycorrhizal rate

The roots were cleared in 10 % (w/v) KOH and stained with 0.05 % (w/v) trypan blue (Thermo Scientific Chemicals) to determine root mycorrhizal rates (Mc Gonigle et al., 1990; Phillips and Hayman, 1970). Root fragments were mounted on microscopic slides, and mycorrhizal structures (arbuscules and vesicles) were counted under a 20x objective lens. Three counts per fragment were performed, and the total mycorrhizal rate was calculated as the number of intersections with mycorrhizal roots multiplied by 100 and divided by the total number of intersections.

7. Angelica seed harvesting

Angelica seeds were harvested using a combine (Dominador 108 SL Maxi, Claas) during the summer when the seeds reached maturity (June 2020, 2021, and 2022). Seed yields were determined based on the following equation (1):

$$\text{Seed yield (kg/ha)} = \frac{\text{Angelica seed amount (kg)}}{\text{Plot surface (ha)}}$$

8. Angelica seed EO distillation

On the day of harvest, the seeds were loaded into a distillation tank (14 m³) along with water steam heated over a wood fire and injected at a pressure of 0.3 bar. The vapors generated were then condensed in a cooling unit, and a receiver/separator was employed to collect the distillate product. The distillation process was repeated in three-hour cycles until no more EO could be extracted. The EO yield obtained after distillation was calculated and expressed as distillation yield. The calculation was based on the following equation (2):

$$\text{Distillation yield (\%)} = \frac{\text{Essential oil amount (kg)}}{\text{Angelica seed amount (kg)}} \times 100$$

The agronomic EO yield was calculated based on the amount of EO produced per hectare and expressed using equation (3):

$$\text{EO yield (kg/ha)} = \frac{\text{EO amount (kg)}}{\text{Plot surface (ha)}}$$

9. Trace element assessment in EO

The EO extracted from angelica seeds was divided into 5 mL portions and subjected to TE analysis using ICP-MS for (Agilent 7500). The analysis process involved mixing 0.5 g of EO with 10 mL of HNO₃ and 3 mL of pure water, followed by digestion in a Mars Xpress microwave oven (CEM) at 180°C for 20 minutes. To assess the quality of angelica EO produced in TE-polluted soil, a sample from commercial EO (Ferrant PHE, France) was also analyzed as control. Each TE analysis was performed three times to ensure accuracy.

10. Analysis of pesticide residues in EO

Multi-residue analyses targeting about 480 active substances were performed by GC-MS/MS (method MOC3/05(S1), version 0) or LC-Q-TOF (detection limit 0.01 mg.kg⁻¹) (method MOC3408(S1), version 2), by Phytocontrol Analytics - France.

11. Phospholipid fatty acid (PLFA) analysis

The analysis of soil fatty acids followed the protocol described by Langrand et al. (2023). Briefly, 3 g of freeze-dried soil was utilized for fatty acid analysis. Lipid extraction was conducted using a mixture of 1 : 2 : 0.8 (v/v/v) chloroform:methanol: 0.15 M citrate buffer (pH 4.0), with stirring for 2 h (Frostegård et al., 1991). The lipid material was fractionated using solid-phase extraction columns (SPE, Interchim, Montluçon, France) through successive elutions with 1:2:1 (v/v/v) chloroform, acetone, and methanol to isolate neutral lipids (NLFA), glycolipids, and polar lipids containing phospholipid fatty acids (PLFA). Following transesterification with 0.2 M KOH in methanol, the resulting fatty acid methyl esters were analyzed using a Shimadzu QP-2010 Ultra gas chromatographic mass spectrometer (GC-MS) (Shimadzu, Japan) equipped with a single quadrupole mass detector and simultaneously coupled to a flame ionization detector (FID). Samples were analyzed in split mode (80: 1 ratio) on a ZB-1MS fast capillary column (10 m length × 0.1 mm inner diameter × 0.1 μm phase thickness, 100% dimethylpolysiloxane, Zebron, Phenomenex, Torrance Calif, CA, USA) using helium as the carrier gas with a constant linear velocity (40 cm s⁻¹). Fatty acid quantification was performed using nonadecanoic acid methyl ester (C19:0, Sigma-Aldrich) as an internal standard. Identification of fatty acids was achieved by comparing their relative retention times with those of commercial fatty acid methyl ester standards (C4-C24:1, Sigma-Aldrich), and referencing spectra from commercial standards and/or literature (NIST Standard Reference Database). Phospholipid fatty acids (PLFA) i15:0, a15:0, i16:0, i17:0, a17:0, and PLFA cy17:0, C18:1ω7, and cy19:0 were used as indicators of Gram-positive and negative bacterial biomass, respectively (Frostegard and Baath, 1996).

12. Ecotoxicity assessment

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The toxicity towards the photobacterium *Aliivibrio fischeri* was measured using the BioTox™ LumoPlate™ Ultimate Matrix kit (EBPI, Mississauga, Ontario, Canada), a widely utilized commercial bioassay in ecotoxicological research and risk assessment of water, sediment, and soil samples. Briefly, sample toxicity was evaluated using a Varioskan™ LUX multimode microplate reader (Thermoscientific, Fisher Scientific SAS, Illkirch, France) by quantifying the inhibition of bioluminescence emitted by the bacteria after a 30-min contact time. Each sample (all tested in triplicate), was mixed with 8 mL of sample diluent for 5 minutes (LumoPlate™ Ultimate Matrix kit, reagent 1243-125). Subsequently, serial dilution were prepared for each sample to obtain concentration-dependent differences in inhibitory effects, which were used to calculate the EC₅₀ (effective concentration causing 50 % inhibition of light output). EC₅₀ values were determined based on the bioluminescence of *A. fischeri* using 7 dilutions of six replicates of each soil samples. Increased toxicity resulted in a decrease in bacterial luminescence.

13. Technical and economic sustainability analysis

To assess the economic sustainability of the EO production chain on contaminated soil, an economic analysis was conducted. The production costs of the EO were estimated separately for each experimental condition (NI or I) per hectare. These costs encompassed various operational expenses including equipment depreciation and maintenance, fuel, labor costs, and inputs such as seed, fertilizer, AMF inoculant, and plant protection products. To estimate these costs, reference guides were consulted. For instance, the “Guide de la protection des grandes cultures”, annually updated by the Chamber of Agriculture in each French region, provided information on phytosanitary products and their costs. Another guide supplied technical details on agricultural equipment, including material and operating costs. The estimated costs covered soil preparation, seeding, inputs, harvesting, and any potential post-harvest expenses. These costs were compared to those associated with other phytoremediation or phytomanagement methods, as well as with the production costs of common field crops such as wheat, winter barley, and rapeseed. A gross profit was then estimated from the EO yields using the following equation (3):

$$\text{Gross profit} = \text{EO yield per Hectare} \times (\text{EO selling price} - \text{Production Costs per Hectare})$$

14. Social acceptability of EO

The social acceptability of growing angelica on TE-polluted soil hinges on the healthiness of the produced EO, its economically profitability, and the acceptance of its origin by consumers. To assess consumer acceptance, in autumn 2019 a survey was conducted by poll. To mitigate costs associated with surveying a representative sample of the French population, three distinct groups with specific characteristics were selected. The first sub-sample (n = 181) comprises environmentalists, including

members of environmental protection associations or individuals actively involved in environmental actions/events. The second sub-sample (n = 161) consists of state or territory employees. Due to the security of their position in the civil service, they are less likely to prioritize employment over strict health precautions, making them potentially less favorable towards such projects. The third sub-sample (n = 162) is made up of Master 1 and 2 students attending universities in the northern half of France. This population is both youthful and educated, wielding significant influence on public opinion in the coming decades. Collectively, the three sub-samples represent a sample size of 504 individuals, providing insights into diverse perspectives on the social acceptability of growing angelica on TE-polluted soil.

Our questionnaire is structured in a funnel-like manner, guiding respondents from broader environmental concerns to the specific topic of crops grown on polluted soils, and finally focusing on EO and their potential marketing. This approach aims to minimize survey bias and other influencing factors on respondents' choices (Wetzel et al., 2016). Data processing primarily involved a flat sorting across the entire sample, enabling the measurement of the hierarchy of perceived risks and the acceptability of EO derived from polluted soils. Subsequently, cross-sorting was employed to examine the socio-demographic profiles of individuals most accepting of this type of cultivation, particularly regarding the industrial utilization of derived products.

15. Statistical Analysis

Statistical analyses were performed using XLSTAT software (v2021.1.1; Addinsoft, Paris, France) and R software (version 4.2.2). To assess the normality of the data, the Shapiro–Wilk test was initially applied. Depending on the outcome of this test, either parametric methods such as ANOVA or non-parametric methods like Kruskal–Wallis test were used, followed by Fisher's post-hoc test (Least Significant Difference, LSD). For data expressed as percentages (mycorrhizal rate) arcsine transformation was performed prior to statistical analysis using the ASIN function in Microsoft® Excel v16.50. This transformation helps meet the assumptions of parametric tests and ensures the validity of statistical analyses.

Results and discussion

This study was conducted as part of a phytomanagement approach, with the objective of cultivating the aromatic plant *Angelica archangelica* L. *in situ* to produce a high-value EO on TE-polluted soils. Additionally, the impact of mycorrhizal inoculation on plant biomass, soil functionality, and EO yields was also evaluated over three successive years.

Angelica grows properly on TE-polluted soil, and AMF inoculation improves its mycorrhizal rate

Table 1. Growth parameters of angelica. Data are presented as means \pm standard error: height and dry weight ($n = 9$), stem diameter ($n=6$). Significant differences between different conditions are indicated by different letters ($\alpha = 0.05$).

Year	Mycorrhizal Inoculation	Height (cm)	Dry weight (g.plant ⁻¹)	Stem diameter (cm)
2020	NI	182 \pm 24 a	216 \pm 55 b	3.5 \pm 0.4 c
	I	179 \pm 15 ab	259 \pm 83 b	4.1 \pm 0.6 b
2021	NI	179 \pm 15 a	118 \pm 42 c	4.1 \pm 0.4 b
	I	187 \pm 17 a	142 \pm 56 c	4.2 \pm 0.6 b
2022	NI	160 \pm 11 bc	483 \pm 28 a	4.7 \pm 0.5 a
	I	158 \pm 12 c	517 \pm 101 a	4.5 \pm 0.4 ab
Year		***	***	***
Mycorrhizal Inoculation		NS	*	NS

Significance: NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ according to multivariate ANOVA in bold. NI: non-inoculated; I: inoculated.

Six weeks after sowing, the emergence rate of angelica was about 26 % in both NI and I conditions (data not shown). Although low, these emergence rates are comparable to those reported by Ojala (1985) in unpolluted soil, confirming the low germination rate characteristic of this plant species. Under our experimental *in situ* conditions, the density of angelica plants was satisfactory, with vegetation completely covering the ground from the first year of cultivation (Figure S1). Among the studied factors (cultivation year, AMF inoculation), the cultivation year exerted the most significant influence on plant growth (Table 1). During the first two harvests (2020 and 2021), angelica reached heights of 179 to 187 cm, dry weights of 118 to 259 g per plant, and stem diameters of 3.5 to 4.2 cm. In the third harvest (2022), angelica height was significantly smaller compared to the first and second harvests. However, the plants exhibited increased branching and significantly higher dry biomass (483 and 517 g per plant in NI and I conditions, respectively) and stem diameters (4.7 and 4.5 cm in NI and I conditions, respectively). Our results showed that soil pollution does not adversely affect plant

growth, and growth parameters remain comparable to those observed in unpolluted soils (Jakab et al., 2014; Kylin, 2012).

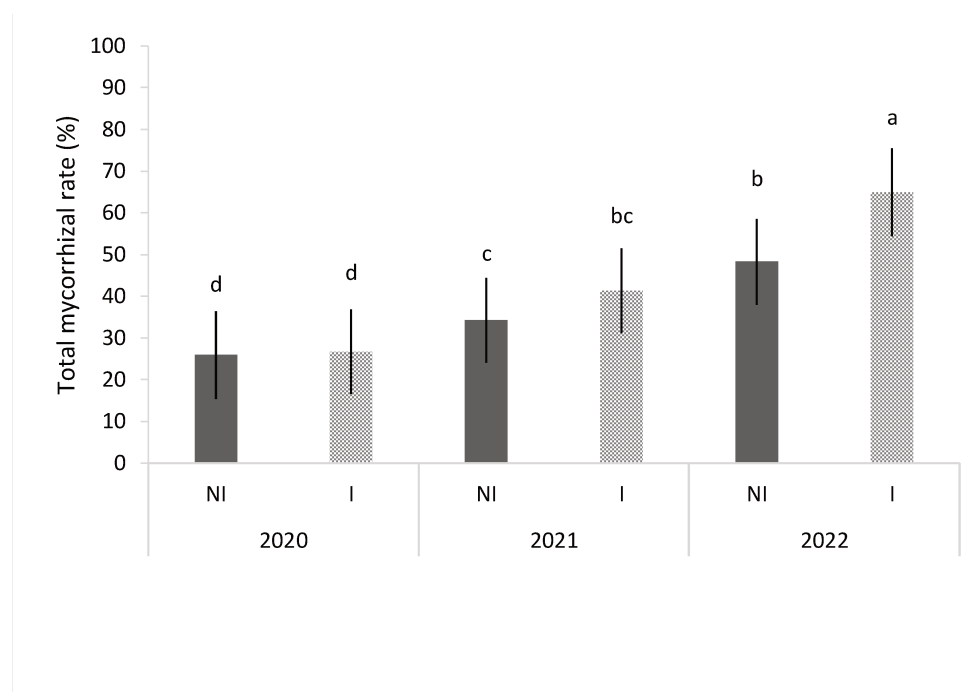


Figure 1. Total mycorrhizal rates of angelica roots. Significant differences between conditions are indicated by different letters ($\alpha = 0.05$). Values are means \pm SE ($n = 18$), NI: non-inoculated; I: inoculated.

The total mycorrhizal rates exhibited significantly increase from 26 to 48 % for NI conditions and from 27 to 65 % for I conditions between 2020 and 2022 (Figure 1). The mycorrhization of angelica in NI conditions can be attributed to the presence of indigenous AMF spores. Indeed, Raveau et al. (2021) identified numerous AMF spores (143 spores/100g of soil) at the same TE-polluted site and also observed spontaneous mycorrhization in other aromatic plants such as *Coriandrum sativum* L. and *Salvia sclarea* L. (Langrand et al., 2023; Raveau et al., 2021). ANOVA analysis of these results indicated that, despite no significant differences in mycorrhizal rates between NI and I conditions during the first two years, inoculation positively impacted angelica dry biomass. These results align with those reported by Zitterl-Eglseer et al. (2015), where mycorrhizal inoculation with two different AMF species, *Funneliformis mosseae* and *Rhizophagus intraradices* and with a commercial inoculum containing a mixture of six AMF species (*F. mosseae*, *R. intraradices*, *Claroideoglossum claroideum*, *Glomus microaggregatum*, *F. caledonium*, *C. etunicatum*) increased *angelica* biomass. Plant growth is likely enhanced by AMF root colonization, which improves water and mineral nutrition, particularly in adverse conditions like polluted soils (Ortaş and Rafique, 2017). Furthermore, Hristozkova et al. (2015)

demonstrated that AMF species such as *Claroideoglossum claroideum* can maintain low TE levels in the aerial parts of *Origanum majorana* L., mitigating the harmful effects of TE. Indeed, AMF can enhance TE immobilization in soil through mechanisms like sequestration by glomalin or immobilization in the cell wall of fungal hyphae and spores or vacuolar storage, limiting TE transfer to plant aerial parts (Azcón et al., 2010). Nevertheless, it is important to remember that the benefits provided by mycorrhizal inoculum in polluted soils depend on the plant-fungus-TE combination and are influenced by soil physicochemical conditions. Moreover, AMF usage appears particularly suitable for severely degraded soils (low in nutrients), especially to support early plant development stages (Siddiqui and Pichtel, 2008).

Angelica produces promising EO yields on TE-polluted soil

Table 2. Seed, distillation and EO yields

Year	Inoculation	Seed yield (kg/ha)	Distillation yield (%)	EO yield (kg/ha)
2020	NI	465	0.4	1.9
	I	179	0.9	1.6
2021	NI	614	0.3	1.8
	I	536	0.2	1.1
2022	NI	344	0.5*	1.2*
	I	136		

*: The small amount of seed harvested in 2022 did not allow separate distillations: NI and I 2022 harvests were pooled before distillation.

The EO yields ranged from 1 to 1.9 kg/ha, with the highest yields obtained in 2020 and 2021. In contrast to Zitterl-Eglseer et al. (2015), who reported a positive effect of AMF on angelica EO production under controlled conditions, our in situ study did not show a significant improvement in EO yield with mycorrhizal inoculation. Furthermore, EO yields typically obtained for angelica seed distillation on unpolluted soil are 5 to 7 times higher than those obtained in our experimental conditions (Jakab et al., 2014). Environmental factors, such as sunshine, geographical location, pedoclimatic conditions, and soil properties strongly influence EO yields (Aćimović et al., 2022). However, in our case, the low yields obtained are likely due to the relatively small cultivated area, where seed losses resulting from optimizing combine settings cannot be overlooked, unlike in larger fields.

Angelica EO is not contaminated by TE and pesticide residues

In verify the quality of the EO produced on TE-polluted soil, the presence of TE and pesticides residues was assessed in angelica EO for each year of production (Table 3), as well as in a commercial EO.

Table 3. Residual pesticides amounts in the distilled EO of angelica over 3 years of cultivation (mg.kg⁻¹ of EO).

Active substances	Target	2020	2021	2022
Pendimethalin	herbicide	0.028	0.160	--
Prosulfocarb	herbicide	0.440	2.000	0.610
Dimethenamid	herbicide	--	<0.010	--
Clomazone	herbicide	--	--	<0.010
Hexachlorobenzene (HCB)	fungicide	--	--	0.029
Metolachlor including S-Metolachlor	herbicide	--	--	0,097
Terbutylazine	herbicide	--	--	0,018
Triallate	herbicide	--	--	0,220

The analysis results indicated that the EO produced in this study are of good quality. Over the three years of cultivation, only 8 active compounds (Prosulfocarb, Dimethenamid, Clomazone, HCB, Metolachlor, Terbutylazine, and Triallate) out of the 480 analyzed were detected, and their concentrations were close to the detection limits. Prosulfocarb, which is used in our crop management system, was the only compound found in each of the 3 harvest. The presence of the other detected active compounds likely resulted from cross-contamination during the treatment of neighboring crops.

Table 4. Concentrations of TE (mg/kg of EO) in EO obtained from angelica seeds cultivated in polluted soil and commercially available

Trace element	2020	2021	2022	Angelica Commercial EO (Control)
Cu	<0.3	<0.3	<0.3	<0.3
Cr	<0.3	<0.3	<0.3	<0.3
Ni	<0.3	<0.3	<0.3	<0.3
Pb	<0.5	<0.5	<0.5	<0.5
Zn	<0.5	0.6 ± 0.2	2.0 ± 2.4	1.7 ± 2.0
Cd	<0.3	<0.3	<0.3	<0.3
As	<0.5	<0.5	<0.5	<0.5
Sb	<1	<1	<1	<1

The presence of TEs in angelica EO produced on polluted soil was examined for each year of production and compared with a control, a commercial angelica EO (Table 4). Across all the EO produced, TE levels were found to be below the limits of quantification, except for Zn in 2021 and 2022 for EO produced on TE-polluted soil and the control EO (0.6, 2.0, and 1.7 mg/kg of EO respectively). Similarly, previous studies have indicated no TE contamination in basil, chamomile, dill, and sage EO derived from biomass

produced on polluted soil (Raveau et al., 2021; Zheljazkov et al., 2008). It is noteworthy that the quality of the angelica seed EO obtained from the polluted soil is equivalent to that of a commercial EO produced on unpolluted soil. Some research has shown that TE accumulation in seeds is limited due to the involvement of specific proteins and transporters (Walker and Waters, 2011). *Apiaceae*, like angelica, exhibit three different behaviors regarding metal accumulation: low, moderate and high accumulation (Angelova et al., 2003). The absence of contamination in EO produced from angelica seeds could therefore be attributed to its probable low accumulator behavior. Additionally, this could be explained by the hydrodistillation process of the seeds, known to limit the quantity of TEs in the EO, with TEs remaining in the plant distillation residues (Scora and Chang, 1997; Zheljazkov et al., 2008; Zheljazkov and Warman, 2003). Finally, it is important to note that there is currently no specific legislation or maximum limit for pesticide or TE residues in EO for non-food use in France or at the European level (Asgari Lajayer et al., 2017; Raveau et al., 2021; Tascone et al., 2014). Therefore, angelica EO produced on the polluted soil in this study could potentially be sold in non-food channels.

Soil ecotoxicity decrease after two years of angelica growth in I condition

The bioluminescence of *A. fischeri* was assessed to determine the ecotoxicity of the polluted soil before and after the three years of angelica cultivation. Following the classification proposed by Bláha et al. (2010), soil toxicity is considered as minor if the $EC_{50} > 80 \text{ mg.mL}^{-1}$, low between 20 and 80 mg.mL^{-1} , intermediate between 5 and 20 mg.mL^{-1} , high between 1 and 5 mg.mL^{-1} , and severe if the $EC_{50} \leq 1 \text{ mg.mL}^{-1}$. Initially, the soil exhibited intermediate toxicity towards *A. fischeri*, with an EC_{50} of approximately $12.2 \pm 3.9 \text{ mg.mL}^{-1}$ (Table 3).

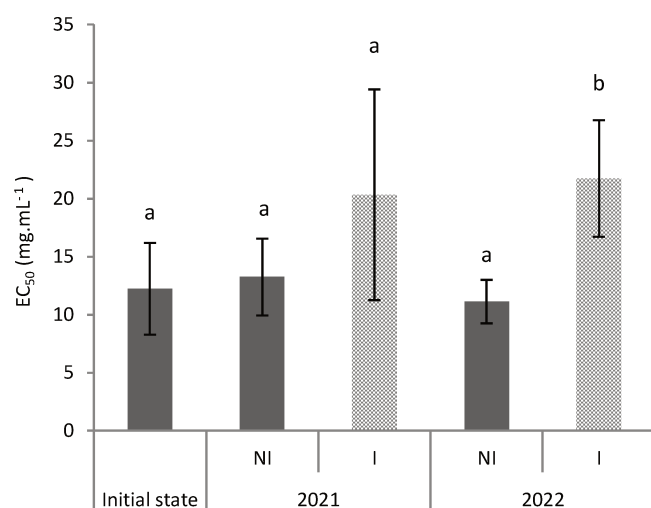


Figure 3. Soil toxicity towards the photobacterium *Aliivibrio fischeri*. Significant differences between conditions are indicated by different letters ($\alpha = 0.05$). Values are means \pm SE ($n = 6$).

The ecotoxicity values for NI soil samples from 2021 and 2022 appeared to be similar to the initial state (13.2 ± 3.3 and 11.1 ± 1.8 , respectively), while AMF inoculation had a positive effect by significantly decreasing soil toxicity three years after seeding (*i.e.* 2022-I, $EC_{50} = 21.7 \pm 5.0 \text{ mg.mL}^{-1}$). Indeed, AMF are known to limit the harmful effects of TEs on plants and help to immobilize them by producing specific proteins such as glomalin (Cicatelli et al., 2014; Ferrol et al., 2016; González-Chávez et al., 2004); therefore, AMF are likely involved in reducing soil toxicity. As comparison and as expected, positive controls of CuSO_4 and ZnSO_4 exhibited high toxicity ($EC_{50} = 1.8 \text{ mg.L}^{-1}$ and $EC_{50} = 6.1 \text{ mg.L}^{-1}$, respectively), while our regional low-contaminated reference soil exhibited lower toxicity ($EC_{50} = 39 \text{ mg.mL}^{-1}$, results not shown).

Angelica culture increases total soil bacterial activity over time

Soil microbial parameters, such as biomass and metabolic potential of soil bacterial communities, were measured to evaluate the benefits of angelica culture and AMF inoculation on soil functioning.

Table 5. Influence of angelica culture and AMF inoculation on total bacterial biomass ($\mu\text{g.g}^{-1}$ of dry weight (DW) soil) and on bacterial metabolic potential expressed as average well color development (AWCD). Data are presented as means \pm standard error. The means were obtained from three replicates ($n = 6$). Means followed by the same letter are not significantly different, as determined by one-way ANOVA comparison and *post-hoc* Fisher test ($\alpha = 0.05$).

Year	Inoculation	Total bacterial biomass ($\mu\text{g g}^{-1}$ of DW soil)	AWCD
Original state	NI	7.1 ± 2.2 c	80.0 ± 7.2 b
2020	NI	10.3 ± 4.7 b	110.1 ± 27.9 a
	I	10.0 ± 4.4 b	118.7 ± 15.9 a
2021	NI	14.1 ± 6.2 a	98.6 ± 23.1 ab
	I	15.8 ± 4.4 a	76.9 ± 7.6 b
Year		***	***
Inoculation		NS	NS
Vegetation		***	**

Significance: NS: not significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ according to multivariate ANOVA in bold. NI: non-inoculated; I: inoculated. AWCD: average well color development

Total soil bacterial biomass increased significantly after each year of cultivation (Table 5). The values increased from 7 to 10 and 16 $\mu\text{g g}^{-1}$ DW soil for the original state, 2020 and 2021, respectively. These values are comparable or even up to two times higher than those reported by Shentu et al. (2014) in

unpolluted soil or Cd-polluted soil at a concentration of 3.5 mg/kg. However, mycorrhizal inoculation had no yield a significant effect on total bacterial biomass. Conversely, in the presence of mycorrhizal inoculum, the metabolic potential of soil bacterial communities, expressed as average well color development (AWCD), increased from 80 in the original state to 119 in 2020. The observed enhancements in bacterial biomass and metabolic potential over time imply a profoundly positive and statistically significant influence of angelica cultivation on soil bacterial communities. It is well-documented that plants release root exudates containing carbon compounds, which serve as readily available sources of nutrients and energy for heterotrophic organisms (Hartmann et al., 2009). Moreover, aromatic plants are also known to release secondary metabolites in their root exudates, attracting diverse microbial communities capable of utilizing both sugars and secondary metabolites, thus enriching the microbiota (Adamovic et al., 2015; Köberl et al., 2013; Misra et al., 2019). Typically, AWCD values ranging from 85 to 120 are anticipated in functional agricultural soils (Alahmad et al., 2019; Nivellet al., 2016). In the present study, the polluted soils studied exhibited a metabolic potential comparable to that of unpolluted agricultural soil (Borymski et al., 2018; Feigl et al., 2017; Gremion et al., 2004; Xie et al., 2016). These results underscore the beneficial contribution of angelica cultivation to the ecological rehabilitation of TE-polluted soils.

EO production distilled from angelica seeds cultivated on TE-polluted soils is an economically profitable phytomanagement channel

One of the objectives of the present study was also to evaluate the economic aspect of EO production distilled from angelica seeds cultivated on TE-polluted soils. Physicochemical methods for soil remediation are costly and generally not applicable to large agricultural areas (Burgess et al., 2018; Kumpiene et al., 2019). In the case of our experimental site, pollution is concentrated in the top 40 cm of soil, requiring the excavation of at least 4,000 m³ of polluted soil for areas exceeding one hectare. With the estimated average cost of around 180 €/m³ for physicochemical remediation of a TE-polluted soil (Zhang, 2019), the projected cost for our site amounts to approximately 720,000€. In comparison, phytoremediation for TEs contamination offers a significantly lower estimated cost, at approximately 34 €/m³ (Wan et al., 2016).

The setup and monitoring of angelica cultivation during the first year incurred a cost of approximately 564 €/ha. Consequently, the total cost of angelica cultivation for the subsequent nine years can be estimated at 749 €/ha, averaging 730 €/ha annually (Table 6). Notably, this estimation encompasses soil preparation and seeding, which are only carried out in the initial year. It's important to remember that angelica cultivation requires a single sowing every decade, as the plant is re-seeded during the

seed harvest. In comparison with clary sage cultivation studied by Raveau et al. (2021), which operates on a three-year cycle, angelica's cultivation period allows for fewer sowings and more harvests.

As of 2023, industry professionals suggest that one kilogram of angelica EO is traded between 620 and 800 €, with an average estimate of 660 €. Under optimal conditions (2020 NI), we obtained an EO yield of 1.9 kg/ha, equivalent to a turnover of 1,254 €/ha. After deducting the cultivation costs of 730 €/ha, this results in a potential gross margin of 814 €/ha.

Alternatively, based on previous works, it is feasible to attain EO yields of 8 kg/ha (Jakab et al., 2014; Kylin, 2012), leading to a turnover of 5,280 €/ha, and a gross margin of 4,550 €/ha. This gross margin surpasses that obtained by Raveau et al. (2021) for coriander aerial parts (100-200 €/ha) and seeds (negative gross margin), and matches or exceeds clary sage (3,024 €/ha). Furthermore, this gross margin exceeds the average results obtained with crops like wheat (averaging 720 €/ha), which are also resource-intensive, potentially enticing farmers to transition to the EO sector (Casdar Project, 2015; Raveau et al., 2021).

Table 6. Estimated production costs for angelica cultivation. Installation cost: estimated cost of setting up the crop; Year n cost: annual cost of cultivation from year 2 to year 10.

Description		Amount installation	Installation cost	Amount Year n	Year n cost
Step 1 : soil preparation					
Tractor + plough	1 intervention: 5 shears plough - 130-hp tractor	1	60	0	
Tractor + rotary harrow	1 intervention: Vibrashank (4 m) - 110-hp tractor	1	60	0	
<i>Total</i>			<i>120</i>		<i>0</i>
Step 2: sowing					
Tractor + beet seeder	8 rows seeder - 80-hp tractor	1	100	0	
Seeds	2 kg/ha	1	60	0	
<i>Total</i>			<i>160</i>		<i>0</i>
Step 3: inputs					
Tractor + fertilizer distributor	2 interventions	2	29	2	29
Fertilizer 1	Sulfate of ammonia	1	60	1	60
Fertilizer 2	Sulfate of ammonia	1	60	1	60
Tractor + sprayer	2 interventions/year	2	30	2	30

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Crop protection products	2 interventions/year	2	105	2	105
<i>Total</i>			284		284
Step 4: harvest					
Combine harvester	Combine harvester 210-hp	0		1	180
<i>Total</i>					180
Step 5: post-harvest					
Grinding		0		1	85
Inter-row hoeing		0		1	60
<i>Total</i>					145
Step 6: distillation process					
Steam production, distillation		0		1	140
<i>Total</i>					140
Total production cost €/ha			564		749

In addition, enhancing the economic return of the crop could involve integrating the valorization of distillation residues into the value chain. Depending on the detected level of metallic contamination in the residues, they could be recycled through processes such as anaerobic digestion or composting. This would not only improve gross margins but also add value to all crop products and by-products. Otherwise, the distillation residues could be returned to the field for use as fertilizer, reducing the requirement for fertilizers and slightly increasing the gross margin.

The production of non-food EO from angelica seeds cultivated on TE-polluted soils receives a good public acceptance

A social acceptability study was conducted to assess the reaction of inhabitants to the production of EO for non-food purposes on TE-polluted soil. It is noteworthy that approximately one in five individuals (18.5%) express opposition to any utilization of products derived from polluted soil (Table 7). While this opposition constitutes a minority viewpoint, it appears deeply ingrained and warrants careful consideration. Particularly in the context of what some characterize as “nature 2.0”, where small activists groups can effectively contest uses and products they perceive as concerning, thereby posing a potential threat to their sustainability (Arts et al., 2015; Büscher et al., 2017).

Table 7. Acceptability of products such as EO from plants grown on polluted soils. Q12: Would you be in favor of growing plants under these conditions to:

Modality	Nb	%
Housing (building materials)	272	54.2
Heating (fuels, ...)	270	53.8
Moving (fuels, ...)	265	52.8
Dressing up (vegetable fibers: t-shirt, pants, ...)	242	48.2
Fitting house interior (carpet, cushion, ...)	198	39.4
Feeding animals	158	31.5
Washing our body (hygiene products: shampoo, ...)	152	30.3
Feeding humans	149	29.7
Grow our indoor and vegetable gardens (herbicides, antiparasitic, ...)	130	25.9
Taking care of our body (beauty products: face cream, ...)	122	24.3
None of this, no agricultural use of polluted soil seems acceptable to me	93	18.5
I don't know	20	4.0
Other	7	1.4
Total	502	

One question sought to measure the acceptability of cultivating angelica on polluted soils when potential economic and employment opportunities were highlighted. Participants were presented with a range of options to choose from (enthusiastic, undecided, worried, angry) or they could suggest their own term or expression (Table 8).

This time, cross-sorting reveals that the responses differ depending on the group to which respondents belong, with a significant square chi-square test at the 5% threshold. This indicates that the 3 sub-samples yield significantly different outcomes. Among these groups, “environmentalists” emerge as the most opposed (+12 points compared to Master’s students) and the least favorable (17 points lower than students and 8 points lower than public sector employees) (Table 8).

Table 8. Consumer attitudes towards the production of essential oils on polluted soil. What do you think about the production of EO from plants cultivated on polluted soils ?

Attitude	Reaction	Group 1 (environmental adherents or supporters)	Group 2 (public sector employees)	Group 3 (master’s students)	Total

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Hostile	Anxious; angry; in a state of misunderstanding; vigilant; sad	45 %	37 %	33 %	38 %
Hesitant	Undecided; not convinced; skeptics; doubters	38 %	38 %	33 %	36 %
Positive	Enthusiastic; curious; interested	17 %	25 %	34 %	25 %
Total		100 %	100 %	100 %	100 %

Firstly, there is widespread rejection of substances produced on polluted soil, including angelic EO and other substances. Resistance is primarily observed for products that come into direct contact with the body (cosmetics, hygiene products), but these are not part of the phytomanagement project.

Regarding the minority opposed to any use of products from polluted soils, these individuals do not envision these soils being reused without prior treatment. Typically, older and less educated than the rest of the sample, age emerged as a more influential factor than environmental commitment, such as voting patterns and participation in environmental events. These opponents exhibit a significant mistrust towards potential stakeholders in this emerging sector of crops on polluted soils. Note that for this question, the chi-square test (at the 5% threshold) indicates that the 3 sub-samples do not yield significantly different results, suggesting that distinctions between respondent populations cannot be made.

Conclusion

In summary, our study demonstrates that angelica represents a viable option for phytomanagement strategies targeting TE-polluted soil, particularly for EO production. Angelica exhibits robust growth even in heavily aged TE-polluted soil without requiring soil amendments. While mycorrhizal inoculum may not be essential for sustaining angelica growth, it could effectively reduce soil ecotoxicity. The EO distilled from angelica seeds shows quality comparable to commercial EO, with minimal traces of TEs and pesticides detected, mostly below or close to the quantification limits. Moreover, angelica cultivation significantly enhances bacterial biomass and metabolic potential in polluted soil, particularly notable in 2020, indicating promising ecological restoration of soil functions. With its single-sowing cycle over ten years, coupled with low input nutrient requirements, angelica cultivation for EO production on polluted soils proves economically advantageous as phytomanagement

approach. Moreover, public acceptance of angelica EO distilled from seeds cultivated on polluted soil is favorable. However, optimizing harvesting practices could further enhance EO yields. Future research should explore the composition and biological properties of EO, as well as the phytostabilizing capacities of angelica, to integrate phytostabilization, valorization of distillation residues, and new outlets in the value chain, such as in plant protection.

Author Contributions:

Conceptualization, J.L., J.F. and A.L.-H.S.; methodology, J.L., J.F., B.T., F.L., N.F., A.V., J.D., D.D., H.F. and A.L.-H.S.; writing—original draft preparation, J.L.; writing—review and editing, J.L., J.F. and A.L.-H.S.; supervision, J.F. and A.L.-H.S.; project administration, A.L.-H.S.; funding acquisition, A.L.-H.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest:

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Publication 1

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Supplemental data



Figure S1. Angelica experimental field Mai 2021

Table S1. List of commercial products used each year for weed control

Commercial name	Active substance	Target	Dose
Challenge 600	Aclonifen	Dicotyledons and grasses	1L/ha
Lentagran	Pyridate	Dicotyledons	0.3kg/ha
Prowl 400	Pendimethaline	Dicotyledons and grasses	1L/ha

Publication 2

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Biocide, antioxidant and anti-inflammatory properties of essential oils distilled from angelica grown on polluted soils

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Présentation de la publication 2

Dans la période de l'après-guerre, l'utilisation de pesticides de synthèse s'est peu à peu imposée dans l'agriculture française comme l'un des piliers de la modernisation agricole, tout comme le recours aux engrais chimiques et à la mécanisation. Connus pour améliorer la production agricole en réduisant les dommages causés par les ravageurs, les pesticides conventionnels sont néfastes pour la santé humaine et l'environnement. En outre, étant peu ou pas biodégradables, certains peuvent persister pendant de nombreuses années dans l'environnement (Rani et al., 2021; Sabarwal et al., 2018). C'est pourquoi, aujourd'hui, à l'heure de la transition agroécologique, le développement de méthodes de lutte alternative devient une priorité pour les pouvoirs publics, les scientifiques ou les agriculteurs, et l'utilisation de molécules biosourcées d'origine naturelle, comme les HE, pourrait constituer une des solutions.

En effet, grâce à la diversité de leur composition et leur richesse en principes actifs, les HE possèdent de nombreuses propriétés biologiques valorisables dans de nombreux domaines tels que l'agriculture, les cosmétiques, ou la santé humaine et animale (Bakkali et al., 2008; Raveau et al., 2020; Shaaban et al., 2012; Turek and Stintzing, 2013).

Toutefois, la composition des HE est fortement influencée par des facteurs environnementaux, tels que la situation géographique, l'ensoleillement, les conditions climatiques et les propriétés pédo-agronomiques du sol, y compris la présence de polluants (Aćimović et al., 2022). En particulier, la présence d'ET dans le sol peut entraîner des pertes de rendement en HE, ainsi que des altérations de leur composition (Biswas et al., 2011; Jezler et al., 2015; Prasad et al., 2014). Néanmoins, de nombreuses études ont rapporté une augmentation de la teneur en HE des plantes aromatiques après inoculation avec un CMA, ce qui pourrait compenser les effets négatifs des polluants du sol (Chaudhary et al., 2008; Khaosaad et al., 2006; Zeng et al., 2013; Zitterl-Eglseer et al., 2015).

Ainsi, pour compléter l'étude précédente portant sur la pertinence de la filière de production d'HE d'angélique pour le phytomanagement d'un sol pollué par les ET, cette publication vise dans un premier temps à caractériser, par GC-MS, la composition chimique des HE issues des graines d'angélique, cultivée sur un sol fortement pollué par les ET, en présence ou non d'un inoculum mycorhizien commercial (AGTIV®; PremierTech). Les propriétés biologiques des HE ont ensuite été évaluées en vue d'une application dans les domaines de la bioprotection des cultures (activités antifongiques et herbicides) ou la santé humaine ou animale (activités antioxydantes et anti-inflammatoires).

Présentation de la publication 2

Le potentiel antifongique de l'HE d'angélique a été évalué contre trois champignons phytopathogènes majeurs des grandes cultures, à savoir *Zymoseptoria tritici*, *Fusarium culmorum*, *Fusarium graminearum*, *Blumeria graminis f. sp. tritici* et un oomycète *Phytophthora infestans*, responsables respectivement de la septoriose chez le blé, de fusarioses chez les céréales, de l'oïdium chez le blé, et du mildiou chez la pomme de terre et, causant d'importantes pertes de rendements (Bellone et al., 2023; Raveau et al., 2022, 2020). En outre, les *Fusarium sp.* sont connus pour produire des mycotoxines qui provoquent des maladies humaines et animales allant d'effets neurotoxiques aux cancers (Ekwomadu et al., 2021).

Le potentiel herbicide de l'HE d'angélique a également été étudié sur deux plantes modèles couramment utilisées dans les tests de phytotoxicité, le ray-grass et la laitue (Park et al., 2016). Enfin, l'activité anti-inflammatoire des HE a été évaluée dans le cadre d'une réponse inflammatoire induite par l'exposition d'une lignée cellulaire bronchiale humaine à des particules atmosphériques. L'activité antioxydante de l'HE d'angélique a été évaluée selon sa capacité à piéger les radicaux libres, d'après un test de piégeage du radical libre DPPH• (Brand-Williams et al., 1995).

Nos résultats ont montré que l'HE d'angélique est composée principalement de monoterpènes (β -phéllandène, α -pinène et α -phéllandène) et de sesquiterpènes (α -copaène). Le composé majoritaire est le β -phéllandène (73 - 80 %), suivi de l' α -pinène (5 - 7 %), puis de l' α -phéllandène (2 - 4 %), et du β -myrcène (0.5 - 2 %). Cette composition est en accord avec les données de la littérature, et similaire à celle d'une HE d'angélique commerciale analysée dans le cadre de ce travail (Jakab et al., 2014; Kylin, 2012; Nivinskiene et al., 2007; Nivinskienė et al., 2005). De plus, la composition de l'HE d'angélique n'est pas impactée par la présence d'ET dans le sol, ni par l'inoculation mycorhizienne.

L'activité antifongique des HE, caractérisée par des valeurs de CI_{50} comprises entre 0,22 et 0,02 g.L⁻¹ étaient supérieures à celle du fongicide (Aviator®) utilisé comme référence. En effet, les CI_{50} obtenues avec ce fongicide étaient comprises entre $3,38 \cdot 10^{-5}$ et $3,54 \cdot 10^{-4}$ g.L⁻¹. Pour l'activité herbicide, les CI_{50} de l'HE d'angélique ont varié entre 0,14 et 5,39 g.L⁻¹ contre le ray-grass et la laitue respectivement. Le glyphosate, utilisé comme référence, a donné des CI_{50} variant de $4,65 \cdot 10^{-4}$ et $1,47 \cdot 10^{-3}$ g.L⁻¹ pour la laitue et le ray-grass respectivement. Par ailleurs, l'HE d'angélique a montré une activité antioxydante modérée par rapport à d'autres HE (Wei and Shibamoto, 2007), mais possède une activité anti-inflammatoire prometteuse.

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Pour cette étude, je me suis chargé de l'analyse des compositions chimiques des HE par GC-MS sous l'encadrement de F. Laruelle (IR à l'UCEIV), de l'ensemble des tests biologiques relatifs aux propriétés antifongiques et herbicides. Les propriétés antioxydantes ont été réalisées dans le cadre d'une collaboration avec S. Fourmentin (équipe CS-UCEIV-ULCO) et les propriétés anti-inflammatoires ont été évaluées en collaboration avec A. Verdin (équipe TCEA-UCEIV-ULCO). Je me suis également chargé des analyses statistiques concernant les parties sus-citées. L'exploitation des données ainsi que la rédaction du manuscrit sont le fruit d'un travail collectif avec les co-auteurs.

Publication 2 : Biocide, antioxidant and anti-inflammatory properties of essential oils distilled from angelica grown on polluted soils

ABSTRACT

The present study aimed to investigate the phytochemical profile and biological properties of essential oil (EO), distilled from the seeds of the aromatic plant, *Angelica archangelica* L., grown on aged trace elements (TE) -polluted soil in a context of phytomanagement. The influence of TE soil pollution, mycorrhizal inoculation and cultivation time on the chemical composition of angelica seed EO was studied, as well as their antifungal, herbicidal, antioxidant and anti-inflammatory properties. The chemical composition of angelica EO (β -phellandrene, α -pinene, α -phellandrene, and β -myrcene) is not altered by the presence of the pollutants in the soil, mycorrhizal inoculation or culture time indicating angelica potential resilience to adverse environmental conditions such as TE pollution, Whereas the antioxidant activity of angelica EO was found to be moderate, its anti-inflammatory activity showed interesting potential compared to others EO, opening up prospects for its use in health products. Moreover, angelica EO presented also antifungal and herbicidal properties, indicating their potential use as alternatives to conventional pesticides. Indeed, angelica EO possessed an antifungal *in vitro* activity against a phytopathogenic agent, *Z. tritici* (IC₅₀ of 21 mg.L⁻¹), responsible of Septoria blotch, against *Fusarium culmorum*, *F. graminearum*, and *Phytophthora infestans* and protected the wheat by 40% against *Blumeria graminis*, a causal agent of powdery mildew, promising antifungal activities was also detected angelica EO also showed an herbicidal activity against ryegrass, (IC₅₀ of 144 mg.L⁻¹). Taken together, our results suggest that TE-polluted soils could be used to produce EO with suitable chemical compositions and interesting biological activities for various non-food applications.

Keywords: Angelica, essential oil, trace elements, polluted soils, biological activities

INTRODUCTION

Essential oils (EO) are sources of beneficial chemical compounds that have potential applications in medicine, food, cosmetics, and other industries (Bolouri *et al.*, 2022). They are mainly composed of terpenes (mono-, and sesquiterpenes), alcohols, acids, esters, epoxides, aldehydes, ketones, amines and sulfides, forming a complex mixture of volatile compounds, with properties as diverse as their compositions (Bakkali *et al.*, 2008; Raveau *et al.*, 2020). Indeed, these compounds are increasingly being studied for their antimicrobial, antioxidant, fungicidal, insecticidal or anti-inflammatory activities (Calo *et al.*, 2015; Sowndhararajan *et al.*, 2017). They are able to scavenge some free radicals and preserve foods from the toxic effects of some oxidants (Miguel, 2010). They can also act as anti-inflammatory agents, because one of the inflammatory responses is the oxidative burst that occurs in diverse cells (monocytes, neutrophils, eosinophils, and macrophages) (Kfoury *et al.*, 2016). They play an important role in some disease prevention such as heart disease, cancer or immune system decline (Sosa *et al.*, 2023).

On the other hand, EO can be used to protect crops. Indeed, the use of EO as biopesticides is one of the main strategies for controlling pests in a more eco-friendly approach (Greff *et al.*, 2023). Due to their relatively low toxicity at low concentration to humans and animals, EO have been classified by the Food and Drug Administration (FDA) as "generally recognized as safe" (GRAS) products, and are therefore considered less harmful to the environment and human health than commercial pesticides (Hyltdgaard *et al.*, 2012; Jannat *et al.*, 2023). They also enjoy public support as environmentally-friendly natural products (De Clerck *et al.*, 2020; Raveau *et al.*, 2021b). This makes them a potentially relevant alternative to phytopharmaceuticals, with promising results against plant pathogens and diseases, as well as weeds (Raveau *et al.*, 2022; Khurshed *et al.*, 2022). For example, in France, clove, spearmint and sweet orange EO are authorized as fungicides, antigerminatives and insecticides respectively, while lemongrass and spearmint are used as herbicides and antigerminatives respectively in Great Britain (Furet & Bellenot, 2013).

However today, the world market for EO is booming and the pressure on arable soils has never been more crucial, and so has the need to increase their productivity (Právělie *et al.*, 2021). In the current and future context, the use of polluted soils for non-food production will become a necessity in order to avoid competition with arable soils, and disrupt global food security. Therefore the production of EO using aromatic plants may appear to be an interesting option within the framework of phytomanagement approaches (Pandey *et al.*, 2019; Raveau *et al.*, 2021a). Indeed, there are more

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than 10 million polluted sites worldwide, nearly half of which are polluted by trace elements (TE) (He *et al.*, 2015). Due to their non-biodegradability, and toxicity, their tendency to accumulate in organisms, they pose a major threat to the environment and human health (Burges *et al.*, 2018; Pandey *et al.*, 2019). For this reason, the ability of certain aromatic plants to tolerate high concentrations of TE and immobilize them at rhizospheric level could prove particularly valuable in a phytomanagement context, while enabling the valorization of high value-added products such as EO (Sá *et al.*, 2015; Pandey *et al.*, 2019). Indeed, growing aromatic plants on polluted soils unsuitable for food crops would minimize the risk of contamination of the food chain, while limiting competition with food crops (Pandey *et al.*, 2019; Raveau *et al.*, 2021a). Finally, one of the main issues limiting the development of phytotechnologies is, in most cases, the lack of economic profitability (Evangelou *et al.*, 2015). In this respect, the production of high value-added biosourced EO, obtained by hydrodistillation, free from TE, could contribute to economic profitability (Asgari Lajayer *et al.*, 2017; Raveau *et al.*, 2021a).

However, EO composition is strongly influenced by environmental factors, such as soil type, geographical location, sunshine, climatic conditions and soil properties, including the presence of pollutants (Aćimović *et al.*, 2022). In particular, the presence of TE in the soil can lead to changes in EO yield, as well as alterations in composition (Biswas *et al.*, 2011; Prasad *et al.*, 2014; Jezler *et al.*, 2015). Nevertheless, numerous studies have reported an increase in the EO content of aromatic plants after inoculation with an arbuscular mycorrhizal fungi (AMF), which could offset the negative effects of soil pollutants (Khaosaad *et al.*, 2006; Chaudhary *et al.*, 2008; Zeng *et al.*, 2013; Zitterl-Eglseer *et al.*, 2015). For example, mycorrhizal inoculation significantly increased the sesquiterpene acid content in the underground parts of valerian (Nell *et al.*, 2010), as well as the percentage of eugenol in basil (Copetta *et al.*, 2006), or the EO content of *Origanum sp.* and in *Artemisia annua* L. leaves (Khaosaad *et al.*, 2006; Chaudhary *et al.*, 2008).

Among aromatic and medicinal plants, producing EO, the *Angelica* L. genus belongs to the *Apiaceae* family, which comprises around 90 species of biennial perennials widely distributed in Europe, Asia and North America (Sowndhararajan *et al.*, 2017). Among these species, *Angelica archangelica* is commonly used in traditional medicine for its antioxidant and anti-inflammatory properties (Wei & Shibamoto, 2007; Jakab *et al.*, 2014; Sosa *et al.*, 2023) and the angelica EO could be of interest in the medical field. Previous studies reported that *Angelica archangelica* extracts has antifungal, or herbicidal properties (Zabka *et al.*, 2011; El-Alam *et al.*, 2020), this could also make it a valuable tool for biocontrol of phytopathogenic fungi, especially considering the increasingly frequent resistance of pests to conventional pesticides (Acheuk *et al.*, 2022; Khursheed *et al.*, 2022).

Thus, the present work aims to investigate the influence of TE soil pollution and mycorrhizal inoculation on the chemical composition of angelica seed EO, distilled from plant cultivated on aged TE-polluted soil for two years (2020 and 2021). Its biological properties were examined so as to determine its potential as alternatives to conventional pesticides (antifungal and herbicidal) and for their beneficial health properties (antioxidant and anti-inflammatory).

MATERIAL AND METHODS

I. Essential oil production

Angelica (*Angelica archangelica* L.) have been cultivated *in situ* during two successive years (2020 and 2021) on a TE-polluted soil (50°25'55.5" N, 3°02'25.5" E) displaying elevated amounts of TE (Pb: 394 ppm, Zn: 443 ppm and Cd: 7.2 ppm (Raveau *et al.*, 2021a)). Experimental plots were inoculated (I) or not (NI) with a commercial mycorrhizal inoculant containing the AMF *Rhizophagus irregularis* (AGTIV® Specialty Crops, 125 g ha⁻¹ Premier Tech, QC, Canada), kindly provided by Premier Tech. The seed distillation was performed during a three-hour cycle until no more EO was recovered on a steam-distillation unit (14 m³ tank-saturated water steam, 0.3 bar). *Angelica* EO were stored at 4°C in tightly closed brown glass vials until their analysis and use. In order to compare the quality of EO produced in polluted soil with that of EO produced in unpolluted soil, a commercial EO kindly provided by Ferrant PHE (France) was analyzed and used as a control.

II. Determination of the chemical composition of angelica EO

The EO composition was determined by gas chromatography simultaneously coupled to a Flame Ionization Detector and a Mass Spectrometer detector (MS - Shimadzu QP 2010 Ultra). Previously diluted in ethyl acetate (1:500 (v/v)), the EO were injected in split mode (ratio 1:80) in a volume of 0.2 µL at 220 °C onto a ZB-5MS capillary column (Phenomenex - 5% Phenylarylene / 95% dimethylpolysiloxane - length 10 m, internal diameter 0.10 mm, phase thickness 0.10 µm). The carrier gas (helium) was maintained at a constant linear velocity of 60 cm.s⁻¹. The temperature was programmed to start at a temperature of 60 °C (30 seconds) and then increase linearly at 25 °C.min⁻¹ to reach a plateau of 315 °C (1 minute).

Mass spectra were determined in the electronic ionization mode at an energy of 70 eV and recorded over a range of 35 to 500 amu (m/z). The temperature parameters of the spectrometer were set at 280 °C for the ion source and 240 °C for the transfer line.

Kovats indices were determined from retention times and co-injection of a series of n-alkanes of a commercial calibration standard with chain lengths between 8 and 40 carbon atoms (C8-C40 Alkanes

Calibration STD - Supelco 40147-U). The identification of the EO compounds was performed by comparing the Kovats indices and mass spectra obtained at the column outlet with those of the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) and Wiley 275 libraries. The relative percentages of the oil constituents were measured from the peak areas.

III. Biological properties of angelica EO

1. Crop protection applications

a. In vitro antifungal activities

In order to explore the potential of angelica EO in crop bioprotection, the antifungal properties were evaluated against three major phytopathogenic fungi of field crops, namely *Zymoseptoria tritici* (strain 2596, kindly provided by Dr Ali Siah - Lille, France), *Fusarium culmorum* (purchased from CBS, Netherlands), *Fusarium graminearum* (MUCL 53451, Mycothèque de l'Université catholique de Louvain, Louvain-la-Neuve, Belgium), *Blumeria graminis f. sp tritici* (kindly provided by Dr M.-P. Latorse from Bayer Crop Science, Lyon, France) and an oomycete *Phytophthora infestans* (MUCL 54981, Louvain-la-Neuve, Belgium). *Z. tritici*, *F. culmorum*, *F. graminearum*, and *P. infestans* are responsible for considerable yield losses in various crops (cereals, potatoes, tomatoes, etc.) (Raveau et al., 2020, 2022; Bellone et al., 2023) and *Fusarium sp.* is known to produce mycotoxins that cause human and animal diseases ranging from neurotoxic effects to cancer (Ekwomadu et al., 2021).

The antifungal activity study on *F. culmorum*, *F. graminearum*, and *P. infestans* was performed in Petri dish on PDA (Potato-Dextrose-Agar, Condalab, Spain) medium according to a protocol adapted from Znini *et al.*, (2013). The medium was cooled to 50°C before introducing the EO, to obtain a range of 5 concentrations from 0.005 to 1.0% (v/v). A positive control was made following the same concentration range using a reference fungicide registered for the use on field crops against many fungal diseases including *Fusarium*, Aviator XPRO (Bixafen 75 g.l⁻¹ and Prothioconazole 150 g.l⁻¹). The dishes were then cut from the circumference of a 7-day-old fungal colony and placed in the center of a 9 cm Petri dish containing PDA medium supplemented with EO. for each species tested. Mycelium growth diameter is measured after 7 days of culture at 20°C. Each test is repeated three times independently. The percentage of inhibition is calculated as follows:

$$\text{Percentage of inhibition} = \frac{x_0 - x_i}{x_0} \times 100$$

" x_0 " is the average diameter of the mycelium of the control.

" x_i " is the average diameter of the mycelium of the different conditions studied.

These values are then used to calculate the concentration required to inhibit mycelial growth by 50 % (IC₅₀) compared to the control.

The antifungal activity of EO against *Z. tritici* was assessed using an *in vitro* microplate assay, adapted from the Fungicide Resistance Committee methods and similar to that developed by De Clerck *et al.*, 2020, with a range of eight concentrations from 0 to 0.8% of EO in the medium. Briefly, in order to produce spores, *Z. tritici* was cultivated for 4-days in PDA. Afterward, 60 µl of suspension calibrated, using Malassez counting cell, at 2 10⁵ spores.ml⁻¹ was inoculated in microplates supplemented by 140 µl of glucose peptone medium with or without test product (Fungicide Resistance Action Committee-2006). A positive control using the same concentration range (0 to 0.8% v/v) was made with the same reference fungicide, Aviator XPRO. The microplates are incubated at 20 °C under agitation (110 RPM) for 6 days. Then, the optical densities (OD) are measured at 620 nm. Each test is repeated three times independently. For each concentration, a net OD value is calculated:

$$OD_{net} = OD_{average\ with\ fungi} - OD_{average\ without\ fungi}$$

IC₅₀ values are obtained using nonlinear regressions (XLStat software) according to the following formula:

$$f(x) = bottom + \frac{top - bottom}{1 + (x \times 10^{-\log IC_{50}})}$$

“Bottom”: is the value to which the curve converges for high HE values (total inhibition in the ideal).

“Top”: corresponds to the value for no inhibition.

“logIC₅₀”: corresponds to the logarithm in base 10 of the IC₅₀.

The net OD values are used as dependent values and the logarithms of the product concentrations as explanatory values. The model gives an R² value associated with log IC₅₀ (named pr3), which is then expressed to obtain the IC₅₀ of each product according to the following formula: IC₅₀ = 10^{pr3}. For each modality, three independent replications are performed (El-Alam *et al.*, 2020).

b. Blumeria graminis f. sp. Tritici (Bgt)

The fungus was grown on wheat cv. Rubisko in a culture room until it was strongly sporulating. Bgt being an obligate biotroph, it is not possible to grow it on PDA medium. However, Bgt spores are able to survive and germinate in 24 to 48 h on agar (1%) agar medium (De Waard, 1971; Reignault *et al.*,

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2001). Antigerminative tests were therefore performed *in vitro* in order to demonstrate a possible direct inhibitory effect on the germination of Bgt spores.

A positive control was carried out using the same range of concentrations of the fungicide previously used and registered for use on field crops against powdery mildew, Aviator XPRO.

c. *In planta* antifungal activity

The *in planta* antifungal tests were carried out on the wheat (Rubisko cultivar, intermediate sensitivity)-Oidium (*Blumeria graminis* f. sp. *tritici*) pathosystem as a preventive treatment. Wheat was grown on trays containing standard horticultural potting soil (3 trays per condition) each containing 24 plants, i.e. 72 plants per condition. Ten days after sowing, the wheat received a foliar treatment based on EO, in order to demonstrate a potential bioprotective effect. The control (distilled water) is not treated and allows us to verify if the infection is normal, a condition with the formulation alone (tween 0.05% and 0% of EO) is also carried out in order to verify the origin of the observed effects. 48 h after these treatments, the wheat is infected by a suspension of powdery mildew spores (1.5×10^5 spores.ml⁻¹ (Malassez counting cell) suspended in FC43 (heptacosafuorotributhylamine, 3M Electronics Materials Solutions Division, Zwijndrecht, Belgium). The evaluation of the protection rate is performed 12 days after infection by counting the number of conidia formed on leaves 1 and 2.

d. *In vitro* herbicidal activity

The herbicidal property of EO was studied on ryegrass (monocotyledonous) and lettuce (dicotyledonous), two plant species which are widely used in phytotoxicity tests (Park *et al.*, 2016). The effects of the EO were evaluated on seedlings' growth of the two plant species in a randomized experimental design.

The protocol for measuring herbicidal activity was adapted from the work of Judd *et al.*, (2015) and Lyu *et al.*, (2018). The water-agar medium (1%) was cooled to 50°C before introducing the EO from 0.005 to 0.5% and casting it into the 12x12 cm square petri dishes. A volume of 60ml of agar medium is prepared for each dish. A positive control prepared in the same way and containing a non-selective systemic herbicide of reference (Glyphosate 1%). 12 seeds are placed at regular spacing on the agar in Petri dishes.

The plates are then placed vertically in growth cabinets (photoperiod of 12 h; daytime temperature of 20 C and nighttime temperature of 12°C; constant hygrometry of 70%). Each trial was repeated three

times independently. After a period of 8 days, root length is measured with a ruler and the IC₅₀ is also calculated relative to the control root length.

2. Healthcare product applications

a. Anti-inflammatory activity

Cell Lines and Culture Conditions

Human bronchial epithelial cells (BEAS-2B) were cultured in CellBIND® surface plastic flasks (Corning - Thermo Fisher Scientific, Illkirch, France) in LHC-9 medium and incubated at 37 °C in a humidified atmosphere containing 5 % of CO₂ (Kfoury *et al.*, 2016).

b. EO cytotoxicity assessment

The EO cytotoxicity was first assessed at different EO concentrations (*i.e.*, 7.5×10^{-4} to 1.5 g.L^{-1}) using the BEAS-2B cell line. BEAS-2B cells, exposed or not to the different EO, were seeded in 96-well CellBIND® microplates at a density of 20 000 or 10 000 cells/200 µL medium for 24 or 48 h of exposure, respectively. Cytotoxicity was then evaluated by measuring the extracellular lactate dehydrogenase (LDH) activity in supernatants (Cytotoxicity Detection Kit LDH, Roche Diagnostics, Neuilly-sur-Seine, France), and mitochondrial dehydrogenase (MDH) activity (Cell Proliferation Reagent WST-1, Roche Diagnostics, France), as previously described (Dergham *et al.*, 2015; Kfoury *et al.*, 2016). Cells not exposed to EO and exposed to Triton X-100 (1 %) were used as negative and positive controls, respectively.

c. Cell Exposure for the Study of EO Anti-Inflammatory Potential

The anti-inflammatory potential of EO was assessed through a method adapted from Abarca *et al.*, 2016. BEAS-2B cells were previously seeded in 96-well CellBIND® microplates, in order to obtain 40,000 cells/well at the end of the exposure time. Cells were then exposed to PM_{2.5}, *i.e.* particulate matter with an aerodynamic diameter inferior to 2.5 µm (15 µg.cm^{-2}) to induce an inflammatory response (Cachon *et al.*, 2014; Dergham *et al.*, 2015). Particles were prepared in the LHC-9 culture medium at the desired concentration in order to reach the final concentration (*i.e.* 15 µg.cm^{-2}) in the well, and sonicated for 5 min before intoxication for a better homogenization (Cachon *et al.*, 2014; Piao *et al.*, 2018). The microplates were then incubated for 48 h, in the presence of EO ($1.5 \times 10^{-3} \text{ g.L}^{-1}$). Cells unexposed to EO and unexposed to both EO and PM_{2.5} were used as positive and negative controls, respectively. After 48 h of exposure, aliquots of cell-free culture supernatants were collected and frozen (-80°C) for the determination of cytokines' concentrations and total protein content analysis.

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Cytokines' concentrations were determined by a sandwich-based Elisa analysis (Quantikine ELISA Kits, R&D Systems Europe, Ltd., Abingdon, UK) and the total protein contents in cell-free culture supernatants were quantified using the BiCinchoninic Acid kit (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Two interleukins, namely IL-6 and IL-8, were selected as cytokines of interest for the assessment of the anti-inflammatory potential of the tested EO, given their known implication as pro-inflammatory mediators in cells exposed to atmospheric particles (Abarca *et al.*, 2016; Kfoury *et al.*, 2016).

d. Antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to study the antioxidant activity of angelica EO. A range of EO concentrations (up to 17 g.L⁻¹) was prepared in ethanol. 1 mL of each ethanolic EO solution was then mixed with 2 mL ethanolic DPPH^{*} solution (0.1 mM). All mixtures were shaken in the dark at 20 ± 1 °C and the absorbance of the solution was measured using a UV/Vis spectrometer (Lambda 2S, Perkin Elmer) at 517 nm after 1, 2, 3, 4 and 5H. The radical scavenging activity was evaluated using the following equation:

$$\text{Antioxidant activity (AA\%)} = \left(1 - \frac{A_S}{A_0}\right) \times 100$$

where A_S and A_0 stand for the absorbance of the sample and of the blank, respectively. The results obtained at each time were expressed as EC₅₀ values, defined as the concentration of antioxidant that causes a 50% decrease in the DPPH^{*} absorbance, determined by graphical interpolation. In addition, EO EC₅₀ values were compared with those obtained for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox[®]), a water-soluble vitamin E analog, with results expressed as Trolox[®] equivalent antioxidant capacity (TEAC).

IV. Statistical analysis

Statistical analyses were performed using XLSTAT software (v2021.1.1; Addinsoft, Paris, France). Normality of the data was first subjected to the Shapiro–Wilk test. Depending on the result of this test, parametric (ANOVA) or non-parametric (Kruskal–Wallis or Mann-Whitney) tests were used, followed by Fisher's post-hoc test (Least Significant Difference, LSD). For biological properties tests, IC₅₀ values were determined using nonlinear regression analysis from three replicates and are expressed as the mean value plus one standard deviation.

RESULTS AND DISCUSSION

In this study, natural plant compounds, EO, distilled from the aromatic plant, *A. archangelica* L. cultivated on TE-polluted soil, were evaluated for their potential valorization as effective alternatives to conventional pesticides (anti-fungal and herbicidal activities), but also for their use as health product (antioxidant and anti-inflammatory properties). The influence of TE soil pollution and mycorrhizal inoculation on the chemical composition of angelica EO was first assessed vs cultivation time (2020 and 2021).

I. Chemical composition of angelica EO

Table 1: Chemical composition of angelica EO (in %) in non-inoculated (NI) and inoculated (I) conditions and commercial EO, RT—retention time; KI*—Retention indices calculated relative to n-alkanes (C-8 to C-40) using capillary column ZB-5MS; KI—Retention indices found in the literature and The Pherobase.

Compounds	RT	KI*	KI	2020		2021		Commercial EO
				NI	I	NI	I	
α -Pinene	1.262	933	918	7.36	7.41	5.1	5.0	7.02
Sabinene	1.467	961	973	0.43	0.40	0.21	0.26	0.45
β -Pinene	1.491	966	980	0.71	0.71	0.61	0.59	0.77
β -Myrcene	1.552	979	991	1.43	1.31	0.61	0.91	1.65
α -Phellandrene	1.644	999	1005	3.26	3.27	2.92	2.87	4.20
β -Phellandrene	1.795	1026	1031	76.27	76.30	74.31	73.92	80.16
β -Ocimene	1.868	1040	1050	0.50	0.55	0.11	0.12	0.40
Ylangene	3.705	1370	1375	0.24	0.24	0.37	0.39	0.15
Copaene	3.735	1376	1377	0.40	0.39	0.56	0.56	0.17
β -Bourbonene	3.782	1385	1384	0.23	0.22	0.22	0.22	0.09
β -Elemene	3.809	1390	1391	0.25	0.29	0.38	0.35	0.14
Humulene	4.154	1457	1440	1.40	1.38	1.59	1.63	1.63
α -Curcumene	4.446	1495	1517	0.12	0.17	0.22	0.24	--
β -Curcumene	4.458	1553	1513	2.06	2.11	3.14	3.21	--
1,8-bis (dimethylamino) naphthalene	4.463	1519	--	0.64	0.67	0.64	0.67	--
Monoterpenes				89.46	89.40	83.76	83.55	94.25
Sesquiterpenes				5.20	4.85	9.01	9.06	3.17
Others				4.70	4.85	9.01	9.06	3.17

The results of Table 1 revealed that the compositions of angelica EO produced in 2020 and 2021 are comparable. Indeed, EO from seeds of angelica contained mostly monoterpenes and sesquiterpene, the major compounds detected were: β -phellandrene (73 - 80%), α -pinene (5 - 7%), α -phellandrene

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(2 - 4%), and β -myrcene (0.5 - 2%) (Table1). These EO compositions were consistent with the literature which describes as main components in angelica seeds EO: β -phellandrene (65 to 76%) followed by α -pinene (2 to 2.6%) and α -phellandrene (2 to 2.6%) (Nivinskienė *et al.*, 2005; Nivinskiene *et al.*, 2007; Kylin, 2010). Interestingly, it is important to note that the EO compositions are not altered by the presence of TE in the soil, nor by mycorrhizal inoculation, or culture time. These results inconsistent with some studies that found that the EO composition of some aromatic plants such as *Coriandrum sativum* L. was modified by mycorrhization (Kapoor *et al.*, 2002; Israel *et al.*, 2022). However, they are in accordance with the findings of Raveau *et al.*, (2021b) which showed that mycorrhizal inoculation of *Coriandrum sativum* L. and *Salvia sclarea* L., did not lead to significant modifications in EO compositions. Although the literature has reported many factors that can influence the EO composition such as the water deficiency, the nature of the soil, or the amount of sunshine, the data are often contradictory (Khalid, 2006; Sowndhararajan *et al.*, 2017).

Moreover, the pollution effect is also controversial. Whereas some studies have shown that the presence of TE in the soil, affected EO chemical composition, some others reported no influence (Biswas *et al.*, 2011; Prasad *et al.*, 2014; Raveau *et al.*, 2021b). In particular, it has been suggested that TE-induced stress in plants which could lead to changes in secondary metabolism (Prasad *et al.*, 2014; Skrypnik *et al.*, 2022). For example, peppermint (*Mentha \times piperita* L.) exposed to 40 mg L⁻¹ of Cd, decreased menthol content from 54.1 to 40.8%, but increased menthofuran and pulegone contents by 5.7 and 1.2%, respectively, compared with the control (Azimychetabi *et al.*, 2021). However, these changes vary considerably depending on the species of aromatic plant, their ability to accumulate TE, the type of TE and their phytotoxicity (Biswas *et al.*, 2011; Prasad *et al.*, 2014). Indeed, reactive oxygen species produced in response to TE can lead to lipid peroxidation, which stimulates the formation of highly active signaling compounds capable of inducing the production of secondary metabolites and thus modifying EO composition (Aqeel *et al.*, 2023).

As the chemical compositions of the EO angelica distilled from 2020 and 2021 cultures, NI and I, are similar (Table 1), one batch of EO (NI 2020) was selected for all the biological activity.

II. Healthcare product applications

1. Antioxidant Activity

This assay determined the ability of an antioxidant compound to donate hydrogens to reduce the stable DPPH^{*} to nonradicals. The EC₅₀ as well as the Trolox[®] equivalent antioxidant capacity (TEAC) values for the angelica EO are presented in Figure 1. Indeed, their efficacy was compared to a well-known antioxidant standard, Trolox[®], and expressed in terms of TEAC, which is inversely proportional

to the EC₅₀ values obtained. These values are <1 μmol eq Trolox[®]/g EO at 1H and close to 2 after 5H. It is difficult to compare the results with literature as the assay are always carried out under the same conditions. For example, in a previous study, values up to 7608 were obtained in *Pimenta racemosa* EO, which contains more phenols than angelica EO (Kfoury *et al.*, 2015). However, a comparative study of 13 EO was performed and angelica seeds exhibited moderate antioxidant activity compared for example to Jasmine EO (Wei & Shibamoto, 2007). In another study, the obtained values are close to those obtained with sage inflorescence and coriander seed EO (Raveau *et al.*, 2021b). Angelica seeds EO is mainly composed of mono- and sesquiterpenes, which are generally described for their antioxidant activities (Kfoury *et al.*, 2016; Forycka & Buchwald, 2019; Bliss *et al.*, 2022). Additionally, sabinene, present in angelica EO at 0.2 to 0.4, is also reported for his strong reducing power and nitric oxide scavenging activities (Sowndhararajan *et al.*, 2017).

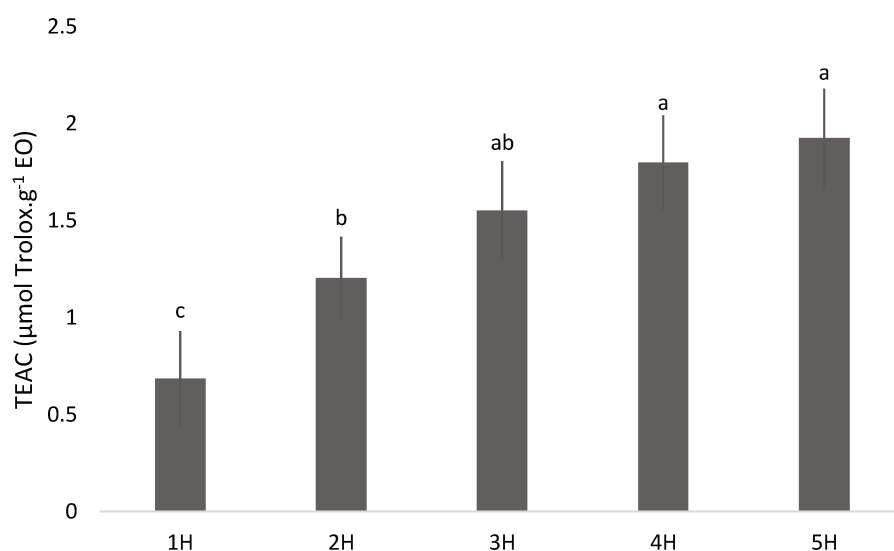


Figure 5: Kinetics of antioxidant activity of angelica EO. Significant differences using a one-way ANOVA comparison ($\alpha = 0.05$), between results are indicated by different letters.

2. Anti-inflammatory activity

Before evaluating the anti-inflammatory potential of our EO, we determined the non-cytotoxic dose to be applied to the BEAS-2B cell line using two complementary cytotoxicity tests: lactate dehydrogenase activity (LDH) and mitochondrial activity (MDH) (data not shown). For the LDH test which allows us to measure the alteration of the membrane, we can notice toxic effects from the concentration of 7.5 mg/L, with high cytotoxic effects after exposure to the highest concentration (1500 mg/L). Concerning MDH test, cytotoxic effects were observed after exposure 15 mg/L of angelica EO. Thus, concentrations of 0.9375, 1.875 and 3.75 mg/L do not present any significant cytotoxic effect on the BEAS-2B cell line. In order to study the potential anti-inflammatory effect of angelica EO, we

therefore decided to retain the concentration 1.5 mg/L which, according to our results, does not present any cytotoxic effect.

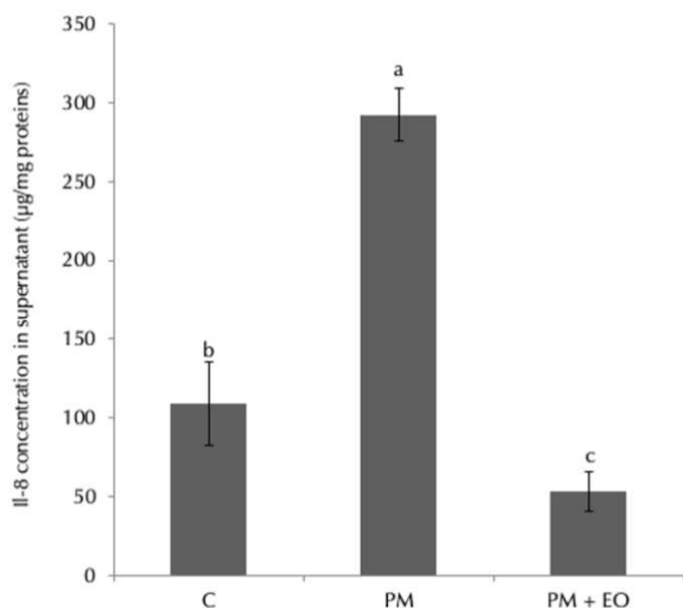


Figure 2: Cytokine levels in BEAS-2B cells exposed for 48 h to PM2.5 in the presence of angelica EO: (C) control, (PM) PM2.5, (PM + EO) Angelica EO NI 2020 - $1.5 \times 10^{-3} \text{ g.L}^{-1}$. Data are presented as mean \pm standard deviation ($n = 3$). Significant differences using a two-way ANOVA comparison ($\alpha = 0.05$), between results are indicated by different letters. BEAS-2B: human bronchial epithelial cells; IL-8: interleukin 8; PM2.5: particulate matter with an aerodynamic diameter inferior to $2.5 \mu\text{m}$.

Then, in order to evaluate the anti-inflammatory effect of angelica EO on the BEAS-2B lung cell line, we previously induced an inflammatory response in the cells by the addition of fine atmospheric particles ($\text{PM}_{2.5}$), a toxic agent known to trigger an inflammatory and oxidative stress on pulmonary cells (Kfoury *et al.*, 2016). The inflammatory response is then evaluated by measuring interleukin 8 (IL-8) which is secreted by cells during exposure to pro-inflammatory stress. The Figure 2 reveals an inflammatory response after PM exposure with a 3-fold increase in the concentration of IL-8 compared to the control. Moreover, the addition of angelica EO caused a significant drop in IL-8 concentration. Indeed, the concentration of IL-8 decreases by 6 for angelica EO. These results therefore underline the anti-inflammatory effect of angelica EO.

Previous studies have shown that several components present in EO such as trans-anethole, estragole, eugenol and isoeugenol, tested alone, had the ability to protect cells against inflammation (Kfoury *et al.*, 2016), and similar effect was observed with coriander EO (Raveau *et al.*, 2021). Regarding angelica EO, Fraternali *et al.* (2018) demonstrated its anti-inflammatory properties with a decrease in the level of the pro-inflammatory interleukin-6 in human umbilical vein endothelial cells, as a model of *in vitro* senescence, after cells exposure to lipopolysaccharide, one of the most powerful pro-inflammatory. In addition, the anti-inflammatory activity of angelica EO may derive from one or a combination of its

components, such as α -phellandrene, which has been identified as having powerful anti-inflammatory and antioxidant effects (Bliss *et al.*, 2022). Indeed, this compound is known to reduce TNF- α -induced cytokine expression, with benefits in inflammatory diseases such as rheumatoid arthritis, osteoarthritis and allergic conditions (Sosa *et al.*, 2023).

III. Crop protection applications

1. *In vitro* tests

a. Growth inhibition of *F. culmorum*, *F. graminearum*, *Z. tritici*, *B. graminis* and *P. infestans*

Numerous studies have demonstrated that the biological activities of EO are the result of synergistic action between their compounds, rather than activity linked to that of a majority compound (Bakkali *et al.*, 2008; Freires *et al.*, 2014). Moreover, the mechanism of action of EO on fungi has not been fully elucidated, studies have highlighted interactions between certain EO compounds and the tyrosine-tRNA ligase of various fungi, suggesting possible alteration of fungal protein synthesis as a mechanism of fungitoxicity (Osorio *et al.*, 2021; Dalcin *et al.*, 2022). Others have suggested that damage occurs in fungal cell envelopes, particularly mitochondria (Nogueira *et al.*, 2010; Oliveira *et al.*, 2016). Several key features of antifungal activity have nevertheless been highlighted, such as inhibition of fungal cell wall formation, inhibition of ergosterol synthesis, cell division, efflux pumps and interference with RNA, DNA and protein synthesis (Lagrouh *et al.*, 2017; Nazzaro *et al.*, 2017; Raveau *et al.*, 2020, 2022). Angelica EO possess antifungal activities with IC_{50} ranging from 21 for *Z. tritici* to 221 $mg.L^{-1}$ for *B. graminis*. (Table 2). At the end of these tests, the subculture of these fungal species (*F. culmorum*, *F. graminearum* and *P. infestans*) on an EO-free medium demonstrated the fungistatic activity of angelica EO on the studied fungal pathogens. However, in all cases studied, the IC_{50} of angelica EO are statistically higher to those of the fungicide, Aviator, used as conventional positive control with values ranging from $3.24 \cdot 10^{-2}$ for *F. graminearum* to $3.54 \cdot 10^{-1} mg.L^{-1}$ for *F. culmorum*. Nevertheless, angelica EO activity remains promising given that unlike the reference plant protection product, the EO have not been formulated. As EO are highly volatile compounds, a formulation aimed at limiting their volatility could both reduce the quantity of EO used, and prolong their activity, thus limiting the need for repeated treatments and increasing their effectiveness.

Table 2: Average IC_{50} ($mg.L^{-1}$) of angelica EO and Aviator against plants fungal pathogens: *F. culmorum*, *F. graminearum*, *Z. tritici*, *B. graminis*, and, *P. infestans*.

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Fungal Phytopathogens	Aviator	Angelica EO
<i>F. culmorum</i>	3.54 10 ⁻¹ ± 7.17 10 ⁻² A	198.0 ± 238 B
<i>F. graminearum</i>	3.24 10 ⁻² ± 8.34 10 ⁻² A	92.5 ± 75.9 B
<i>Z. tritici</i>	5.50 10 ⁻² ± 3.83 10 ⁻² A	21.0 ± 6.19 B
<i>B. graminis</i>	1.93 10 ⁻¹ ± 2.59 10 ⁻² A	221.0 ± 186 B
<i>P. infestans</i>	3.38 10 ⁻⁵ ± 1.83 10 ⁻⁶ A	128.0 ± 6.78 B

Mann-Whitney, $\alpha=0,05$ performed independently for each pathogen

To date, fungistatic effects of *Cinnamomum cassia* and *C. zeylanicum* EO have been demonstrated on *F. culmorum* and *F. graminearum* and to a lesser extent on *P. Infestans*, including a fungistatic effect of *angelica* seed and roots EO against *F. culmorum* (El-Alam *et al.*, 2020; De Clerck *et al.*, 2020; Raveau *et al.*, 2022). However, the biological effects of EO are mostly dose-dependent (De Clerck *et al.*, 2020). Indeed, selecting the appropriate concentration is crucial to maximize efficacy while minimizing adverse effects (Tisserand & Young, 2014). A thorough understanding of the dose-response relationship is essential for optimal use of EO (Raut & Karuppaiyil, 2014). It has been described that, angelica EO has strong antibacterial activity against *Clostridium difficile*, *C. perfringens*, *Enterococcus faecalis*, *Eubacterium limosum*, *Peptostreptococcus anaerobius*, and *Candida albicans* (Sowndhararajan *et al.*, 2017). In the present study, antifungal activity of angelica EO against *F. culmorum*, *F. graminearum*, *P. Infestans* and *Z. tritici* has been evidenced. For example, sabinene, α -pinene, β -elemene, cis- β -ocimene, and curcumene which represent around 8% of total angelica EO composition are reported for antifungal activity against *Aspergillus*, *Trichophyton* or *Candida spp* (Roh & Shin, 2014; Cavaleiro *et al.*, 2015; Sowndhararajan *et al.*, 2017; Zhan *et al.*, 2021). In addition, Zhan *et al.* 2021 showed a decrease in *Saccaromyces cerevisiaea* membrane fluorescence when treated with EO from *Chrysanthemum morifolium cv. Fubaiju*, suggesting that the above-mentioned EO components caused severe damage to the cytoplasmic membrane, up to and including its total destruction. This characteristic could be valuable in preventive applications as a means of controlling fungal diseases before and after harvest. Although EO IC₅₀ are higher than those of marketed chemical fungicides, the EO tested still showed interesting antifungal activity. Furthermore, EO are known to be less harmful to the environment, human health and non-target organisms than conventional fungicides (De Clerck *et al.*, 2020). In particular, because of their low persistence in soils, as well as their volatile nature (De Clerck *et al.*, 2020). What is more, unlike pesticides, they do not lead to the development of resistance in target pest species (Acheuk *et al.*, 2022; Khursheed *et al.*, 2022). This

could be explained by the complexity, diversity and large number of components in EO, or their less frequent use.

b. Herbicidal activity

In addition to antifungal properties, the angelica EO revealed a significant herbicidal activity (Table 3), on both lettuce and ryegrass, two plant species frequently used in phytotoxicity tests (Park *et al.*, 2016). For the lettuce, the IC₅₀ of angelica EO was significantly higher than that obtained with the control (glyphosate). On the other hand, we note an interesting herbicidal activity of angelica EO against ryegrass, since the IC₅₀ of EO and the positive control are not statistically different.

Table 3: Average IC₅₀ (mg.L⁻¹) of angelica EO against lettuce and ryegrass.

Plants	Glyphosate	Angelica EO
Ryegrass	1.47 ± 6.91 10 ⁻¹ A	144 ± 82.9 A
Lettuce	4.65 10 ⁻¹ ± 4.26 10 ⁻¹ B	5390 ± 4760 A

Letters indicate a significant difference (Student, α= 0,05) performed independently for each plant species

Regarding the phytotoxic effects of EO, some authors have concluded that EO affect membrane lipid organization and domain formation, particularly in the case of monoterpenes, while phenylpropanoids interact with plasma membrane receptors (Lins *et al.*, 2019). For example, Citronella EO components reduced germination of *Digitaria horizontalis* and *Cenchrus echinatus*, and decreased their chlorophyll and total protein content (Ootani *et al.*, 2017). Citronella EO also caused cuticular wax rupture, stomatal obstruction and rapid electrolyte leakage, as a consequence of disrupted membrane integrity (Singh *et al.*, 2006). In addition to its activity on the membrane, Citronella EO has also been shown to affect the structure of microtubules in plant cells (Bakkali *et al.*, 2008; Dhifi *et al.*, 2016). Similarly to angelica seed EO, lemongrass EO contains a majority of monoterpenes, which are the source of its biological activity (Kakaraparthi *et al.*, 2014).

2. In planta tests

Tests of protection wheat against powdery mildew by preventive treatments of angelica EO were performed *in planta*. The application of 5 % angelica EO resulted in a significant decrease, estimated to 35 %, in the number of colonies per plant by comparison to the non-treated wheat control. Thus, angelica EO allowed a wheat protection of 40% (Figure 3). To our knowledge, there is few studies evaluating EO antifungal activity *in planta*. Only, Hafez (2008) demonstrated that formulated *Nigella sativa* EO, rich in monoterpenes and sesquiterpenes (Benkaci–Ali *et al.*, 2007) like angelica EO, was

effective as a curative treatment on barley and cucumber powdery mildew. These authors showed a strong toxic effect on hyphal growth and spore germination treated with *Nigella sativa* EO, with damaged conidia and an inhibition of hyphal formation (Hafez, 2008). The inhibition of spore germination and hyphal growth could be explain by an increase of H₂O₂ and O₂, as well as ascorbate peroxidase and glutathione-S-transferase activities in cucumber and barley treated with *Nigella sativa* EO (Hafez, 2008). Our results show a lesser efficacy, which may be explained by the fact that in the present study angelica EO was used as a preventive treatment 48 h before infection, and not as a curative, which may tend to minimize its potential as a curative treatment for wheat powdery mildew.

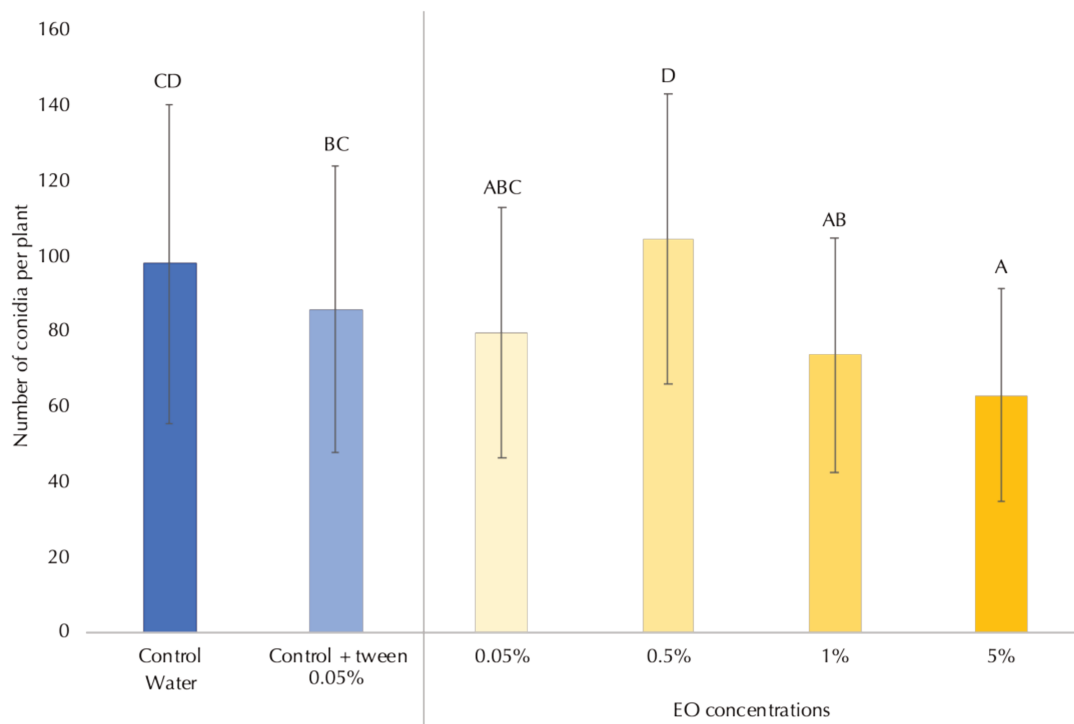


Figure 3: Bioprotection tests of wheat against powdery mildew. Mean values \pm standard deviation ($n=72$). Letters indicate a significant difference (Kruskal-Wallis, $\alpha = 0.05$). Control: water.

CONCLUSION

Analysis of the chemical composition revealed that both TE soil pollution mycorrhizal inoculation and cultivation time did not alter the chemical composition of angelica EO. Different biological activities for angelica EO were highlighted in this study and are probably attributed to the presence of sabinene, α -pinene, β -elemene, cis- β -ocimene and β -curcumene, as well as to their synergistic effects. Indeed, angelica EO showed moderate antioxidant and a significant anti-inflammatory properties, suggesting potential applications in the health sector. In addition, this EO is particularly promising for its potential use as an alternative to conventional pesticides. *In vitro* tests revealed promising antifungal and herbicidal activities for angelica EO against *F. culmorum*, *F. graminearum*, *Z. tritici*, *B. graminis*, and *P. infestans*. Furthermore, significant herbicidal activity against ryegrass suggests its potential use in weed management strategies. . This study highlights the potential of angelica EO distilled from plants grown on TE polluted soil, in the protection of culture and health sector. To complete this work, further studies are needed to explore its efficacy under real conditions and to optimize formulations for practical applications.

Author Contributions:

Conceptualization, J.L., A.L.-H.S. and J.F.; methodology, N.F., F.L., J.L., S.F., A.V., A.L.-H.S., and J.F.; writing—original draft preparation, J.L.; writing—review and editing, J.L., A.L.-H.S., S.F., A.V., and J.F.; supervision, A.L.-H.S. and J.F.; project administration, A.L.-H.S.; funding acquisition, A.L.-H.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest:

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Publication 3

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Angelica cultivation and mycorrhizal inoculation improve microbial diversity, functions and network complexity of trace elements-polluted soil: a three-year field study

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Présentation de la publication 3

La qualité biologique d'un sol dépend de la diversité et de l'abondance des différents organismes qui le composent. Par exemple, les communautés microbiennes jouent un rôle fondamental dans le fonctionnement du sol puisqu'elles sont impliquées dans les cycles géochimiques du carbone et de l'azote, la nutrition des végétaux ou même l'immobilisation des ET dans le sol (Saccá et al., 2017). Aussi, la modification de la structure des communautés microbiennes telluriques par les ET peut affecter durablement la fonctionnalité du sol et par conséquent les services écosystémiques rendus par celui-ci (Antoniadis et al., 2017; Gong et al., 2018).

Par ailleurs, le microbiote du sol est susceptible d'être façonné par la présence d'un couvert végétal. En effet, l'espèce végétale a une importance prépondérante, puisque la structure des communautés microbiennes du sol rhizosphérique évolue en fonction de la nature et de la quantité des exsudats racinaires libérés dans le sol (Broeckling et al., 2008; Dagher et al., 2019). De même, les CMA en modifiant la nature des exsudats racinaires de leur plante hôte ou en produisant directement leurs propres exsudats sont également susceptibles d'influencer les communautés microbiennes (Gui et al., 2017).

C'est pourquoi, le travail présenté dans ce troisième article, a pour principal objectif d'étudier l'impact de la culture de l'angélique, sur le fonctionnement microbiologique du sol. Cet aspect, fait partie des critères d'évaluation de la filière angélique-HE puisque les méthodes de phytomanagement visent à maintenir ou améliorer la qualité biotique des sols pollués en augmentant la biodiversité et l'activité microbienne. Pour ce faire, nous avons suivi différents indicateurs de fonctionnalité du sol pendant trois années successives (état initial avant végétalisation, première année et deuxième année de récolte de l'angélique cultivée en présence et en absence d'un inoculum mycorhizien) : (i) la biomasse microbienne (bactérienne et fongique) en quantifiant les marqueurs lipidiques spécifiques des différents groupes microbiens (acides gras associés aux phospholipides (AGPL)), (ii) la structure de ces communautés microbiennes par une approche de métagénomique, en ciblant spécifiquement les régions V3-V4 de l'ADNr 16S bactérien, et les régions ITS3-ITS4 fongiques, et (iii) le potentiel métabolique globale du sol (Biolog Ecoplates).

Nos résultats ont montré que la biomasse bactérienne était majoritaire dans notre sol pollué ; la biomasse fongique représentant moins d'un tiers de la biomasse microbienne totale. Avant la mise en place de la culture, les phyla bactériens majoritaires étaient les Actinobacteriota (57 %), Proteobacteria (24 %), et Gemmatimonadota (7 %), ainsi que les Ascomycota (98 %) pour les phyla fongiques. Certains

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de ces phyla (Actinobacteriota, Proteobacteria et Ascomycota) sont décrits comme des taxons tolérants aux ET (Chen et al., 2018; Cui et al., 2018; Lin et al., 2019). Les indices de richesse (Chao1) et de diversité (Shannon) pour les bactéries et les champignons étaient respectivement de 750,5 ; 4,8 ; 540,9 et 3,6, et étaient comparables à ceux obtenus sur des sols non pollués (Gui et al., 2017; Morvan et al., 2020).

La végétalisation du site pollué avec l'angélique a permis d'augmenter significativement la biomasse microbienne, la richesse et capacité métabolique bactériennes, ainsi que la complexité des interactions entre les différents micro-organismes du sol. Après trois années de culture, une sélection d'espèces fongiques et bactériennes impliquées dans la dégradation de la matière organique a été observée, probablement en lien avec un enrichissement observé de celle-ci dans le sol. L'inoculation mycorhizienne a également eu une influence positive sur l'abondance des gènes bactériens liés au cycle du carbone et la complexité du réseau microbien. Ce travail fournit de nouvelles informations sur l'évolution du microbiote d'un sol historiquement pollué par des ET après trois années de végétalisation et sur sa requalification écologique.

Dans cette étude, j'ai participé aux prélèvements et le conditionnement des échantillons de sols. J'ai réalisé l'ensemble des étapes d'extraction, et d'amplification d'ADN. Le séquençage des amplicons a été réalisé par Génome Québec. Le traitement bio-informatique des données de séquençage a été réalisé par mes soins. Les analyses Biolog ecoplate ont été effectuées en collaboration avec J. Duclercq (UPJV). Je me suis également chargé des analyses statistiques concernant les parties sus-citées. L'exploitation des données ainsi que la rédaction du manuscrit sont le fruit d'un travail collectif avec les co-auteurs.

Publication 3 : *Angelica* cultivation and mycorrhizal inoculation improve microbial diversity, functions and network complexity of trace elements-polluted soil: a three-year field study

Abstract

Soil microbial communities play a central role in natural and agricultural systems by providing fundamental ecosystem functions such as nutrient cycling and plant productivity. However, the bacterial and fungal rhizospheric microbiota of aromatic plants, such as angelica (*Angelica archangelica* L.), remains unexplored, particularly in phytomanagement strategy for aged trace elements (TE)-polluted soils. Thus, the present study aims to investigate the biomass, composition, functional diversity, and network complexity of soil microbial communities (fungi and bacteria) and their evolution after 3 years of *in situ* angelica cultivation, inoculated or not with arbuscular mycorrhizal fungi (AMF). The results showed that although the cultivation of angelica had little influence on fungal richness and diversity, the biomass and diversity of bacterial communities increased significantly, as did the complexity of interactions between various microorganisms in the polluted soil compared to the initial state (before cultivation). Saprotrophic fungi became significantly more abundant after angelica cultivation, contributing to increased soil organic carbon (C) and organic matter content. AMF inoculation enhanced microbial network complexity from the second year of angelica cultivation. Finally, a significant improvement in the abundance of functional genes linked to the C cycle was demonstrated for 2020, and 2021 compared to the initial state. This study provided new insights into the ecological requalification of TE-polluted soil and the evolution of fungal and bacterial microbiota after 3 years of growing angelica in aged TE-polluted soil.

Key words: trace elements, heavy metals, angelica, polluted soil, ecological requalification.

Introduction

Soil microbiota performs fundamental ecosystem functions and plays an important role in nutrient cycling and food production (Qu *et al.*, 2020). Unfortunately, increasing pollution, often originating from human activities, has rendered vast areas unsuitable for cultivation, posing danger to wildlife and humans, and rendering them uninhabitable for many living microorganisms (Jiang *et al.*, 2023). Indeed, besides inhibiting biological processes in ecosystems, TE are known to affect the abundance, diversity, activity, and functions of soil microorganisms (Jiang *et al.*, 2023). Furthermore, TE are also responsible for soil acidification and the depletion of organic matter (OM), humic acids, and, by extension, nutrients (Gupta & Germida, 1988; Epelde *et al.*, 2014; Gutiérrez *et al.*, 2016). It is therefore essential to reduce TE toxicity in polluted soils to restore soil ecosystem services. Plant management technologies, such as phytostabilization, are viable options for managing TE-polluted soils from both ecological and economic standpoints (Kidd *et al.*, 2015; Mench *et al.*, 2019; Raveau *et al.*, 2021). Consequently, the selection of TE-tolerant plant species that grow rapidly and produce significant, valuable biomass is crucial to successful phytomanagement (Kidd *et al.*, 2015; Deng *et al.*, 2020; Raveau *et al.*, 2021).

In that view, the cultivation of aromatic plants for the reclamation of TE-polluted lands appears as a promising approach (Perlein *et al.*, 2021; Raveau *et al.*, 2021). Indeed, in addition to producing high value-added molecules such as essential oils (EO), aromatic and medicinal plants release root exudates capable of selecting microbial communities from polluted soils, offering potential TE phytostabilization (Qu *et al.*, 2020; Greff *et al.*, 2023).

Belonging to the Apiaceae family, angelica (*Angelica archangelica* L.) possesses characteristics that make it a promising candidate for phytostabilization of TE-polluted soils. This plant species is known for its ability to thrive in challenging environments (Kylin, 2010; Chauhan *et al.*, 2016; Rautio *et al.*, 2016). Additionally, it exhibits strong growth, and its seeds are rich in active EO, high value-added compounds that can potentially make angelica cultivation on polluted soils economically viable (Kylin, 2010).

Moreover, it is known that arbuscular mycorrhizal fungi (AMF) inoculation can promote the growth and development of medicinal plants. Indeed, these symbiotic fungi significantly contribute to the uptake of soil nutrients, particularly phosphorus (Javot *et al.*, 2007), as well as nitrogen, calcium, sulphur, and potassium, thereby increasing plant biomass but also enhancing plant tolerance to soil pollution (Smith & Read, 2008; Meier *et al.*, 2012). AMF enable host plants to thrive under stressful conditions, especially in TE-polluted soils (Cicatelli *et al.*, 2014), by reducing the bioavailability of

metals through the production of insoluble glycoproteins with soil-binding properties (glomalin) or by sequestering TE within hyphae (González-Chávez *et al.*, 2004; Ciatelli *et al.*, 2014; Ferrol *et al.*, 2016). Additionally, AMF are reported to enhance the production of secondary metabolites such as EO in aromatic plants (Zhao *et al.*, 2022). Furthermore, AMF are considered to play a pivotal role in rhizosphere microbial community assembly and succession, contributing significantly to ecological restoration efforts (Lenoir *et al.*, 2016; Ciadamidaro *et al.*, 2022).

However, the influence of the aromatic plants, particularly angelica cultivation, on the microbial communities of a polluted soil and the restoration of ecosystem services has received little attention. Therefore, to optimize angelica potential as a phytomanagement tool, the current work aims to investigate the impact of angelica cultivation, with or without AMF inoculation, on the microbial communities of aged TE-polluted soil. To achieve this objective, we examined and monitored the biomass (phospholipid fatty acid, PLFA), diversity, and network complexity of soil bacterial and fungal communities (using meta-barcoding approach), as well as the soil metabolic potential prior and after angelica cultivation during three years.

Materials and Methods

In Situ Experimental Design

The study was conducted on an agricultural soil located in northern France (50°25'55.5" N, 3°02'25.5" E; elevation 23 m), historically polluted by TE. The experimental site was situated 500 m away from a former Pb and Zn smelter, Metaleurop Nord, which emitted substantial amounts of TE-rich dust, leading to TE-pollution of the topsoil (0–30 cm) (Sterckeman *et al.*, 2007). The topsoil is a slightly alkaline clayey silt (pH 7.9) (Table S1). It contains high amounts of Cd, Pb, and Zn (7, 394 and 443 ppm, respectively - Raveau *et al.*, 2020), which are approximately 17-, 11-, and 6-fold higher than the regional background levels for agricultural soils (Sterckeman *et al.*, 2007; Raveau *et al.*, 2020).

Angelica (*Angelica archangelica* L.), a perennial aromatic plant species, was sown in September 2018 at a rate of 300,000 seeds per hectare and cultivated for 3 years on the polluted site. Certified seeds were obtained from the French research institute ITEIPMAI. The polluted plot, covering an area of 7,100 m², was divided into two sections: a non-inoculated (NI) plot of 4,300 m² and an inoculated (I) plot of 2,800 m². The mycorrhizal inoculum AGTIV[®] (kindly provided by Premier Tech) containing spores of *Rhizophagus irregularis* (12,000 viable spores per gram of product) was used. During sowing, the mycorrhizal inoculum was directly mixed with seeds and applied at a rate of 125 g/ha.

Sample Collection

At the onset of the experiment in 2018, before sowing, 6 soil samples representing the initial state were collected. As angelica follows a biennial development cycle, soil samples (12 in total - 6 from the NI condition and 6 from the I condition) were collected in 2020, before first seed harvest, and again in 2021, marking the second year of cultivation. To ensure comprehensive coverage of the experimental conditions, soil samples were collected using a stratified sampling method with an auger in the 0 - 20 cm soil horizon. Additionally, the samples were geo-referenced using the TDC100[®] (Trimble) and stored at - 20°C until analysis.

Phospholipid fatty acid (PLFA) Analysis

After removing plant debris from the soil samples, 3 g of freeze-dried soil were utilized for fatty acid analysis (3 replicates per sample). Lipid extraction was performed according to Frostegård *et al.* (1991), employing a solution 1:2:0.8 (v/v/v) chloroform:methanol:citrate buffer (0.15 M, pH 4.0) with agitation for 2 h. The lipid material was fractionated using Solid Phase Extraction columns containing silica (6 mL volume, 500 mg sorbent, Interchim, Montluçon, France). Successive elutions with chloroform, acetone, and methanol (1:2:1, v/v/v) separated the material into neutral lipids (NLFA), glycolipids and polar lipids containing phospholipid (PLFA) (Frostegård *et al.*, 1991). The NLFA and PLFA were trans-esterified with 0.2 M KOH in methanol to obtain free fatty acid methyl esters. The resulting fatty acid methyl esters were analyzed using a gas phase chromatography-mass spectrometer (GC-MS) Shimadzu QP-2010 Ultra (Shimadzu, Japan) equipped with a single quadrupole mass detector and simultaneously coupled with a flame ionization detector (FID). Samples were analyzed in split mode (80:1 ratio) on a ZB-1MS fast capillary column (10 m length x 0.1 mm inner diameter x 0.1µm phase thickness, 100 % dimethylpolysiloxane, Zebron, Phenomenex, Torrance Calif, U.S.A.) using helium as the carrier gas at a constant linear velocity (40 cm sec⁻¹). The injector temperature was set at 280°C, and the detector temperatures were 330°C (FID) and 280°C (ion source). The temperature program began at 175°C and increased by 25°C per minute until reaching a final temperature of 275°C, held for 0.5 min. Electronic impact at 70 eV was used as ionization mode, scanning a mass range between 50.0 and 400.0 u was scanned. The single impact monitoring (SIM) mode was used simultaneously. Fatty acid quantification utilized nonadecanoic acid methyl ester (C19:0, Sigma-Aldrich) as an internal standard. Identification of fatty acids was based on relative retention time compared with commercial fatty acid methyl ester standards (47080-U Bacterial Acid Methyl Ester Mix, Sigma Aldrich) and spectra obtained from commercial standards and/or literature (NIST Standard Reference Database). Phospholipid fatty acid (PLFA) markers, i15:0, a15:0, i16:0, i17:0, a17:0 and PLFA cy17:0, C18:1ω7, and cy19:0, were employed as indicators of Gram-positive and negative bacterial biomasses, respectively (Frostegård & Baath, 1996).

DNA Extraction

Genomic DNA was extracted in triplicate directly from 250 mg fresh soil ($n = 60$) using the NucleoSpin Soil® kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The quality of the extracted DNA was assessed using 1% (w/v) agarose gels, DNA quantification was performed using a SpectraMax® iD3 spectrophotometer (Molecular Devices LLC, Sunnyvale, CA, United States). Extracted DNA was stored at -20°C until use.

For bacterial 16S rRNA gene amplification, hypervariable regions V3–V4 were targeted with the forward primer CS1_341_F (ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCAG) and the reverse primer CS2_805_R (TACGGTAGCAGAGACTTGGTCTCTGACTACCAGGGTATCTAATC) (Herlemann *et al.*, 2011). For fungal ITS region amplification, PCR was conducted using degenerated primers: forward primer CS1_ITS3_Kyo2_F (ACACTGACGACATGGTTCTACAGATGAAGAACGYAGYRAA) and reverse primer CS2_ITS4_Kyo3_R (TACGGTAGCAGAGACTTGGTCTCTBTTVCKCTTCACTCG) (Toju *et al.*, 2012). PCR amplifications were performed from 1 ng of extracted DNA in a PCR thermal cycler (Surecycler 8800, Agilent, Technologies, Les Ulis, France). CS1 (ACACTGACGACATGGTTCTACA) and CS2 (TACGGTAGCAGAGACTTGGTCT) adapters with molecular barcodes are displayed in bold. Two independent PCR reactions were performed per DNA sample.

Illumina MiSeq Sequencing

All PCR products were pooled ($n = 60$) and sent to the Genome Quebec Innovation Centre (Montreal, QC, Canada) for sequencing, using Illumina MiSeq platform, producing paired-end 2 bp \times 300 bp.

Bioinformatic Processing

The sequences data were uploaded to the Galaxy instance (v.2.3.0) of the Genotool bioinformatics platform (<http://sigenae-workbench.toulouse.inra.fr>) and processed using the FROGS (Find Rapidly OTU with Galaxy Solution) pipeline (Escudié *et al.*, 2018). Sequences containing ambiguous bases (N) or lacking specific primers were removed. Primer sequences with less than 10% difference were trimmed using Cutadapt software (Martin, 2011). Sequence clustering was performed using the SWARM algorithm (v2.1.5) (Mahé *et al.*, 2014). A two-step denoising process was applied, initially clustering sequences with minimal differences ($d=1$), followed by a second step with an aggregation distance of 3. Representative sequences for each cluster or OTU (Operational Taxonomic Units) were subjected to chimera detection using the VSEARCH algorithm (Rognes *et al.*, 2016), and those with abundance less than 2 relative to the total abundance were removed. Taxonomic classification of each OTU was performed against the Unite Fungi (v8.2) (ITS) and Silva (v138.1) (16S rRNA) databases was performed using RDP Classifier (Wang *et al.*, 2007). OTU with uncertain genus-level assignments were classified at phylum level and noted as Unclassified_phylum. Rarefaction of OTU was carried out by

setting the rarefaction value for each dataset to the abundance of the lowest sample using the *rrarefy* function in the *vegan* R package (Oksanen *et al.*, 2023). Richness (Chao1) and diversity (Shannon) indexes were calculated for each community (16S rRNA and ITS) using the *Phyloseq* R package (v1.36.0). Beta-diversity of bacterial and fungal communities were computed by principal coordinate analyses (PCoA) using dissimilarity matrices calculated with the Bray-Curtis method. For bacterial communities, the relative abundance of biogeochemical cycling genes (C, N, S) was determined using FAPROTAX (Louca *et al.*, 2016), and for fungal communities, functional groups were determined using the FUNguild classification tool (Nguyen *et al.*, 2016). The 16S rRNA and ITS sequences have been deposited in the NCBI Sequence Read Archive (SRA) database under accession number PRJNA1006032.

Soil Bacterial Metabolic Potential

The metabolic potential of soil communities was assessed from community-level physiological profiles (CLPPs) using Biolog EcoPlates (Biolog Inc) (Alahmad *et al.*, 2019). Each soil sample was inoculated in triplicate into an EcoPlate containing 31 different carbon sources (along with one blank) and incubated for 196 h at 25°C in an OmniLog® system (Biolog Inc). Carbon source utilization was monitored by the reduction of tetrazolium, a redox color indicator that changes from colorless to purple, detected at a wavelength of 590 nm. Data were recorded every 15 min of incubation and saved in OmniLog® units generated by Biolog Data Analysis software (v1.7). Values for each well were calculated by subtracting the blank values from each well of the plate. A single absorbance time point at 50 h was selected for the comparisons, following the recommendation of Weber *et al.* (2007). The metabolic potential of soil microbial communities was quantified by calculating the average well color development (AWCD) at the determined time point, obtained by dividing the sum of the optical density data by 31 (the number of substrates). To determine functional richness, the total number of wells in a replicate with an absorbance above 25 OmniLog® units was counted.

Statistical Analysis

Statistical analyses were performed using XLSTAT software (v2021.1.1; Addinsoft, Paris, France). Normality of the data was first subjected to the Shapiro–Wilk test. Depending on the result of this test, parametric (ANOVA) or non-parametric (Kruskal–Wallis) tests were employed, followed by Fisher’s post-hoc test (Least Significant Difference, LSD). For data expressed as percentages (e.g., AMF colonization rate), arcsine transformation was performed using the ASIN function in Microsoft® Excel (v16.50) prior to statistical analysis. To evaluate the significance of variation in bacterial and fungal communities structure among different modalities, Permutational multivariate analysis of variance (PERMANOVA) was conducted based on the Bray–Curtis dissimilarity. This analysis was performed with 1,000 permutations using the *adonis* function of the *vegan* R package (Oksanen *et al.*, 2023).

Additionally, a co-occurrence network analysis was carried out to explore potential relationship among microbial community members (bacteria and fungi), considering only OTUs with a relative abundance greater than 0.1%. Pairwise correlations between OTUs were assessed using the Spearman method, and significant correlation ($r > 0.7$ or $r < -0.7$ and $p < 0.05$) were considered as effective connections. Network visualization was performed using Gephi software (v0.10.1) (Bastian *et al.*, 2009b).

Results and discussion

Numerous studies have underscored the pivotal role of soil microorganisms in soil fertility and biogeochemical cycles (Saccá *et al.*, 2017; Misra *et al.*, 2019). However, This study represents the first investigation which examines the impact of 3 years of *A. archangelica* cultivation, under inoculated and non-inoculated AMF conditions, on rhizospheric bacterial and fungal communities in polluted soil. While the microbiota of some aromatic plant species, such as *A. simensis*, *Salvia sclarea* L., *Coriandrum sativum* L., *Ocimum basilicum* L., *Mentha x piperita*, *Anethum graveolens* L., *Calendula officinalis* L., *Mentha arvensis* L., *Artemisia annua* L., and *Panax ginseng* Meyer, have already been examined in agricultural soils, to our knowledge, the rhizosphere microbiota of *A. archangelica* remains unexplored, both in polluted and non-polluted soils (Adamović *et al.*, 2015; Misra *et al.*, 2019; Raveau *et al.*, 2020; Langrand *et al.*, 2023; Peng *et al.*, 2023). Hence, to investigate the influence of angelica cultivation on soil microbial communities, we compared the biomass, structure, richness, diversity, metabolic potential, and network complexity of bacterial and fungal communities between initial state (non-vegetated soil, 2018) and after two and three years of angelica cultivation (2020 and 2021).

Angelica vegetation increased bacterial biomass in the TE-polluted soil after three years of culture

In the initial state, the total microbial biomass was approximately 9.65 µg PLFA.g⁻¹ of dry soil, with bacterial biomass being predominant at 7.2 µg PLFA.g⁻¹ of dry soil. Fungal biomass was equivalent between saprotrophic fungi and AMF at 1.4 and 1.0 µg PLFA.g⁻¹ of dry soil, respectively. This microbial biomass profile aligns with findings commonly observed in agricultural soils (Borymski *et al.*, 2018). Typically, arable soils contain lower quantities of total microbial biomass, ranging from 4 to 56 µg.g⁻¹ in France, while soils under forestry and grassland use are often richer in microbial biomass, ranging from 39 to 179 µg.g⁻¹ in France (Francisco *et al.*, 2016).

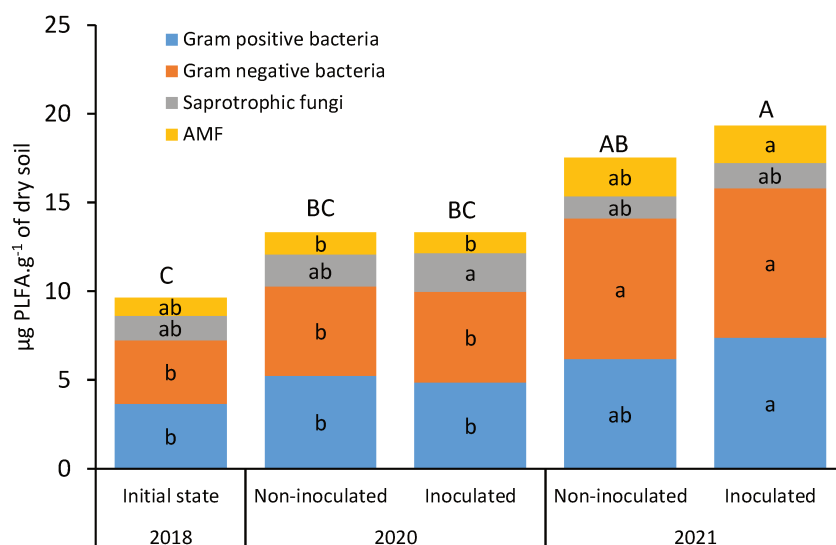


Figure 1: Influence of mycorrhizal inoculation and angelica cultivation on soil bacterial and fungal PLFA content per g of dry soil. Each parameter (Gram positive bacterial biomass, Gram negative bacterial biomass, Saprotrophic fungal biomass, AMF biomass, and total microbial biomass) was tested independently. One-way ANOVA ($n=12$). Different letters indicate a significant difference. NI: non-inoculated; I: inoculated.

In our study, total microbial biomass increased significantly from the initial state to the third year of angelica cultivation (2021), under both NI and I conditions. While fungal biomass (AMF and saprotrophic fungi) remained stable over the three years studied, there was a significant increase in the biomass of Gram-negative bacteria in 2021, observed in both I and NI conditions, as well as an increase in Gram-positive bacteria in the I condition (**Figure 1**). Although bacteria and fungi interact constantly in soils, these components of the microbial community have a different ecological characteristics, such as growth rate, nutrient requirements, and respond differently to environmental conditions (Bourceret *et al.*, 2016). Gram-negative bacteria tend to utilize relatively labile C sources and adapt more readily to unfavorable conditions like pollution (Carrasco *et al.*, 2010; Fernández *et al.*, 2012), whereas Gram-positive bacteria typically utilize more recalcitrant C sources derived from soil organic matter (Fanin *et al.*, 2019). In our study, the fungal:bacterial (F:B) ratio was 0.33 for the initial state, 0.31 and 0.33 for NI and I 2020, and 0.24 and 0.22 for NI and I 2021, respectively, falling within the lower range for French soils. Indeed, recently Djemiel *et al.*, (2023) demonstrated F:B ratios ranging from 0.24 to 12.15 across different geographical areas in France, with the majority falling between 1 and 5. This variation in F:B ratios is often associated with soil disturbance due to factors such as intensive tillage or substrates with low C:N ratios (Zhang *et al.*, 2016). Furthermore, it appears that this parameter is strongly correlated with specific environmental parameters such as pH or C:N

ratio, as well as influenced by land use, suggesting that F:B ratios could serve as bioindicator of soil condition (Rousk *et al.*, 2010; Egan *et al.*, 2018; Djemiel *et al.*, 2023).

It is also well established that plants can secrete specific root exudates, such as carbohydrates and proteins, which shape the structures of microbial communities in the rhizosphere. Similarly, aromatic plant species, like angelica, are known to release secondary metabolites with antimicrobial properties into the soil, potentially playing an important role in shaping microbial communities (Adamovic *et al.*, 2015; Wu *et al.*, 2015; Misra *et al.*, 2019). While it's understood that plants can modify soil microbiota and have both positive or negative feedback effects on these communities, the underlying mechanisms are still not fully understood (Bever *et al.*, 2012; Hu *et al.*, 2018). For instance, sesquiterpene found in root exudates of *Lotus japonicus* where identified to trigger hyphal branching in dormant mycorrhizal fungi, revealing a novel role for root exudates in cross-communication between roots and mycorrhiza (Akiyama *et al.*, 2005). In a recent review, the biological activities of various phytochemical constituents of the genus angelica were summarized (Batiha *et al.*, 2023). Compounds like sabinene, α -pinene, or β -elemene, present in angelica roots, exhibit potent antimicrobial activity (Cavaleiro *et al.*, 2015; Sowndhararajan *et al.*, 2017; Batiha *et al.*, 2023).

Time and AMF inoculation shaped the alpha diversity of bacterial communities in TE-polluted soil.

To specifically investigate the impact of angelica cultivation and mycorrhizal inoculation on soil bacterial and fungal communities, we employed a molecular approach targeting conserved genetic sequences but with sufficient variation to differentiate species, coupled with high-throughput sequencing. A total of 1,013,199 sequences were obtained from the 30 samples (6 per condition) and analyzed to study the bacterial communities. Following bioinformatic processing, including denoising and chimera elimination, we retained 12,303 OTUs with an abundance of two or more. For fungal communities, 446,998 sequences were identified from the 30 samples. After bioinformatic processing, 8,481 OTUs present at least twice were retained.

Table 1: Diversity and richness indices of soil bacterial and fungal communities. Data are means \pm SD ($n = 6$) for each condition. Means followed by the same letter do not differ significantly, according to the Kruskal-Wallis test supplemented by Dunn's post-hoc test ($\alpha = 0.05$). NI: non-inoculated; I: inoculated.

Year	Mycorrhizal inoculation	Bacteria		Fungi	
		Shannon (diversity)	Chao1 (richness)	Shannon (diversity)	Chao1 (richness)
Initial state (2018)	--	4.84 \pm 0.24 b	750.50 \pm 100.84 a	3.59 \pm 0.35 a	540.92 \pm 53.65 a
2020	NI	5.02 \pm 0.15 ab	851.81 \pm 50.99 a	3.97 \pm 0.31 a	610.08 \pm 42.41 a
	I	5.03 \pm 0.15 ab	839.42 \pm 38.26 a	3.98 \pm 0.21 a	574.18 \pm 107.36 a
2021	NI	5.04 \pm 0.10 ab	786.39 \pm 66.23 a	4.00 \pm 0.29 a	528.80 \pm 78.46 a

I	5.22 ± 0.16 a	788.30 ± 77.05 a	3.31 ± 0.48 a	428.03 ± 100.24 a
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Globally, soil bacterial diversity, as indicated by the Shannon index, increased during the culture duration, ranging from 4.84 to 5.22. Significant increases were observed between the initial state and the inoculated condition in 2021 (**Table 1**). In contrast, bacterial richness, ranging from 750.50 to 851.81, did not show any significant differences between the initial state and the years 2020 and 2021 (both I and NI conditions). Soil bacterial communities are known to be influenced by toxic TE pollution, which can alter their composition (Wu *et al.*, 2022). Previous studies have reported higher Shannon bacterial diversity indices, around 8, in non-permanent crops (Labouyrie *et al.*, 2023). Our study revealed a relatively low bacterial diversity in the initial state, likely attributed to TE-pollution or agricultural practices (Luo *et al.*, 2018). Understanding bacterial richness and the responses of specific bacterial taxa (e.g. phyla or classes) to metal toxicity remains limited, with contradictory findings reported in the literature (Berg *et al.*, 2012). Our results suggest that vegetation, such as angelica cultivation, plays a crucial role in increasing bacterial community diversity, consistent with previous findings (Luo *et al.*, 2018). Principal coordinate analyses (PCoA) ordination revealed changes in microbial community structure following angelica cultivation and AMF inoculation (**Figures 2**). For bacterial communities (**Figure 2A**), the first two dimensions of the PCoA explained 30% of the total variance in soil bacterial communities. Notably, soil bacterial communities sampled in 2021 exhibited a significant separation from those sampled at the initial state (2018) and in 2020, forming a distinct cluster along PCo1 (PERMANOVA, $R^2= 0.3$, $F=2.63$ and $p < 0.001$), highlighting a temporal effect on soil bacterial composition. However, there was no clear separation between I and NI soils in both 2020 and 2021, suggesting a limited effect of mycorrhizal inoculation effect on bacterial community structure (**Figure 2A**).

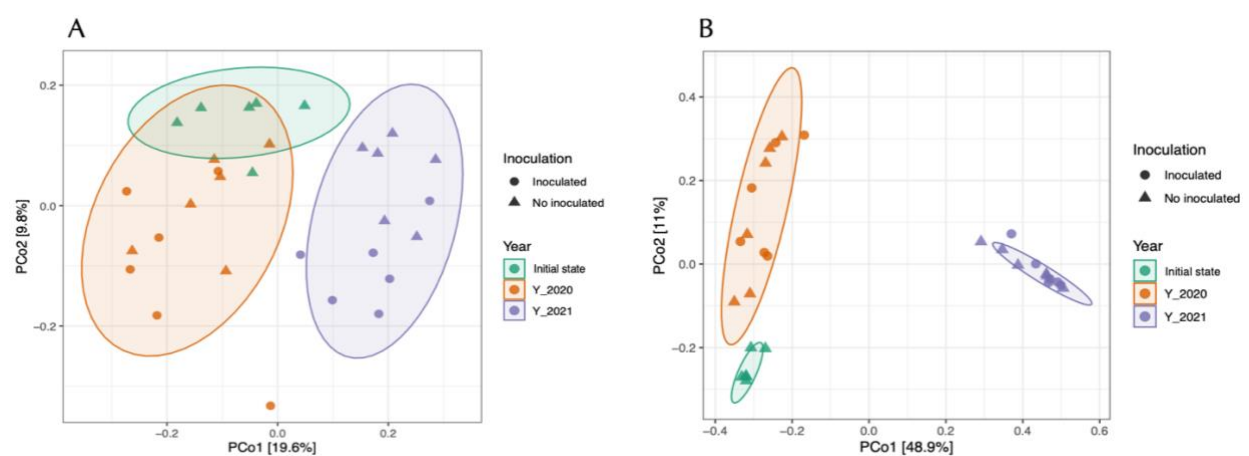


Figure 2: Principal coordinate analysis based on Bray-Curtis dissimilarity of soil bacterial A/ and Fungal B/ OTU. Ellipses confidence interval =0.95. NI: non-inoculated; I: inoculated.

The soil fungal Shannon diversity, ranging from 3.31 to 4.00, and fungal richness, ranging from 428.03 to 610.08, remained consistent between the initial state and the years 2020 and 2021 (both I and NI conditions) (**Table 1**). These Shannon diversity indices are comparable to those reported in some studies on unpolluted agricultural soils, which range from 2 to 7 (Morvan *et al.*, 2020; Liu *et al.*, 2023). The fungal PCoA indicated that the two first axes together explained 60% of the total variance in soil fungal communities (**Figure 2B**). The structure of soil fungal communities in 2021 was clearly distinct from that of the initial state and 2020 along the PCo1 (PERMANOVA, $R^2= 0.6$, $F=9.42$ and $p < 0.001$). Both I and NI soils were clustered together in 2020 and 2021, indicating no effect of AMF inoculation on soil fungal community structure (**Figure 2B**).

Angelica culture shaped bacterial and fungal communities in the TE-polluted soil

To assess the composition of bacterial communities, each OTU was categorized into phyla, classes, families and genera. In our TE-polluted soil, *Actinobacteriota*, *Proteobacteria*, *Gemmatimonadota*, *Myxococcota* and *Acidobacteriota* phyla account for over 85% of soil bacterial communities across all the conditions studied, with *Actinobacteriota* being the most represented phylum (**Figure 3A**). While the relative abundances of these dominant phyla may vary slightly, our findings align with previous studies conducted in agricultural TE-contaminated soils and vegetated with other aromatic plant species such as *C. sativum*, *S. sclarea*, *Bahia xylopoda*, or *Viguiera linearis* (Navarro-Noya *et al.*, 2010; Raveau *et al.*, 2020; Langrand *et al.*, 2023). In this work, an increase in *Actinobacteriota* abundance (coupled with a reduction in *Proteobacteria*) was observed only in 2020 under both NI and I conditions compared to the initial state. However, by 2021, after three years of cultivation, there was an increase in *Proteobacteria*, *Gemmatimonadota*, *Acidobacteriota*, *Verrucomicrobiota* and *Bacteriodota* under both NI and I conditions compared to the initial state. Some genera within *Actinobacteriota*, such as *Pseudarthrobacter*, *Proteobacteria*, *Sphingomonas*, or *Gemmatimonadota*, are known for their TE-tolerance and have been studied for potential remediation applications (Chen *et al.*, 2014; Waigi *et al.*, 2017; Fekih *et al.*, 2018; Yu *et al.*, 2023). For instance, *Gemmatimonadota* have been recognized for their role in increasing enzyme activities (*e.g.*, urease, sucrase, and acid phosphatase) in the plant rhizosphere, thereby enhancing plant growth and reducing Cd levels in soils (Yang *et al.*, 2013; Wang *et al.*, 2022). Similarly, the *Sphingomonas* genus is ubiquitous in vegetation grown in TE-polluted soils due to its ability to induce plant growth-promoting traits and remove TE from soil, such as promoting TE uptake in roots (Waigi *et al.*, 2017). The observed increase in the abundance of these bacterial genera is likely attributable to the nearly three-year cultivation of angelica in the soil.

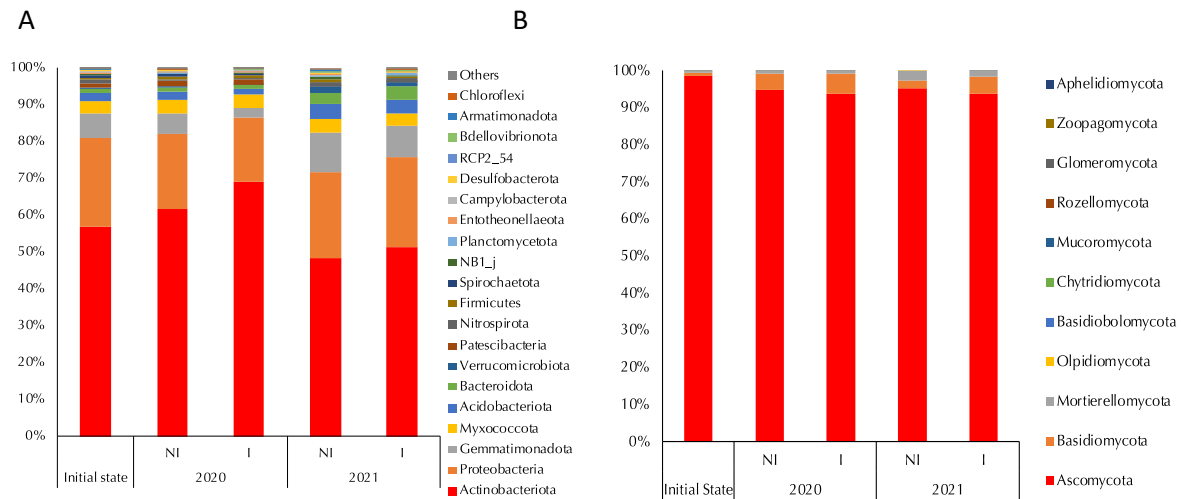


Figure 3: Relative abundances of dominant bacterial A/ and fungal B/ phyla in soils. NI: non-inoculated; I: inoculated.

Analysis of the fungal beta diversity reveals the dominance of the *Ascomycota* phylum, accounting for relative abundances ranging from 94 to 98%. When combined with *Basidiomycota*, these two phyla collectively represent 99 to 100% of the soil fungal communities (**Figure 3B**). The *Basidiomycota* phylum significantly increased in both NI and I conditions in 2020 and 2021 compared to the initial state. These phyla are commonly found in agricultural soils (Egidi *et al.*, 2019; He *et al.*, 2022). A recent study highlighted the ability of *Ascomycota* and *Basidiomycota* members to colonize soils globally, suggesting that these generalist phyla share a high number of genes related to stress tolerance and resource uptake, enabling them to colonize diverse environments (Egidi *et al.*, 2019; He *et al.*, 2022). The *Ascomycota* taxon is known for its tolerance to TE (Lin *et al.*, 2019; Sun *et al.*, 2022). While a significant decrease in the abundance of *Ascomycota* was observed in 2020 and 2021 under both NI and I conditions compared to the initial state, increases in *Basidiomycota* and *Mortierellomycota* phyla were detected after two and three years of cultivation. It has been reported that soil quality generally tends to attenuate the dominance of *Ascomycota* in favor of other phyla such as *Mortierellomycota* (Yuan *et al.*, 2020).

The fungal community structure at the genus level follows a similar trend as observed for phyla (**Figure 3B**). A total of 16 and 17 differentially abundant bacterial taxonomic clades were identified based on treatment and time, with an LDA score greater than 3. Additionally, 14 and 30 differentially abundant fungal taxonomic clades were identified with an LDA score exceeding than 3 (**Figure 4**). Among the 20 most abundant genera detected in the TE-polluted soil, several key genera were prominent. These included the *Basidiomycota* genus *Typhula*, and several *Ascomycota* genus such as *Malbranchea* (up to 5%), *Fusicolla* (up to 7%), *Penicillium* (up to 6%), *Gibellulopsis* (up to 5%), and *Ramophialophora* (**Figure S1**).

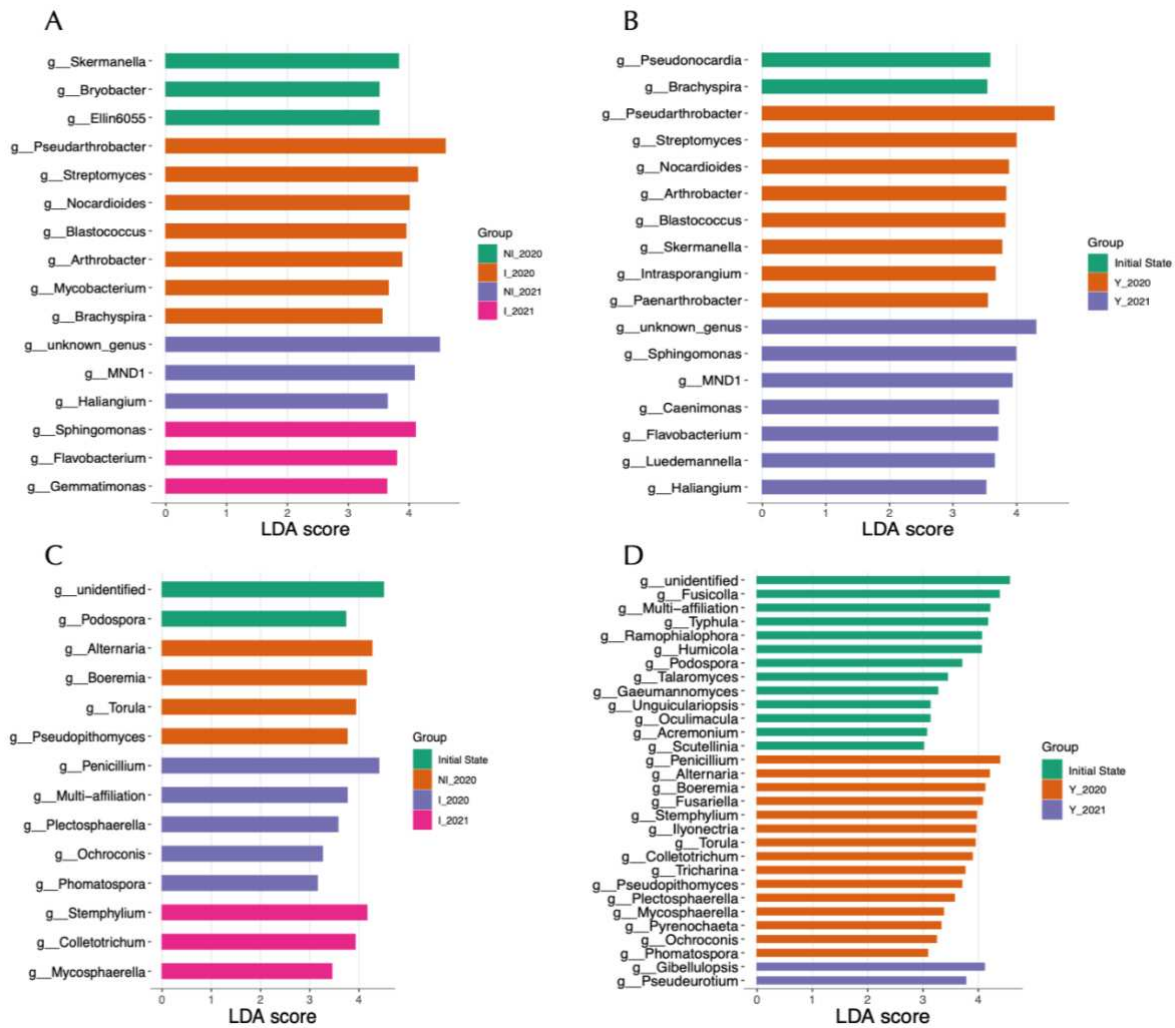


Figure 4: Linear discriminant effect size plot displaying specific bacterial (A and B) and fungal (C and D) genera that are differentially abundant across soil treatments (A and C) and sampling times (B and D).

Angelica culture enhanced microbial co-occurrences network in TE-polluted soil

The impact of angelica cultivation and AMF inoculation on soil microbial communities was investigated through the construction of inter-kingdom networks. The network visualization indicated an increase of microbial co-occurrences over the years of cultivation and with AMF inoculation (**Figure 5**). In 2021, the network exhibited greater heterogeneity (**Table S2**), with a notable increase in the importance of bacteria nodes, particularly *Actinobacteriota*, *Proteobacteria*, and *Bacteriodota*. Network topology analysis revealed an overall increase in complexity over the years of cultivation, with a positive influence of AMF inoculation (**Table S2**). Specifically, the number of nodes and edges (47 nodes and 35 edges at the initial state) expanded in 2020 (NI 2020: 76 nodes and 60 edges; I 2020: 82 nodes and 59 edges) and further in 2021 (NI 2021: 577 nodes and 719 edges; I 2021: 920 nodes and 3597 edges). The proportion of positive edges, which was 82.9% in the initial state, decreased in the inoculated

treatment in 2020 (76.3%) before surpassing its initial value in 2021 (88.9%). Literature suggests an increase in the diversity and stability of co-occurrence networks correlated with global warming (Yuan *et al.*, 2021). However, it appears that the presence of plants, as well as the duration of cultivation, contribute to the increase in the proportion of positive interactions between microorganisms (Luo *et al.*, 2021; Lan *et al.*, 2023).

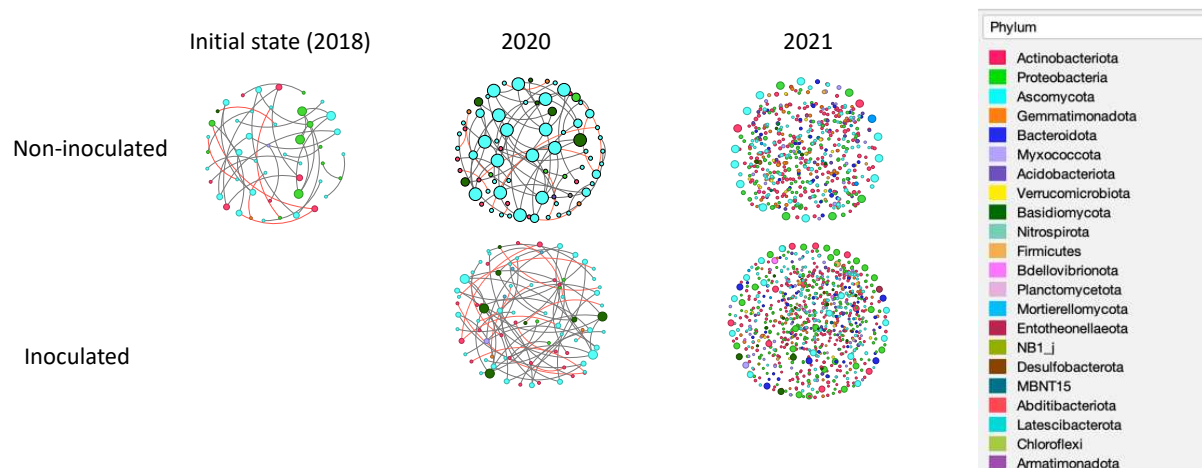


Figure 5: Co-occurrence network of soil bacterial and fungal communities. Nodes are colored by Phylum. Each connection stand for a significant correlation ($R < -0.7$ or $R > 0.7$, $p < 0.05$) between tow nodes. Positives links are colored in grey, negative links are colored in red.

Angelica culture improved carbohydrates metabolism and C cycle related genes in the TE-polluted soil

- *Microbial metabolic potential*

The microbial metabolic potential of five substrates classes (carbohydrates, carboxylic and acetic acids, amino acids polymers and amide or amines) was determined. Carbohydrates were the most utilized substrates, with values ranging from 64 for the initial state to 1494 for condition I 2020 (**Figure 6**). These values are consistent with those generally found in unpolluted soils (Gremion *et al.*, 2004; Xie *et al.*, 2016; Borymski *et al.*, 2018), suggesting that presence of TE in the soil does not significantly affect the metabolic potential of soil bacterial communities. A significant increase in the metabolic potential of all substrates was observed compared to the initial state. This increase may has been caused by the establishment of angelica, which, by maintaining soil moisture or through its root exudates, improved microbial functional richness (Deng *et al.*, 2019). Indeed, during its growth, the plant modifies the composition of its root exudates according to its needs (Emmett *et al.*, 2020), thereby altering microbial communities (Zhang *et al.*, 2020), which in turn influence the soil's metabolic capacity. The analysis of biogeochemical cycling gene abundance revealed a predominance of C cycling genes in all

soil treatments (**Figure 7**), with an increase in their abundance from the initial state (40%) to I 2021 (51%). Specifically, AMF inoculation significantly increased the abundance of C cycling genes in both 2020 and 2021 soils compared to the initial state and the NI soils in 2021.

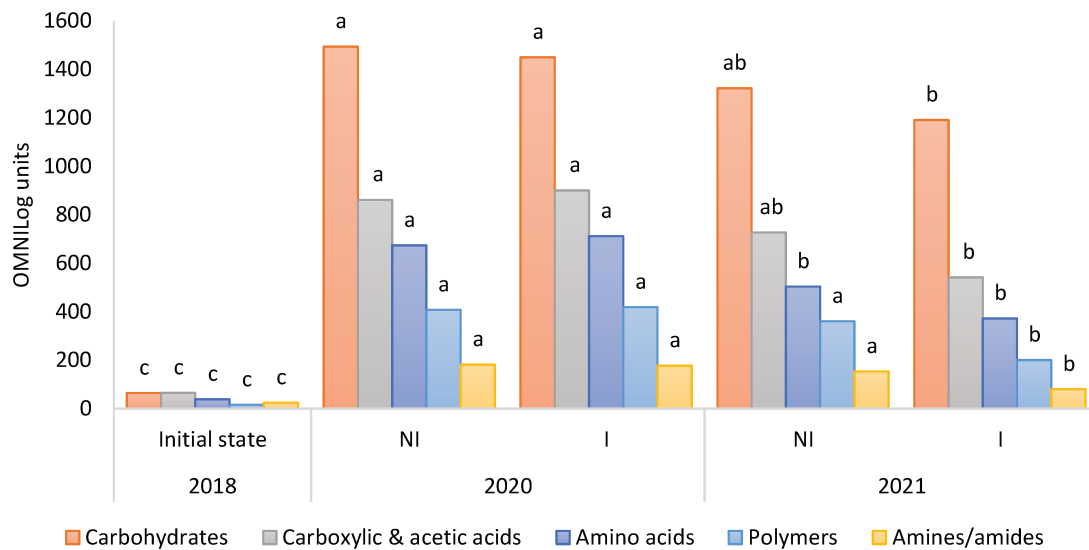


Figure 6: Influence of angelica cultivation and AMF inoculation on the soil bacterial metabolic potential, expressed as OMNILog units for five substrates classes ($n = 6$). Means followed by the same letter are not significantly different, by Kruskal-Wallis comparison ($\alpha = 0.05$).

For N cycling genes, a higher relative abundance was observed in I 2020 (9%) compared to the initial state soil, and the NI and I 2021 (10-11%) (**Figure 7**). However, no significant difference was observed in the relative abundance of S cycling genes between the different treatments. Among the different groups of cycling genes (*i.e.* C, N, and S), specific genes were differentially abundant according to angelelica cultivation or AMF inoculation. Indeed, soils inoculated with AMF in 2021 exhibited a higher relative abundance of genes involved in aerobic chemoheterotrophy, indicating a greater potential for organic matter degradation in the presence of O_2 , whereas in the initiale state, the soils showed a higher relative abundance of genes involved in the anaerobic pathway of this process, suggesting a preference for anaerobic conditions. AMF inoculation led to a higher relative abundance of genes involved in fermentation and degradation of aromatic compound in 2020. Some studies have indicated that once AMF reached the decomposing litter, bacteria, rather than fungi, become the main transformers of litter-derived carbon (Herman et al., 2012; Nuccio et al., 2013; Xu et al., 2018). Mycorrhizal fungi seem to modify the physicochemical environment for the microbial community by exporting N from the decomposing litter (Nuccio et al., 2013). This selection likely influenced the relative abundance of genes involved in fermentation and degradation of aromatic compounds, and thus contributing to the observed increase in C metabolism under our experimental conditions.

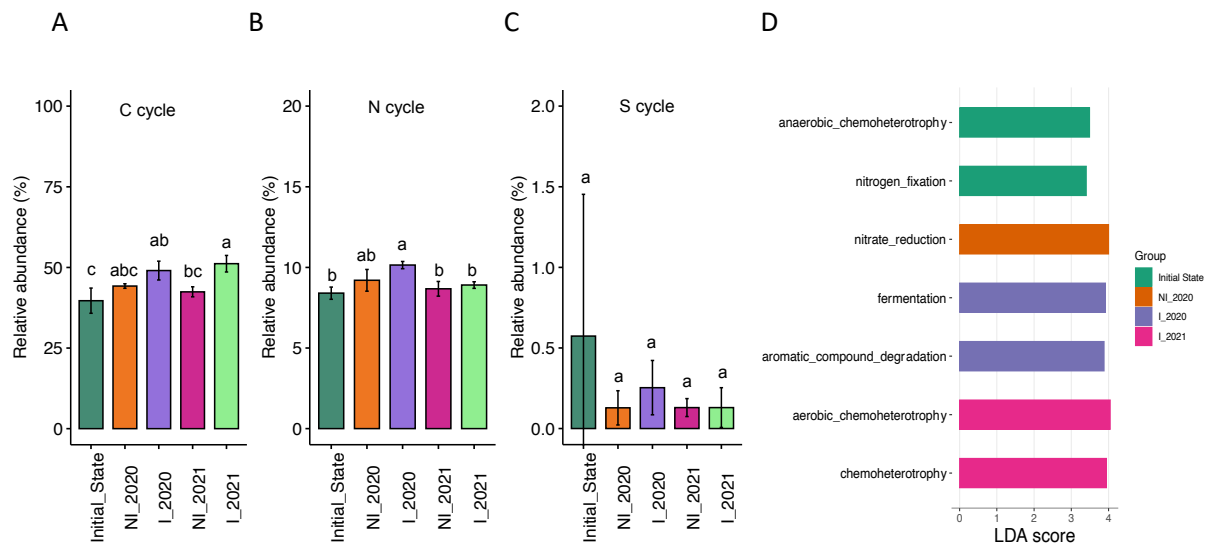


Figure 7: Functional gene content in different soils treatments. A/ relative abundance of C cycling genes. B/ Relative abundance of N cycling genes. C/ Relative abundance of S cycling genes. D/ Linear discriminant effect size plot showing specific genes differentially abundant in the different soil treatments.

The analysis of the main fungal trophic guilds (symbiotrophs, saprotrophs, and pathotrophs) did not reveal significant differences between the different conditions (**Figure 8**). However, within these groups, we observed notable variations. For instance, the relative abundance of the fungal parasite group was higher in initial state soils and but decreased significantly in NI and I conditions in 2020 and 2021 (**Figure 8**). Conversely, sub-groups like wood saprotrophs, soil saprotrophs, and dung saprotrophs showed a significant increase in 2020 I and NI soils. Similarly, the relative abundance of ectomycorrhizal and litter saprotroph sub-groups rose significantly in I and NI 2021 soils. Some studies indicates that *Ascomycota* and *Basidiomycota* fungi play key roles as decomposers of lignocellulosic organic matter in soils (Vandenkoornhuysen *et al.*, 2002; Osono & Takeda, 2006; Yelle *et al.*, 2008; Bastian *et al.*, 2009a; Ma *et al.*, 2013). Soil analyses conducted at the outset and conclusion of the study revealed an enrichment in organic matter (27, 40, and 38 g.kg⁻¹ for the initial state, NI 2021 and I 2021 respectively) and organic C (16, 23, and 22 g.kg⁻¹ for the initial state, NI 2021 and I 2021 respectively) (**Table S1**). We hypothesize that angelica residues, incorporated into the soil each year after harvest, may contribute to the increased abundance of saprotroph fungi, which in turn enhance soil quality by enriching it with organic matter and C. This enrichment likely aids in the immobilization of metals through chelation, thereby improving phytostabilization. Previous studies have shown that copper and lead tend to strongly bind to organic matter (Palansooriya *et al.*, 2020).

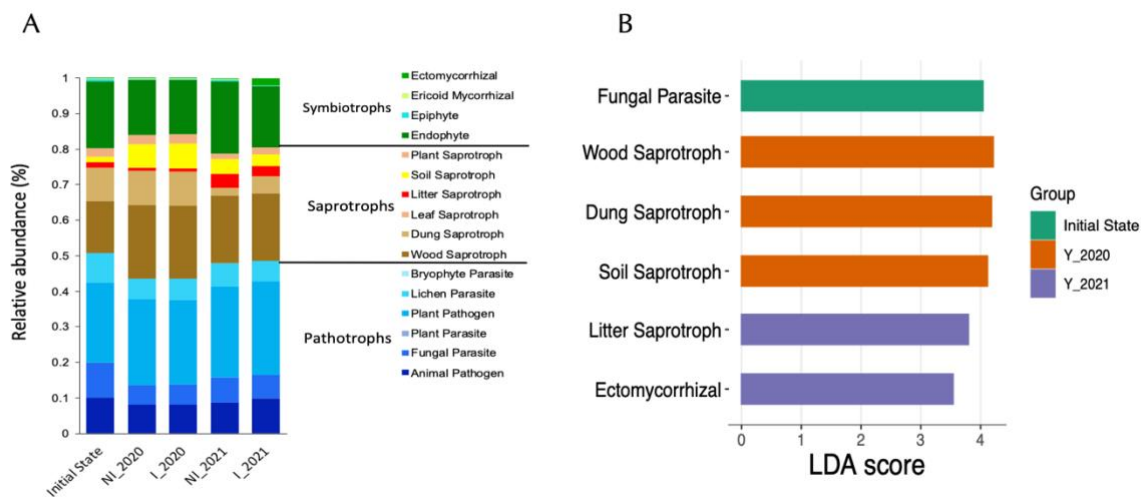


Figure 8: Trophic guilds of fungal communities. A/ Relative abundance of functional fungal guild. B/ Linear discriminant effect size showing the fungal group characterizing the different years of soil sampling.

Conclusion

The main objectives of this field study were to analyze the contribution of angelica cultivation and mycorrhizal inoculation to the composition and structure of bacterial and fungal communities in a polluted soil over three successive years, within a phytomanagement context. *Actinobacteriota*, *Proteobacteria* and *Ascomycota* were the dominant phyla in the initial state of the TE-polluted soil, as well as during the two years of angelica cultivation. Angelica cultivation significantly increased bacterial biomass and diversity, but did not significantly affect fungal diversity or richness. However, the relative abundance of saprotrophic fungi increased significantly over the time cultivation. Additionally, cultivation time significantly enhanced the complexity of interaction networks between bacterial and fungal communities, as well as the number of positive interactions among microbial communities in TE-polluted soil. Moreover, an increase in the relative abundance of genes linked to C metabolism was highlighted, along with saprotrophic fungi involved in OM degradation (*i.e.* litter saprotrophs). Inoculated conditions significantly improved both vertex and edge numbers, as well as positive edge compared to the initial state. The parameters studied in this work—angelica cultivation, cultivation duration and mycorrhizal inoculation—positively influenced the microbial communities of TE-polluted soil by selecting certain trophic guilds and enhancing interactions among soil microorganisms, thereby likely contributing to the ecological rehabilitation of the TE-polluted soil.

Author Contributions:

Conceptualization, J.L., A.L.-H.S. and J.F.; methodology, J.L., P.M.S.N., J.D., F.L., N.F., A.L.-H.S., and J.F.; writing—original draft preparation, J.L., P.M.S.N.; writing—review and editing, J.L., P.M.S.N., F.L., N.F.,

A.L.-H.S., and J.F.; supervision, A.L.-H.S. and J.F.; project administration, A.L.-H.S.; funding acquisition, A.L.-H.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest:

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Table S1 data are mean and expressed as g.kg⁻¹, except for pH.

Modality	OM	Organic C	N	C/N	pH	P	K	Ca	Mn	Total limestone	Arbuscular rate (%)
Initial state	27.58	16.02	1.52	10.00	7.05	0.13	0.35	6.76	0.18	4.00	--
NI 2021	40.40	23.50	2.15	10.00	7.98	0.06	0.26	6.90	0.26	12.00	32.2
I 2021	38.40	22.30	1.95	10.50	7.97	0.09	0.31	7.17	0.23	13.50	38.0

Table S2 Topological parameters of microbial network.

	<i>Initial_State</i>	<i>NI_2020</i>	<i>I_2020</i>	<i>NI_2021</i>	<i>I_2021</i>
Vertex	47	76	82	577	920
Edge	35	60	59	719	3597
Positive edge (%)	82.9	81.6	76.3	77.3	88.9
Module	20	32	36	214	241
Average_degree	1.49	1.58	1.44	2.49	7.82
Heterogeneity	0.44	0.52	0.55	1.24	1.79

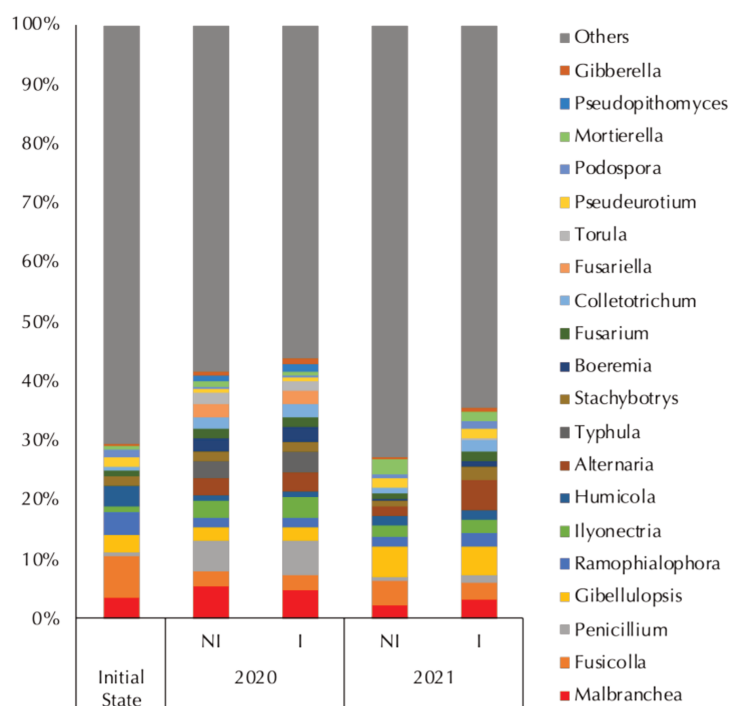


Figure S1: Relative abundances of the 20 most abundant fungal genera in soil. NI: non-inoculated; I: inoculated.

Publication 4

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Coriander (*Coriandrum sativum*) Cultivation Combined with Arbuscular Mycorrhizal Fungi Inoculation and Steel Slag Application Influences Trace Elements-polluted Soil Bacterial Functioning

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Présentation de la publication 4

Afin d'optimiser les performances de la phytostabilisation, diverses stratégies, dont l'utilisation d'amendements organiques, inorganiques et biologiques, ont été développées ces dernières années ; on parle alors de phytostabilisation aidée (Sarwar et al., 2017). Outre la réduction de la mobilité et de la biodisponibilité des polluants, ces amendements ont pour effet de favoriser l'installation et le développement du couvert végétal et parfois d'améliorer la qualité biotique du sol (Shah and Daverey, 2020).

Par exemple, dans le respect de l'économie circulaire, l'incorporation d'amendements minéraux à base de laitiers sidérurgiques dans le sol, pourrait améliorer les caractéristiques physico-chimiques du sol, tels que le pH, la mobilisation des nutriments ou la structure et l'activité microbienne du sol (Agnello et al., 2018; Branca and Colla, 2012; Das et al., 2020). En effet, les amendements minéraux, tels que les laitiers sidérurgiques, peuvent contribuer à l'immobilisation des ET, en particulier le Cd, le Zn et le Pb (Agnello et al., 2018; Branca and Colla, 2012; Kumpiene et al., 2008; Ning et al., 2016). Les laitiers sidérurgiques ont été récemment décrits comme augmentant la biomasse microbienne et l'activité enzymatique du sol (Das et al., 2020). Il a également été rapporté que l'utilisation d'amendements biologiques à base de CMA, permettent un meilleur développement des plantes, en particulier dans des conditions de stress comme dans le cas des sols pollués par les ET (Cicatelli et al., 2014; Meier et al., 2012). Ils contribuent aussi à améliorer l'absorption des nutriments du sol, en particulier le phosphore (Javot et al., 2007). Jusqu'à présent, peu de travaux scientifiques traitent de l'application combinée d'inoculum mycorhizien et d'amendements minéraux (laitiers sidérurgiques), et leurs effets sur la qualité microbiologique des sols phytomanagés (Hu et al., 2019). Néanmoins, des études récentes ont décrit un effet synergique de l'inoculation mycorhizienne avec des amendements minéraux (boues de phosphate provenant de déchets de marbre) sur la croissance des plantes dans les sols pollués par les ET (Liu et al., 2018; Rakkami et al., 2021).

C'est pourquoi, dans le cadre de cette étude, nous avons vérifié si un apport d'amendements minéraux et/ou biologiques à base de CMA, pouvait améliorer la croissance de la coriandre et le fonctionnement biologique du sol. L'apport de ces amendements dans la phytostabilisation des ET et le transfert problématique du Cd dans les parties aériennes sera traité par l'un des partenaires du projet DEPHYTOP.

Présentation de la publication 4

Pour cela, nous avons testé deux types d'amendements, des laitiers sidérurgiques (laitier de poche et de four, utilisés à 1,5 ou 45 t.ha⁻¹) ainsi qu'un inoculum mycorhizien (Symbivit® - INOCULUM plus, Bretenièrre, France) et cultivé la coriandre sur un dispositif de microparcelles de 9 m² sur le site d'Evin - Malmaison. Au total 20 conditions expérimentales ont été étudiées reprenant toutes les combinaisons (en présence ou non de coriandre, d'inoculum mycorhizien ou de laitiers sidérurgiques – Figure 3). Nous avons étudié les paramètres de croissance de la coriandre, la biomasse bactérienne du sol (acide gras phospholipidique, AGPL), le potentiel métabolique du sol (Biolog Ecoplates) et la structure (métabarcoding de l'ARNr 16S) des communautés bactériennes du sol pollué par les ET.

Après 7 semaines de culture, la coriandre mesurait entre 62 et 72 cm. Sa croissance n'a pas été significativement impactée par l'ajout d'amendements minéraux. Par ailleurs, les taux de mycorhization de la coriandre étaient similaires dans les conditions NI et I, ce qui confirme la présence de CMA autochtones dans les sols fortement pollués par les ET, mis en évidence par Raveau *et al.*, (2021) sur le même site.

Comme dans l'article précédent, les phyla bactériens les plus abondants détectés dans notre sol pollué sont les Actinobacteria, les Proteobacteria, Gemmatimonadota, Myxococcota et les Acidobacteriota. Les laitiers sidérurgiques ou l'inoculum mycorhizien, ont modifié la structure des communautés bactériennes de la rhizosphère sans affecter son potentiel métabolique (AWCD). L'introduction des amendements minéraux dans le sol a provoqué un changement dans la structure des communautés bactériennes, la plus faible dose d'amendement minéral a augmenté la biomasse bactérienne et l'abondance relative des Gemmatimonadota. Il a été démontré que les agrégats minéraux agissent comme un support physique pour la fixation des micro-organismes (bactéries, champignons, etc.) et des plantes, ainsi que comme une réserve de nutriments, et augmente pH du sol (Wang, 2019). À cet égard, et en raison de la nature alcaline des scories, le pH et la minéralosphère peuvent être des facteurs importants influençant les communautés microbiennes (Uroz *et al.*, 2015; Wang, 2019).

La végétalisation du sol a significativement augmenté la biomasse de bactéries à Gram négatives. En outre les indices de richesse (Chao1) et de diversité (Shannon) restent stables et ne sont impactés ni par l'inoculation, ni par la présence de laitiers sidérurgiques, ni par la végétalisation. L'abondance relative des Actinobacteria était significativement supérieure dans les conditions végétalisées par la coriandre. Par exemple, les genres *Pseudarthrobacter*, *Arthrobacter* et *Streptomyces*, appartenant au phylum des Actinobacteria étaient plus abondants dans les conditions de végétalisées, tandis que les genres appartenant au phylum des Gemmatimonadota étaient favorisés dans des conditions non

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végétalisées. Le potentiel métabolique global (AWCD) microbien et la richesse métabolique était plus élevé dans les conditions végétalisées que dans les conditions non végétalisées.

Nos résultats suggèrent que les laitiers sidérurgiques, qui par leur recyclage contribuent à l'économie circulaire, utilisés en combinaison avec la végétalisation du sol par des plantes aromatiques telles que la coriandre, offrent potentiellement des avantages écologiques à long terme aux sols pollués par les ET, et pourraient être considérés comme un outil supplémentaire dans les futures approches de remédiation des sols historiquement pollués par les ET.

Dans cette étude, les prélèvements et le conditionnement des échantillons ont été réalisés avant mon arrivée en thèse. J'ai réalisé l'ensemble des étapes d'extraction et d'amplification de l'ADN par PCR. Le séquençage a été réalisé par Génome Québec. J'ai également réalisé le traitement bio-informatique des données de séquençage. Les analyses Biolog ecoplate ont été réalisées en collaboration avec J. Duclercq (UPJV). L'ensemble des analyses statistiques a été réalisé par mes soins. L'exploitation des données est issue d'un travail collectif, ayant donné lieu à la rédaction de cette publication.








Publication 4 : *Coriander (Coriandrum sativum) Cultivation Combined with Arbuscular Mycorrhizal Fungi Inoculation and Steel Slag Application Influences Trace Elements-Polluted Soil Bacterial Functioning*

Publication 4

Publication 4

Article

Coriander (*Coriandrum sativum*) Cultivation Combined with Arbuscular Mycorrhizal Fungi Inoculation and Steel Slag Application Influences Trace Elements-Polluted Soil Bacterial Functioning

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Abstract: The cultivation of aromatic plants for the extraction of essential oils has been presented as an innovative and economically viable alternative for the remediation of areas polluted with trace elements (TE). Therefore, this study focuses on the contribution of the cultivation of coriander and the use of arbuscular mycorrhizal fungi (AMF) in combination with mineral amendments (steel slag) on the bacterial function of the rhizosphere, an aspect that is currently poorly understood and studied. The introduction of soil amendments, such as steel slag or mycorrhizal inoculum, had no significant effect on coriander growth. However, steel slag changed the structure of the bacterial community in the rhizosphere without affecting microbial function. In fact, *Actinobacteria* were significantly less abundant under slag-amended conditions, while the relative proportion of *Gemmatimonadota* increased. On the other hand, the planting of coriander affects the bacterial community structure and significantly increased the bacterial functional richness of the amended soil. Overall, these results show that planting coriander most affected the structure and functioning of bacterial communities in the TE-polluted soils and reversed the effects of mineral amendments on rhizosphere bacterial communities and their activities. This study highlights the potential of coriander, especially in combination with steel slag, for phytomanagement of TE-polluted soils, by improving soil quality and health.

Keywords: steel slag; phytomanagement; trace elements; coriander; arbuscular mycorrhizal fungi

1. Introduction

Soils provide fundamental ecosystem functions and play an important role in water and nutrient cycling, food production and the provision of renewable materials. Their ability to store carbon is essential for coping with climate change, and soil biodiversity is critical for soil fertility and biodiversity in general. Unfortunately, increasing pollution has made large areas of soil unsuitable for cultivation, a hazard to wildlife and humans, and uninhabitable for many living microorganisms. It is widely recognized that trace elements (TE), such as lead (Pb), cadmium (Cd), and zinc (Zn), can inhibit biological processes in ecosystems and affect the biodiversity, abundance, activity and function of soil microorganisms. These pollutants originate from human activities and, in particular, from smelters that have contributed to the production of significant amounts of dusts,

resulting in severe contamination of soils with TE [1]. They represent an increased toxicity for microorganisms living in the soil and are also responsible for soil acidification, small size of soil aggregates and poor soil structure due to the lack of organic matter (OM), humic acids and, by extension, nutrients [2–4]. Therefore, it is crucial to reduce TE-toxicity in polluted soils to restore soil ecosystem services. Phytomanagement technologies, such as phytostabilisation, are suitable options to manage agricultural soils polluted with TE ecologically and economically [5–7]. Therefore, a crucial point for successful phytomanagement is the selection of TE-tolerant plant species that grow rapidly and produce enough biomass to allow recovery [6,8]. Previous studies have shown that the cultivation of aromatic plants, such as coriander (*Coriandrum sativum*), should be a promising option for phytomanagement of TE-polluted soils [6,9]. Indeed, this aromatic belonging to the *Apiaceae* family is cultivated for the production of essential oils (EO), which are an important way of valorizing plant biomass. The EO produced on TE-polluted soils have antibacterial, antifungal and antioxidant activities, and can be used for various applications [10]. Although it has been demonstrated that coriander can grow in TE-polluted soils, this plant species accumulates Zn and Cd in its aboveground parts, which makes it impossible to recycle residues from the distillation of coriander (shoots or seeds), in the methanization process, or as compost [6,9,11].

For example, in the context of circular economy, the incorporation into the soil of mineral basic amendments could improve soil parameters, such as pH, nutrient mobilization or soil structure and microbial composition [12–14]. In addition, mineral basic amendments, such as slags, can contribute to mineral nutrition and thus improve crop yields, but also play an important role in stabilizing soil aggregates and resistance to plant diseases [12,13,15]. Some studies have shown the role of slag in TE immobilization, especially Cd, Zn and Pb [13,16,17]. Furthermore, steel slag has been described to increase microbial biomass, soil enzyme activity and the number of copiotrophic bacterial taxa, and is believed to provide beneficial ecosystem services to plants [14].

Therefore, an adequate supply of mineral, organic or biological amendments could be interesting, first to reduce TE-mobility and bioavailability, and also to improve the growth of coriander and soil function [11].

Biological amendments, such as arbuscular mycorrhizal fungi (AMF) inoculum, have also been shown to be useful for plant protection. Indeed, AMF enable host plants to grow vigorously under stressful conditions, especially in TE-polluted soils [18]. They contribute significantly to the uptake of soil nutrients, especially phosphorus [19], but also nitrogen, calcium, sulfur, potassium and zinc, thereby increasing plant biomass and improving plant tolerance to soil pollution [20,21]. In addition, AMF produce glomalin, an insoluble glycoprotein that has soil binding properties, but also melanin and chitin, which reduce metal bioavailability by metal immobilization into hyphae [18,22,23]. Soil microbial communities are indeed crucial for soil structure, OM degradation and C cycling [3,24]. They are often used to assess the degree of soil remediation [8,25].

Many recent studies have described a synergistic effect of AMF inoculation and biological and mineral amendments (olive residues, biochar, phosphate sludge from marble waste, compost, manure) on plant growth in TE-polluted soils [26–30]. So far, there are few scientific works dealing with the combined application of AMF and mineral amendments (steel slag), and their effects on microbial communities and their activities are generally poorly studied [16].

Therefore, under *in situ* conditions, this study focuses on the potential benefits of adding amendments (mineral amendment alone or in combination with AMF inoculum) to improve coriander growth and microbial function of historically TE-polluted soils. To this end, we investigated plant growth parameters, soil bacterial biomass (phospholipid fatty acid, PLFA), the functional activity (community-level physiological profile) and structure (16S rRNA metabarcoding) of rhizosphere bacterial communities.

2. Results

2.1. Coriander Growth Parameters

Although the plants had the same height after 11 weeks of cultivation regardless of the modality, we found that plants inoculated with AMF had a lighter aboveground dry weight than those non-inoculated (Table 1). In addition, the type and dose of the amendment did not alter the dry weight of coriander.

Table 1. Growth parameters and mycorrhizal rate of coriander. Data are presented as means \pm standard error. The means were obtained from five replicates ($n = 5$) for height and dry weight of plants, and three replicates ($n = 3$) for mycorrhization rate. Means followed by the same letter are not significantly different, by one-way ANOVA comparison and post-hoc Fisher test ($\alpha = 0.05$).

Amendment	Dose (t ha ⁻¹)	Inoculation	Plant Aboveground Height (cm)	Dry Weight (g)	Mycorrhizal Rate (%)
Unamended		NI	69.6 \pm 7.0 ab	4.1 \pm 1.1 bcd	40.8 \pm 10.7 abc
		I	71.6 \pm 2.5 ab	4.7 \pm 0.6 cd	35.6 \pm 4.9 abc
Ladle slag	1.5	NI	68.0 \pm 4.0 ab	7.0 \pm 1.4 abc	31.7 \pm 0.8 c
		I	65.0 \pm 2.5 ab	3.2 \pm 0.3 d	35.8 \pm 1.8 abc
	45	NI	75.0 \pm 9.8 a	8.7 \pm 2.1 a	33.1 \pm 3.9 bc
		I	72.7 \pm 4.0 ab	5.1 \pm 1.1 abcd	37.0 \pm 1.3 abc
Oven slag	1.5	NI	70.0 \pm 5.1 ab	6.1 \pm 1.4 abcd	37.5 \pm 4.1 abc
		I	69.0 \pm 4.3 ab	4.9 \pm 0.9 bcd	51.0 \pm 4.6 a
	45	NI	73.8 \pm 4.1 a	8.0 \pm 0.7 ab	48.6 \pm 7.5 ab
		I	62.0 \pm 2.6 b	6.0 \pm 1.9 abcd	47.9 \pm 4.3 ab
Dose			NS	NS	NS
Amendment			NS	NS	**
Mycorrhizal inoculation			NS	*	NS
Dose * Amendment			NS	NS	NS
Dose * Mycorrhizal inoculation			NS	NS	NS
Amendment * Mycorrhizal inoculation			NS	NS	NS
Dose * Mycorrhizal inoculation * Amendment			NS	NS	NS

Significance: NS: not significant; *: $p < 0.05$; **: $p < 0.01$; according to multivariate ANOVA in bold. NI: not inoculated; I: inoculated.

2.2. Mycorrhizal Rate

The mycorrhizal rate of coriander roots ranged from 31.7 to 51%, and was significantly higher in plots amended with 1.5 t ha⁻¹ oven slag (51%) than in plots amended with 1.5 t ha⁻¹ ladle slag (31.7%). No difference was observed between NI and I conditions, indicating the presence of native AMF. The dose of amendment did not affect the mycorrhization rate.

2.3. Soil Microbial Biomass

Total bacterial biomass was not significantly different between the two unamended conditions. Analysis of variance (ANOVA) of the Gram-positive bacteria biomass showed an increase with the lower dose of amendment (1.5 t ha⁻¹) and with a combined effect of dose (1.5 t ha⁻¹) and vegetation (Table 2). Moreover, the biomass of Gram-positive bacteria was significantly higher (8.14 $\mu\text{g g}^{-1}$ of dry weight (DW) of soil) in soil amended with 1.5 t ha⁻¹ oven slag and under vegetated conditions than under the same non-vegetated conditions (2.54 $\mu\text{g g}^{-1}$ of DW of soil) (Table 2).

A significant effect of vegetation was observed on Gram-negative bacterial biomass. In fact, this increase was significantly higher in vegetated soils and NI condition supplemented with 1.5 t ha⁻¹ ladle slag (5.30 $\mu\text{g g}^{-1}$ soil) than under unvegetated modality (2.31 $\mu\text{g g}^{-1}$ soil, Table 2).

Table 2. Influence of amendments (ladle slag and oven slag used at 1.5 and 45 t ha⁻¹), inoculation and vegetation on Gram-positive, Gram-negative and total bacterial biomass ($\mu\text{g g}^{-1}$ of dry weight (DW) soil), and Gram-positive/Gram-negative ratio in trace element-polluted soil. Data are presented as means \pm standard error. The means were obtained from three replicates ($n = 3$). Means followed by the same letter are not significantly different, by one-way ANOVA comparison and post-hoc Fisher test ($\alpha = 0.05$).

State	Amendment	Dose (t ha ⁻¹)	Inoculation	Gram-Positive Biomass $\mu\text{g g}^{-1}$ of DW Soil	Gram-Negative Biomass $\mu\text{g g}^{-1}$ of DW Soil	Total Bacteria $\mu\text{g g}^{-1}$ of DW Soil	Gram-Positive/Gram-Negative Bacteria	
Unvegetated	Unamended		NI	3.64 \pm 3.64 abcd	2.96 \pm 1.58 abc	6.60 \pm 5.20 abc	1.02 \pm 0.60 bcd	
			I	5.50 \pm 4.26 abcd	2.85 \pm 3.21 bc	8.35 \pm 7.34 abc	2.93 \pm 2.39 a	
	Ladle slag	1.5	NI	2.43 \pm 0.57 d	2.31 \pm 0.94 c	4.74 \pm 0.40 c	1.30 \pm 0.92 bcd	
			I	4.30 \pm 0.95 abcd	4.11 \pm 0.95 abc	8.41 \pm 1.88 abc	1.05 \pm 0.09 bcd	
		45	NI	4.08 \pm 1.76 abcd	3.58 \pm 0.85 abc	7.65 \pm 1.76 abc	1.22 \pm 0.64 bcd	
			I	2.05 \pm 1.44 d	2.82 \pm 0.86 bc	4.88 \pm 1.85 c	0.76 \pm 0.47 cd	
	Oven slag	1.5	NI	6.97 \pm 1.35 abc	2.86 \pm 0.77 bc	9.83 \pm 5.86 abc	2.42 \pm 0.65 ab	
			I	2.54 \pm 1.13 bcd	2.51 \pm 1.41 bc	5.05 \pm 2.45 c	1.17 \pm 0.49 bcd	
		45	NI	4.70 \pm 0.86 abcd	2.82 \pm 0.91 bc	7.52 \pm 0.08 abc	1.87 \pm 0.98 abcd	
			I	3.26 \pm 1.81 bcd	2.82 \pm 0.77 bc	6.08 \pm 2.57 abc	1.09 \pm 0.42 bcd	
	Vegetated	Unamended		NI	3.21 \pm 2.40 bcd	2.20 \pm 1.15 c	5.41 \pm 3.54 c	1.35 \pm 0.40 abcd
				I	2.42 \pm 0.71 cd	4.06 \pm 1.29 abc	6.47 \pm 1.94 abc	0.60 \pm 0.09 d
Ladle slag		1.5	NI	3.65 \pm 1.96 abcd	5.30 \pm 0.36 a	8.96 \pm 1.63 abc	0.71 \pm 0.43 cd	
			I	7.30 \pm 3.49 ab	4.82 \pm 1.98 ab	12.12 \pm 5.45 a	1.47 \pm 0.20 abcd	
		45	NI	1.77 \pm 0.35 d	2.31 \pm 0.77 c	4.08 \pm 1.12 c	0.78 \pm 0.09 cd	
			I	3.97 \pm 3.09 abcd	4.37 \pm 2.89 abc	8.34 \pm 5.95 abc	0.88 \pm 0.18 bcd	
Oven slag		1.5	NI	7.34 \pm 6.21 ab	4.83 \pm 1.05 ab	12.16 \pm 5.49 a	1.72 \pm 1.75 abcd	
			I	8.14 \pm 4.66 a	3.78 \pm 0.34 abc	11.92 \pm 4.47 ab	2.21 \pm 1.29 abc	
		45	NI	3.21 \pm 1.92 bcd	4.15 \pm 2.18 abc	7.36 \pm 3.15 abc	0.91 \pm 0.80 bcd	
			I	2.83 \pm 0.59 bcd	2.86 \pm 0.78 bc	5.70 \pm 0.81 bc	1.05 \pm 0.35 bcd	
Vegetation				NS	*	NS	NS	
Amendments				NS	NS	NS	NS	
Dose				*	NS	*	NS	
Mycorrhizal inoculation				NS	NS	NS	NS	
Vegetation * Amendment				NS	NS	NS	NS	
Vegetation * Dose				NS	NS	*	NS	
Vegetation * Mycorrhizal inoculation				NS	NS	NS	NS	
Mycorrhizal inoculation * Amendments				NS	NS	NS	NS	
Mycorrhizal inoculation * Dose				NS	NS	NS	NS	
Vegetation * Mycorrhizal inoculation * Amendments				NS	NS	NS	NS	

Significance: NS: not significant; *: $p < 0.05$; according to multivariate ANOVA in bold. NI: non-inoculated; I: inoculated.

The total bacterial biomass followed the same trend as the Gram-positive bacterial biomass. Indeed, a significant increase in total bacterial biomass was observed in the amended soil with 1.5 t ha⁻¹ oven slag and under vegetated conditions (11.92 $\mu\text{g g}^{-1}$ of DW soil) than in the same non-vegetated plots (5.05 $\mu\text{g g}^{-1}$ of DW soil). Examination of the ratio of the biomass of Gram-positive to Gram-negative bacteria showed no significant difference between diverse modalities (Table 2).

2.4. Bacterial α -Diversity of Soil Biotopes

To further investigate the effects of slags, vegetation and AMF inoculation on rhizospheric bacterial communities, the bacterial OTUs (Operational Taxonomic Units) were taxonomically assigned. The bacterial OTUs in the soil were assigned to 993 genera belonging to 33 phyla. *Actinobacteria* was the most represented phylum in each modality and accounted for between 41 and 67% of the total bacterial abundance (Figure 1).

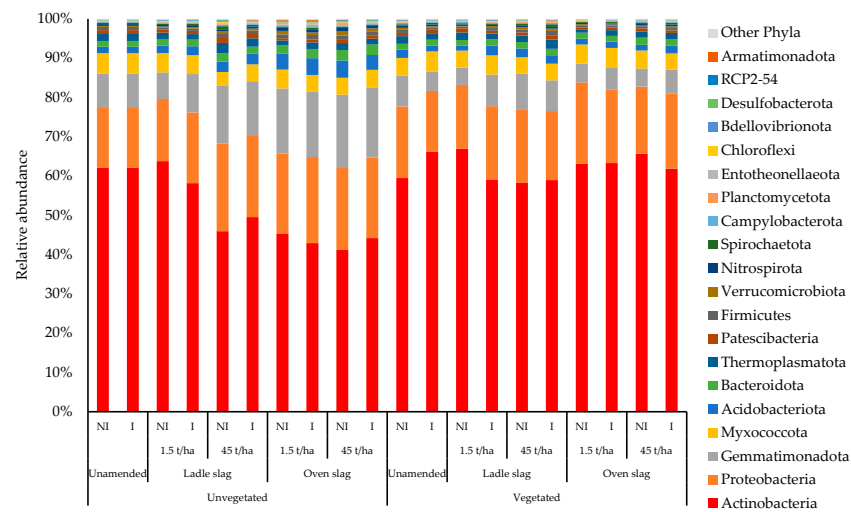


Figure 1. Relative abundance of bacterial operational taxonomic units (OTUs) grouped at phylum taxonomic rank in soil. NI, non-inoculated; I, inoculated.

Bacterial α -diversity was calculated independently for each modality. According to the Chao1 index, soil bacterial species richness remains stable across modalities (average 325.3). Similarly, both bacterial richness and diversity (Shannon index) were not affected by the addition of slag, mycorrhizal inoculation or vegetation (Table 3).

Table 3. Influence of amendments (ladle slag and oven slag used at 1.5 and 45 t ha⁻¹), inoculation and vegetation on bacterial richness and diversity indexes. Data are mean \pm SD ($n = 3$) for each condition. Means followed by the same lowercase letter are not significantly different, by one-way ANOVA comparison and post-hoc Fisher test ($\alpha = 0.05$).

State	Amendment	Dose (t ha ⁻¹)	Inoculation	Chao1	Shannon
Unvegetated	Unamended		NI	298.7 \pm 28.5 cd	4.3 \pm 0.1 a
			I	305.1 \pm 48.8 cd	4.4 \pm 0.1 a
	Ladle slag	1.5	NI	333.3 \pm 53.7 abc	4.5 \pm 0.1 a
			I	318.3 \pm 13.5 bcd	4.5 \pm 0.1 a
		45	NI	288.1 \pm 11.1 a	4.5 \pm 0.1 a
			I	343.1 \pm 17.7 abc	4.5 \pm 0.0 a
	Oven slag	1.5	NI	335.5 \pm 31.8 abc	4.4 \pm 0.0 a
			I	329.9 \pm 16.7 abcd	4.4 \pm 0.0 a
45		NI	312.5 \pm 19.3 bcd	4.3 \pm 0.2 a	
		I	334.1 \pm 28.6 abc	4.4 \pm 0.3 a	
Vegetated	Unamended		NI	321.7 \pm 25.7 abcd	4.4 \pm 0.2 a
			I	324.0 \pm 23.4 abcd	4.4 \pm 0.0 a
	Ladle slag	1.5	NI	320.2 \pm 1.6 bcd	4.5 \pm 0.1 a
			I	365.4 \pm 27.9 a	4.4 \pm 0.0 a
		45	NI	335.4 \pm 28.2 abc	4.5 \pm 0.1 a
			I	302.8 \pm 13.3 cd	4.5 \pm 0.0 a
	Oven slag	1.5	NI	350.7 \pm 26.9 ab	4.4 \pm 0.1 a
			I	310.2 \pm 5.0 bcd	4.5 \pm 0.1 a
45		NI	335.7 \pm 33.5 abc	4.4 \pm 0.1 a	
		I	340.9 \pm 24.3 abc	4.5 \pm 0.1 a	
Vegetation				NS	NS
Amendments				NS	NS
Dose				NS	NS
Mycorrhizal inoculation				NS	NS
Vegetation * Amendment				NS	NS
Vegetation * Dose				NS	NS
Vegetation * Mycorrhizal inoculation				NS	NS
Mycorrhizal inoculation * Amendments				NS	NS
Mycorrhizal inoculation * Dose				NS	NS
Vegetation * Mycorrhizal inoculation * Amendments				NS	NS

Significance: NS: not significant; *: $p < 0.05$; according to multivariate ANOVA. NI: non-inoculated; I: inoculated.

Actinobacteria associated with *Proteobacteria*, *Gemmatimonadota*, *Myxococcota* and *Acidobacteriota* accounted for over 85% of bacterial OTUs. In general, we found that the abundance of the different bacterial phyla, and thus the structure of the bacterial community, was affected by the conditions tested. One of the most important changes concerned the high abundance of *Actinobacteria*, which was even more pronounced under the vegetated conditions, than under the unvegetated conditions, except for the not inoculated and unamended condition (Table S2). In contrast, a decrease of the *Gemmatimonadota* and *Acidobacteriota* abundances was observed under all vegetated conditions (Figure 1).

In addition to these changes in the abundance of the bacterial phyla, we also noted changes within phyla (Figure 2). For example, within the phylum *Actinobacteria*, we found that the genera *Pseudarthrobacter*, *Arthrobacter* and *Streptomyces* were enriched under vegetated conditions, whereas the genus *Gemmatimonadota* was favored under non-vegetated conditions.

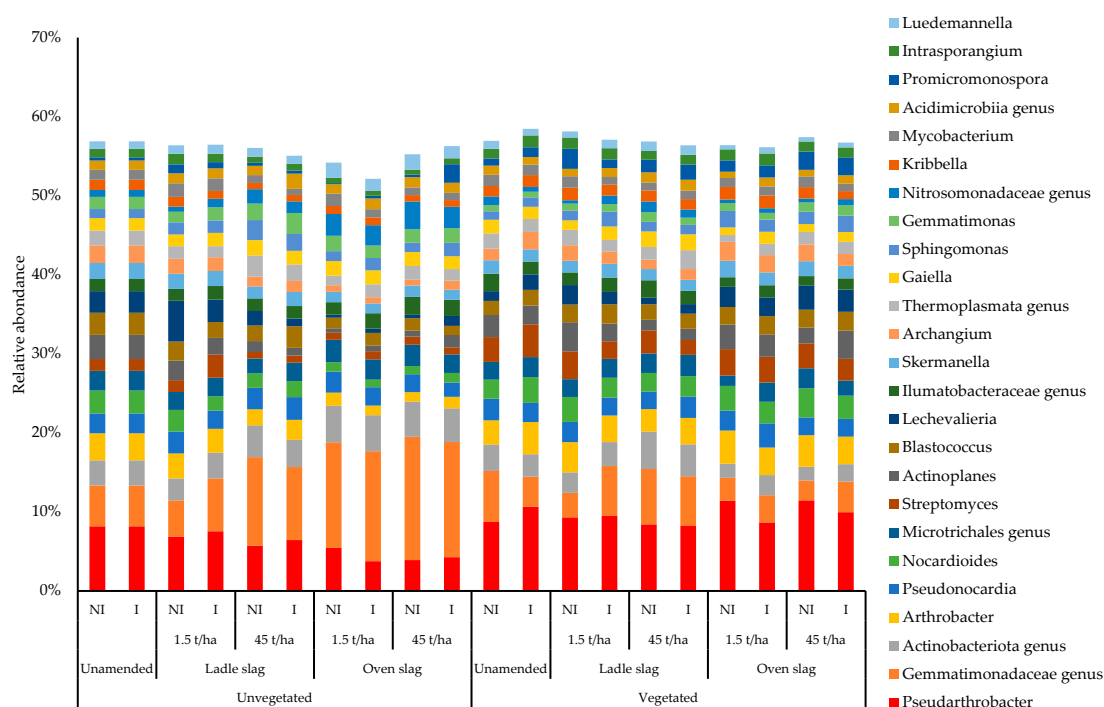


Figure 2. Abundances of the 25 most represented bacterial genus in soil. NI, non-inoculated; I, inoculated.

2.5. Metabolic Potential of Soil Microbial Communities

To understand the impact of taxonomic changes on the functionality of these microbial communities, a study was conducted on their ability to degrade different carbon sources. Overall, the metabolic potential of the soil microbial communities of each sample, expressed as average well color development (AWCD), was significantly higher under vegetated conditions than under the unvegetated conditions. The lowest value was found under the not inoculated, unvegetated and oven slag amended condition at 45 t ha^{-1} . In addition, there was no significant effect of inoculation, type of amendment or dose on AWCD level (Table 4).

A multivariate ANOVA showed that functional richness was significantly higher in vegetated conditions than in the unvegetated conditions. Neither the inoculation, nor the type or dosage of amendment had a significant influence on the values of functional richness (Table 4).

Table 4. Richness and diversity indexes of the 20 soil modalities. Data are mean \pm SD ($n = 3$) for each condition. Means followed by the same lowercase letter are not significantly different, by one-way ANOVA comparison and post-hoc Fisher test ($\alpha = 0.05$).

State	Amendment	Dose	Inoculation	AWCD	Functional Richness	
Unvegetated	Unamended		NI	100.27 \pm 18.70 ef	23.00 \pm 1.63 cde	
			I	109.00 \pm 16.49 bcdef	23.50 \pm 2.65 bcde	
	Ladle slag	1.5	NI	101.51 \pm 29.86 ef	21.50 \pm 4.43 de	
			I	131.28 \pm 21.44 abcd	25.25 \pm 1.71 abc	
		45	NI	105.58 \pm 5.46 def	24.50 \pm 1.00 bcd	
			I	99.88 \pm 17.08 ef	21.50 \pm 2.65 de	
	Oven slag	1.5	NI	107.53 \pm 15.34 cdef	23.75 \pm 1.26 bcde	
			I	98.86 \pm 18.16 ef	21.00 \pm 2.16 e	
		45	NI	87.99 \pm 23.07 f	20.75 \pm 2.87 e	
			I	96.76 \pm 14.04 ef	21.00 \pm 2.83 e	
	Vegetated	Unamended		NI	134.96 \pm 6.02 ab	26.50 \pm 1.91 ab
				I	148.19 \pm 20.41 a	26.50 \pm 1.29 ab
Ladle slag		1.5	NI	141.52 \pm 15.20 a	25.75 \pm 0.50 ab	
			I	136.81 \pm 25.96 a	26.75 \pm 1.50 ab	
		45	NI	128.84 \pm 25.97 abcd	25.00 \pm 3.65 abc	
			I	122.11 \pm 19.30 abcde	25.50 \pm 2.52 abc	
Oven slag		1.5	NI	135.67 \pm 18.88 ab	25.75 \pm 1.71 abc	
			I	133.32 \pm 19.25 abc	25.25 \pm 3.30 abc	
		45	NI	147.66 \pm 12.12 a	28.00 \pm 1.63 a	
			I	145.17 \pm 24.64 a	26.75 \pm 1.89ab	
Vegetation				***	***	
Amendments				NS	NS	
Dose				NS	NS	
Mycorrhizal inoculation				NS	NS	
Vegetation * Amendments				*	NS	
Vegetation * Dose				NS	NS	
Vegetation * Mycorrhizal inoculation				NS	NS	
Mycorrhizal inoculation * Amendments				NS	NS	
Mycorrhizal inoculation * Dose				NS	NS	
Vegetation * Mycorrhizal inoculation * Amendments * Dose				NS	NS	

Significance: NS: not significant; *: $p < 0.05$; ***: $p < 0.001$ according to multivariate ANOVA. NI: non-inoculated; I: inoculated.

3. Discussion

The cultivation of aromatic plants for the EO extraction has been presented as an innovative and economically viable alternative for the phytoremediation of TE-polluted areas [6]. However, the effects of growing aromatic plants, such as coriander, in combination with mineral (steel slag) and microbial (AMF) amendments introduced into the soil on the structure and potential function of bacterial communities in the TE-polluted rhizosphere, have never been studied. Thus, our results showed that coriander was able to establish and develop well on heavily TE-polluted soil, confirming previous work [6]. The addition of steel slag had no overall effect on plant growth. This result could be explained by the low nutrient requirement of coriander, but also by the OM content of this agricultural soil [6,31]. Consequently, it is likely that the slag did not provide any supplemental nutrient gain to the plant. Indeed, the beneficial effect of slag is controversial. Some studies have shown that these mineral amendments could contribute to mineral supply and consequently improve crop yields, while others showed no change or diverse responses, depending on the experimental conditions [13,15,32–34].

We have shown that coriander was able to form a mycorrhizal symbiosis with the native AMF present in our TE-polluted soil. Therefore, no significant difference in root mycorrhizal colonization rates was found between NI and I conditions, while AMF inoculation had no significant effect on shoot growth. In contrast to our results, some studies have shown that the introduction of AMF inoculum into TE-polluted and unpolluted soils can lead to a significant improvement in overall mycorrhizal rates, indicating different responses in experiments depending on the plant species, but also on the type of mycorrhizal inoculum [6,35,36]. We cannot exclude that the natural presence of native AMF in the TE-polluted soils masked the contribution of the exogenous mycorrhizal inoculum on coriander growth. In contrast, an increase in mycorrhizal colonization rates was observed in the presence of oven slag. This increase could be due to a reduction in TE-bioavailability or changes in pH or soil texture, as suggested by Hu et al. [16].

Although no benefits were found for the growth of coriander after mineral amendment and inoculation with AMF, several studies reported the crucial role played by these mineral and microbial amendments, as well as vegetation, in restoring soil functions that might be disturbed by TE-pollution [16,26]. In our results, the high bacterial diversity and richness indexes were comparable to some indexes obtained on unpolluted soils [37,38]. Moreover, despite the soil TE pollution, the functionality of the AWCD-based microbial communities indicated that the studied soils had a high metabolic capacity, which is typical of functional soils [39–42]. However, numerous studies have also shown a reduction in the abundance, activity, and diversity of soil microbial communities following TE exposure [42–44]. For example, Fontaine et al. [45] demonstrated a relatively low ratio of fungi-to-bacteria in this agricultural polluted soil, indicating an extensively managed soil in combination with tillage, high fertilization and a low C:N ratio favored bacteria. In this study, more Gram-positive than Gram-negative bacteria were observed under all conditions. Adaptation to an environment rich in TE leads microorganisms to exhibit activities of biosorption, bioprecipitation, extracellular sequestration, transport mechanisms and/or chelation [46]. Gram-positive bacteria are known for their diverse physiological and metabolic properties that allow them to thrive in a wide range of environments. Compared to Gram-negative bacteria, they appear to accumulate higher TE-concentrations on their cell walls [47]. Therefore, these bacteria are able to adapt easier to adverse conditions, such as pollution, and should even be used for TE-removal, which could explain their frequent presence in this soil [44,48]. Specifically, *Actinobacteria*, *Proteobacteria* and *Gemmatimonadota*, were the most abundant bacterial phyla in our TE-polluted soils. The *Actinobacteria* phylum has been highlighted for its TE-tolerance and even investigated for potential applications in remediation [49]. It plays a role in the degradation of polysaccharides, e.g., cellulose, xylan and chitin, OM turnover and in nutrient cycling [50]. *Proteobacteria*, the second most abundant phylum, is known to contain a wide range of functional bacteria, including fast-growing bacteria and many plant promoters, as well as growth-promoting bacteria [51]. Furthermore, *Proteobacteria* taxa have been described to be more abundant in heavily TE-polluted soils [44,52]. The latter phylum, *Gemmatimonadota*, is widespread in ecosystems around the world, making it an important member of soil bacterial communities [53]. Therefore, the long-term response of the bacterial community to TE results from the ability of these microorganisms to develop TE-tolerance without affecting overall community structure, as proposed by Brandt et al. [54].

The addition of amendments, such as steel slags or mycorrhizal inoculum, altered the structure of the rhizosphere bacterial community without affecting its metabolic potential, which was assessed by the AWCD parameter. In fact, the low dose of the mineral amendment increased bacterial biomass and caused a shift in the bacterial community structure. The *Actinobacteria* phylum was significantly less abundant under the slag-amended conditions, while the relative proportion of *Gemmatimonadota* increased. These results are in agreement with those of Gremion et al. [40] on a TE-polluted soil, but also with the functional redundancy of these communities [55]. Mineral granulometry has been shown to act as a physical support for the fixation of microorganisms (i.e., bacteria, fungi, etc.)

and plants, as well as a nutrient reserve, and that the abundance of *Acidobacteria* decreases with increasing pH [56]. In this respect, and due to the alkaline nature of slags, the pH and mineralosphere can be important factors influencing microbial communities [56,57].

Cultivation of coriander also resulted in a change in the bacterial biomass and community structure. A significant increase in Gram-negative bacterial biomass under vegetated conditions and in the proportion of *Actinobacteria* phylum was detected. It is well known that plants secrete specific root exudates to shape the microbial community structures in the rhizosphere. These compounds, including sugars, amino acids and organic acids, have various effects on bacterial community composition and microbiome assembly [58]. In addition, aromatic plants are also known to be able to secrete secondary metabolites in their root exudates [59–61]. Due to their anti-microbial properties, some of these metabolites probably influence bacterial richness and diversity by targeting specific communities [62]. Some compounds may therefore affect microbial communities in the rhizosphere [60]. This is especially true for mono- and sesquiterpenes found in aromatic plants [60].

We noted an increase in the genus *Streptomyces* under vegetated conditions, a genus that has been described for its abilities as plant growth-promoting bacteria, but also for TE biosorption, making it particularly interesting in a phytostabilization context [63,64]. Moreover, the relative abundance of some genera (*Sphingomonas*, *Gemmatimonas*, *Kribbella*, ...) was very low, but they could play an important role in bio-geochemical cycles [65,66]. In fact, rare taxa are more modulated in abundance than dominant taxa [66], and therefore could be important factors in the resilience and adaptability of soil microorganisms [66]. Interestingly, some bacterial phyla have shown the ability to limit the accumulation of TE in plants. Indeed, some *Actinobacteria* and *Proteobacteria* can alter the bioavailability of heavy metals through redox, precipitation or sorption and desorption reactions [49]. These phyla, such as *Firmicutes* and *Bacteroidetes*, are known to possess TE-tolerance genes encoding proteins involved in efflux and sequestration of TE ions, such as AT-Pases, D-protein or B-protein (copper tolerance and transport, respectively) and oxidative stress attenuation proteins, such as superoxide dismutase, alkyl hydroperoxide reductase or mycothiol reductase [67,68].

Therefore, coriander in TE-polluted soil activated the resilience of bacterial communities. This resilience of microbial communities and functions in TE-polluted soils is usually related to a shift from sensitive to tolerant species, genetic modifications leading to TE-resistance, a transfer of genes encoding resistance or tolerance to TE, or a decrease in TE-bioavailability [69]. The recruitment of bacterial species belonging to the genera *Pseudarthrobacter*, *Arthrobacter* and *Streptomyces* by root exudates of coriander to the detriment of *Gemmatimonadota* bacteria, was associated with an increase in soil functionality that corresponds to such a shift. In this case, species belonging to *Gemmatimonadota* could be considered as indicator species for resistance to TE. Similarly, species belonging to *Pseudarthrobacter*, *Arthrobacter* and *Streptomyces* could be more sensitive to TE, but can characterize the resistance of TE-polluted soils when suitable substrates are available, such as those provided by root exudates in phytomanagement.

4. Materials and Methods

4.1. In Situ Experimental Design

Study Area.

The study took place on an agricultural soil, historically polluted by TE (50°25'55.5" N, 3°02'25.5" E; elevation 23 m) in the "Hauts de France" region (northern France). The experimental site was located 600 m away from a former Pb and Zn smelter, Metaleurop Nord, whose activity generated significant amounts of TE-rich dust, resulting in TE-pollution of the topsoil (0–30 cm) [70,71]. The topsoil is characterized as silt loam, with a slightly alkaline water pH (7.9). It contains high TE total concentrations of Cd, Pb and Zn (7, 394 and 443 ppm, respectively), of which the NH_4NO_3 extractable fractions account for 0.114, 0.078, and 0.930 ppm, respectively [6]. These concentrations were approximately 17, 11 and 6-fold higher, respectively, than the regional background values for agricultural soils [72].

4.2. Experimental Design

The *in situ* experimental design was divided into 20 equal plots of 9 m², to investigate the effects of one biological (AMF) and two mineral amendments on soil function, with or without planting an annual herbaceous aromatic plant species, coriander (*Coriandrum sativum* L.). The mineral amendments, ladle and oven slags, were by-products of steel production, often used in agriculture due to their buffering capacity [73]. Their main constituent was CaO and Fe₂O₃, respectively. The biological amendment was the commercial mycorrhizal inoculum SYMBIVIT[®] (INOCULUMplus, Bretenière, France), which contained six AMF species: *Rhizophagus irregularis*, *Funneliformis mosseae*, *Glomus microaggregatum*, *Glomus claroideum*, *Claroideoglomus etunicatum* and *Funneliformis geosporum*. The surface of each test area was 9 m² (3 m × 3 m), and each test area was randomly arranged and separated from each other by a 1 m wide corridor. A total of 20 conditions were designed (Table S1). Before sowing, the soil of the test plots was amended or not with 2 different concentrations of 1.5 and 45 t ha⁻¹ mineral amendment (ladle and oven slags) and/or the biological amendment at a rate of 10 kg ha⁻¹. These plots were then sown (7.5 kg ha⁻¹) or not (unvegetated condition) with coriander (seeds were provided by Iteipmai, France) in March 2019. The unvegetated plots were kept in this condition by regular manual weeding.

4.3. Sample Collection

At the beginning of the *in situ* experiment, 5 soil samples representing the original condition were taken with a soil auger in the 0–20 cm soil horizon, and stored at –20 °C until analysis. Three weeks after sowing, germination rate was assessed by counting the number of coriander plants in 3 random squares (1 m²) for each vegetated plot. After 11 weeks of cultivation, 5 new soil samples were collected in each plot from the 0–20 cm soil horizon and stored at –20 °C until analysis; the aboveground biomass and roots of 5 plants were also harvested from the vegetated area. The height of the aerial parts was measured first, before the plants were dried and weighed, while the fresh roots were washed and stored at 4 °C until stained for AMF root colonization determination. The roots were cleared in 10% (*w/v*) KOH and stained with 0.05% (*w/v*) trypan blue (Alfa Aesar) [74] to determine root colonization [75]. Briefly, root fragments were placed on microscopic slides and mycorrhizal structures (arbuscules and vesicles) were counted for each fragment observed. Three counts per fragment were performed. The percentage of total colonization was equal to the number of intersections with mycorrhizal roots × 100/number of total intersections. This percentage of colonization was expressed as the percentage of arbuscules, vesicles and total colonization.

4.4. Fatty Acid Analysis

After removing plant debris from the soil samples, 3 g of freeze-dried soil (3 samples per test area) were used for the analysis of fatty acid content. Lipid extraction was performed according to Frostegård et al., with a mixture of chloroform:methanol:citrate buffer—0.15M, pH 4.0—(1:2:0.8 *v/v/v*), under agitation for 2 h [76]. The lipid material was fractionated on Solid Phase Extraction (SPE) columns containing silica (6 mL volume, 500 mg sorbent, Interchim, Montluçon, France) by successive elutions with chloroform, acetone and methanol (1:2:1, *v/v/v*) into neutral lipids (NLFA), glycolipids and polar lipids containing phospholipid (PLFA) [76]. The NLFA and PLFA were trans-esterified with 0.2 M KOH in methanol to obtain free fatty acid methyl esters. The resulting fatty acid methyl esters were analyzed using a gas phase chromatography-mass spectrometer (GC-MS) Shimadzu QP-2010 Ultra (Shimadzu, Japan) equipped with a single quadrupole mass detector and simultaneously coupled with a flame ionization detector (FID). Samples were analyzed in split mode (80:1 ratio) on a ZB-1MS fast capillary column (10 m length × 0.1 mm inner diameter × 0.1 µm phase thickness, 100% dimethylpolysiloxane, Zebron, Phenomenex, Torrance Calif, CA, USA) using helium as the carrier gas at a constant linear velocity (40 cm s⁻¹). The injector temperature was 280 °C, and the detector temperatures were 330 °C and 280 °C for FID and for the ion source, respectively. The temperature program

started with an initial temperature of 175 °C and increased by 25 °C every minute to reach a final temperature of 275 °C, which was maintained for 0.5 min. The ionization mode was electronic impact at 70 eV and the mass range between 50.0 and 400.0 u was scanned. The single impact monitoring (SIM) mode was used simultaneously. Quantification of fatty acids was performed using nonadecanoic acid methyl ester (C19:0, Sigma-Aldrich) as an internal standard. Fatty acids were identified by comparing their relative retention time with that of commercial fatty acid methyl ester standards (C4–C24:1, Sigma-Aldrich), and by comparing them with spectra either obtained from commercial standards and/or published in the literature (NIST Standard Reference Database). Phospholipid fatty acid (PLFA) i15:0, a15:0, i16:0, i17:0, a17:0 and PLFA cy17:0, C18:1 ω 7 and cy19:0 were used as indicators of Gram-positive and negative bacterial biomasses, respectively [77].

4.5. DNA Extraction

Genomic DNA was extracted in triplicate directly from 250 mg fresh soil ($n = 60$) using the NucleoSpin Soil[®] kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The quality of the extracted DNA was checked using 1% (w/v) agarose gels. Quantification of the extracted DNA was performed using a SpectraMax[®] iD3 spectrophotometer (Molecular Devices LLC, Sunnyvale, CA, United States). The extracted DNA was stored at -20 °C until use.

Polymerase Chain Reaction and sequencing of bacterial 16S rRNA gene Hypervariable regions V3–V4 of the 16S rRNA gene were amplified from 1 ng of genomic DNA using a PCR thermal cycler (Agilent Surecycler 8800) with the forward primer CS1_341_F (ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCAG) and the reverse primer CS2_805_R (TACGGTAGCAGAGACTTGGTCTCTGACTACCAGGGTATCTAATC) [78]. Two independent PCR reactions were performed per DNA sample.

4.6. Illumina MiSeq Sequencing

All PCR products were pooled ($n = 60$) and sent to the Genome Quebec Innovation Centre (Montreal, QC, Canada) for sequencing, using Illumina MiSeq platform, producing paired-end 2 bp \times 300 bp.

4.7. Data Analysis–Bioinformatic Processing

Sequences were loaded into the Galaxy instance (v.2.3.0) of the Genotool bioinformatics platform (<http://sigenae-workbench.toulouse.inra.fr>, accessed on 27 December 2022) to be processed in the pipeline FROGS (Find Rapidly OTU with Galaxy Solution, [79]). Sequences with ambiguous bases (N) or without the specific primers were removed. The Cutadapt software [80] was used to search and cut primer sequences with less than 10% difference. The number of sequences identified was 2,660,014 and sequence clustering was performed using the SWARM algorithm (v2.1.5; [81]). A first denoising step was performed to build very fine clusters with minimal differences ($d = 1$), and a second one with an aggregation distance of 3. The resulting representative sequences for each cluster, or OTUs (Operational Taxonomic Units), were subjected to chimera detection using the VSEARCH algorithm [82] and eliminated, as well as those with an abundance $<0.001\%$ of the total abundance. In the end, 48,251 OTUs were retained, corresponding to 2,264,807 sequences. The taxonomic classification of each OTU against the Silva database (v138.1) was done using RDPClassifier [83]. The abundance of the different samples ranged from 65,070 to 16,243. As a result, a rarefaction of OTUs at species level was performed with 16,243 as the rarefaction value (“rrarefy” function of the vegan package). Following this, diversity indices were determined. Comparisons of means were made (Figures Shannon, and Chao1). As the Shapiro–Wilk group tests for the different indices indicated significant p -values (samples not following a normal distribution), comparisons via Wilcoxon–Mann–Whitney and Kruskal–Wallis tests were performed.

4.8. Nucleotide Sequence Accession Numbers

The 16S rRNA gene sequences of the whole dataset have been deposited in NCBI Sequence Read Archive (SRA) database and can be found under accession number PRJNA918629.

4.9. Soil Bacterial Metabolic Profiles

The metabolic potential of soil communities was determined from community-level physiological profiles (CLPPs) using Biolog EcoPlates (Biolog Inc.; [55]). For each soil sample, an EcoPlate containing 31 different carbon sources (and one blank with no carbon source) in triplicate was inoculated, and incubated for 196 h at 25 °C in an OmniLog® system (Biolog Inc.). The rate of carbon source utilization was recorded by the reduction of tetrazolium, a redox color indicator that changes from colorless to purple and is detected at a wavelength of 590 nm. Data were recorded every 15 min of incubation and saved in OmniLog® units generated by Biolog Data Analysis software (v1.7). Values for each well were calculated by subtracting the blank values from each well of the plate. A single absorbance time point at 50 h was used for the comparisons, as recommended in [84]. The metabolic potential of the soil microbial communities in each sample, expressed as the average well color development (AWCD), was calculated at the determined time point by dividing the sum of the optical density data by 31 (number of substrates). The total number of wells in a replicate with an absorbance above 25 OmniLog® units was counted to determine the functional richness of the soil microbial community.

4.10. Statistical Analysis

Statistical analyses were performed using XLSTAT software (v2021.1.1; Addinsoft, Paris, France). Normality of the data was first subjected to the Shapiro–Wilk test. Depending on the result of this test, parametric (ANOVA) or non-parametric (Kruskal–Wallis) tests were used, followed by Fisher’s post-hoc test (Least Significant Difference, LSD). Data expressed as percentages (AMF colonization rate) were converted to arcsine values (ASIN function in Microsoft® Excel v16.50) before statistical analysis. Bacterial community analysis was performed using the R environment (v4.2.2; <http://www.r-project.org/>, accessed on 27 December 2022). Permutational multivariate analysis of variance (PERMANOVA), based on the Bray–Curtis dissimilarity, were performed with 1000 permutations using the “adonis” function of the vegan [85] R package. Bacterial richness (Chao1) and diversity (Shannon) indexes were calculated using the Phyloseq (v1.36.0; [86]) R package.

5. Conclusions

This study investigated the effect of coriander cultivation and introduction of AMF in combination with mineral amendments (steel slags) on the bacterial function of the rhizosphere, an aspect that is poorly understood and still poorly studied. The introduction of amendments, such as steel slags or mycorrhizal inoculum, into the soil had no significant effect on coriander growth. However, these amendments altered the structure of bacterial communities in the rhizosphere, without affecting their metabolic potential. In fact, *Actinobacteria* were significantly less abundant under the slag-amended conditions (55% on average), while the relative proportion of *Gemmatimonadota* increased (10% on average). In contrast, soil vegetation by coriander increased Gram-negative bacterial biomass (mean 2.96 to 3.87 µg g⁻¹ of DW soil) and the proportion of *Actinobacteria* phylum (62%). Thus, the vegetation improved soil quality and health by increasing the diversity of bacterial metabolic functions and diversity. Our results showed that coriander activated the resilience of bacterial communities in TE-polluted soils. Consequently, our results suggest that steel slag, which contributes to the circular economy through its recycling, in combination with soil vegetation that includes aromatic plants such as coriander, potentially provides long-term ecological benefits to TE-polluted soils and should be considered as a reliable tool for future TE-polluted soil remediation. Further studies are needed to confirm the contribution of steel slag to assisted TE-phytostabilization and its persistence in soil.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/plants12030618/s1>, Table S1: Experimental design of plots describing all 20 modalities studied; Table S2: Influence of amendments and vegetation on most abundant phyla relative abundance, Table S3: Influence of amendments and vegetation (V: vegetated, UV: unvegetated, NI: not-inoculated, I: inoculated, LS: ladle slag and OS: oven slag, NA: not-amended, 1.5 and 45 t ha⁻¹) on biolig substrats family.

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Partie 4 : Discussion générale et perspectives

Synthèse générale et perspectives

Parmi les filières de phytomangement présentant un haut potentiel de rentabilité, la filière PPAM-HE s'est révélée être une option prometteuse dans le cadre d'un précédent projet, PHYTEO financé par l'ADEME (APR Graine 2016). Ce projet avait pour principal objectif de développer un mode de gestion reposant sur la phytostabilisation des ET et la production d'HE extraites à partir de PPAM. Trois espèces végétales ont été testées dans le cadre de ce projet, la coriandre, la sauge sclérée et partiellement l'angélique. Les essais exploratoires réalisés dans le cadre du projet PHYTEO ont permis d'obtenir des résultats encourageants et de démontrer la faisabilité technique et le bien-fondé de ce mode de gestion.

Celui-ci s'est poursuivi par le projet DEPHYTOP (APR Graine 2019, ADEME) dans lequel s'inscrivent mes travaux de thèse. Le projet DEPHYTOP avait pour objectifs d'optimiser cette filière PPAM-HE en testant différents amendements, biologiques (inoculum mycorhizien), ou minéraux (laitiers sidérurgiques) chez la coriandre et d'étudier la requalification écologique des sols pollués grâce à la culture de PPAM, telles que l'angélique et la coriandre ou l'emploi de ces amendements. D'autre part, ce projet avait également pour but d'évaluer la capacité phytostabilisatrice de l'angélique (étude de la mobilité des ET dans le sol et/ou éventuel transfert dans les parties aériennes) et les filières de valorisation des résidus de distillation. Ces derniers points ne seront pas abordés dans ce manuscrit, puisque ce travail a été réalisé par l'un des partenaires du projet, l'INERIS.

Aussi, ma contribution dans le cadre du projet DEPHYTOP a consisté à participer (i) au suivi et à la récolte (2021, 2022) de l'angélique cultivée depuis 2018 sur le site d'Evin - Malmaison, (ii) à la mise en place du dispositif expérimental en microparcelles afin de tester l'apport d'amendements minéraux et/ou biologiques chez la coriandre (iii) à l'étude de la composition chimique des HE d'angélique distillées par la société Ferrant PHE, producteur d'HE situé dans la Région Hauts-de-France, (iv) à la détermination de leurs qualités (contaminations par les ET et résidus de pesticides) (v) à l'étude de leurs propriétés biologiques (antifongiques, herbicides, antioxydantes et anti-inflammatoires) (vi) à l'étude de l'impact de la culture de PPAM et des amendements sur le microbiote rhizosphérique et la requalification écologique du sol pollué avant et après phytoremédiation (vii) et à l'étude de la viabilité économique de la filière Angélique-HE.

À l'échelle nationale, la région Hauts-de-France est particulièrement touchée par la problématique de la pollution des sols par les ET, en raison de son passé industriel et minier. La gestion de ces sols pollués (SSP) représente un enjeu majeur d'ordre sanitaire, environnemental, économique et social. En

répondant à la double exigence de performances environnementales et économiques, le phytomanagement, qui combine à la fois la phytoremédiation et la valorisation de la biomasse produite sur ces SSP, compte parmi les méthodes innovantes de gestion des SSP (Cundy et al., 2016). Jusqu'à présent, les filières de valorisation étudiées ont porté principalement sur l'utilisation de la biomasse à des fins énergétiques et à l'élaboration d'éco-matériaux (Moreira et al., 2021; Perlein et al., 2021a). La production de molécules à haute valeur ajoutée telles que les HE extraites des PPAM, suscite de plus en plus d'intérêt et pourrait s'avérer être une alternative intéressante et rentable (Pandey et al., 2019; Raveau et al., 2021a). En effet, grâce à leurs nombreux principes actifs, les HE peuvent être utilisées dans de nombreux domaines à vocation non alimentaire tels que la parfumerie, la cosmétique, l'industrie pharmaceutique ou encore comme alternative aux produits phytosanitaires (Raveau et al., 2022). À l'heure actuelle, peu de travaux ont décrit la faisabilité technico-économique de la filière PPAM-HE dans un contexte de phytomanagement (Raveau et al., 2021a) et à notre connaissance, aucune étude n'a porté sur l'angélique.

En effet, le choix du couvert végétal le plus adapté au contexte de la pollution des sols, est un enjeu important dans la gestion d'un site pollué, et doit reposer sur des critères précis (Mench et al., 2019). Ainsi, dans le cadre d'une filière de phytomanagement où l'objectif est de proposer aux agriculteurs une alternative aux cultures conventionnelles, des espèces végétales ayant une rentabilité économique importante et nécessitant peu d'intrants pour leur développement doivent être privilégiées. Les semences doivent être facilement disponibles et les pratiques culturales doivent être proches de celles utilisées par les agriculteurs. Enfin, la tolérance aux ET est également un élément important dans le choix des espèces végétales. Aussi, dans le cadre de mes travaux de thèse, deux PPAM ont été retenues : l'angélique (*Angelica archangelica* – Apiacées) et la coriandre (*Coriandrum sativum* L. - Apiacées). L'angélique est une espèce bisannuelle pouvant être cultivée pendant une dizaine d'années sur la même parcelle, et ce sont les graines qui sont récoltées et distillées (Aćimović et al., 2022). La coriandre quant à elle, est une espèce annuelle à croissance rapide, capable d'établir en quelques semaines un couvert végétal dense (Ghobadi and Ghobadi, 2010), ce sont les parties aériennes (tiges et feuilles) ou les graines qui peuvent être utilisées pour produire les HE (Mandal and Mandal, 2015).

Les deux espèces végétales sélectionnées dans le cadre de ce projet ont été peu étudiées dans un contexte de phytomanagement, en particulier l'angélique, ce qui constitue en partie, l'originalité de ce travail (Figure 5).

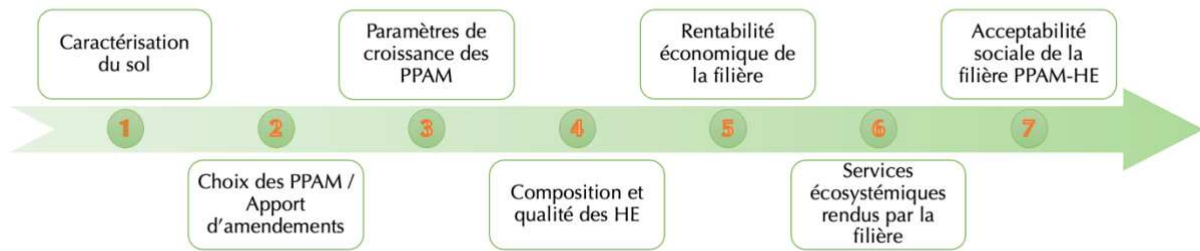


Figure 7 : Schéma récapitulatif du développement de la filière PPAM-HE dans un contexte de phytomanagement d'un sol pollué par les ET

I. Pertinence de la filière angélique-HE pour le phytomanagement des sols pollués par les ET

A. Dispositif expérimental

Dans le cadre du projet DEPHYTOP, une parcelle expérimentale du réseau SAFIR, située à Evin - Malmaison, dans les Hauts-de-France, a été mobilisée. Ce site expérimental se trouve à 500 m environ de l'ancienne usine métallurgique Metaleurop Nord, dans la zone 3, à la limite de la zone 2 du périmètre défini autour du site Evin Malmaison (Figure 1). Les sols de cette parcelle expérimentales sont caractérisés par des teneurs en Cd (7 ppm), Pb (376 ppm) et Zn (429 ppm) dépassant respectivement de 17, 11 et 6 fois le fond géochimique régional (Sterckeman et al., 2007).

Pour le dispositif angélique, la parcelle expérimentale mesurait 7 100 m² et était divisée en deux conditions, l'une inoculée par un inoculum mycorhizien composé d'une seule espèce de CMA (*Rhizophagus irregularis* - inoculum AGTIV® cultures spécialisées, Premier Tech), l'autre non inoculée (Figure 2). Le semis a été réalisé en 2018, et les premières récoltes d'angélique ont été réalisées en 2020, 2021 et 2022. Des échantillons de sols ont été prélevés avant le semis (état initial) et avant chaque récolte afin d'obtenir un suivi sur quatre années de culture successives. Les HE étudiées dans le cadre de ce travail de thèse ont été obtenues par hydrodistillation réalisée par la société Ferrant PHE à partir des graines d'angélique récoltées sur notre dispositif expérimental de Metaleurop.

B. Croissance de l'angélique, rendements en biomasse et en HE satisfaisants

Synthèse générale et perspectives

Six semaines après le semis de l'angélique, le taux de levée de l'angélique était d'environ 26 % dans les conditions NI et I. En effet, les graines d'angélique ont un taux de germination assez faible qui se situe en moyenne autour de 24 % (Ojala, 1985). Les taux de mycorhization étaient similaires les deux premières années de culture, et significativement plus élevés dans la condition inoculée en quatrième année de culture. Cependant, l'inoculation n'a pas significativement amélioré les rendements en graines ou en HE. En effet, les plants d'angélique ont atteint une hauteur comprise entre 158 à 187 cm, un poids sec de 118 à 517 g/plante et un diamètre de tige de 3,5 à 4,7 cm. La production de graines d'angélique était comprise entre 136 et 614 kg.ha⁻¹, ce qui a permis d'obtenir un rendement maximal d'HE de 1,9 kg.ha⁻¹ ([Publication 1 - Langrand et al., 2024, à soumettre](#)). Nos résultats montrent que les paramètres de croissance sont similaires à ceux mesurés sur des sols non pollués (Jakab et al., 2014; Kylin, 2012), ce qui indique que la pollution des sols n'a pas affecté la croissance des plantes.

⇒ **L'angélique est capable de germer et de se développer sur un sol fortement pollué par les ET de la même façon que sur un sol non pollué.**

Cependant, les rendements en HE généralement obtenus pour la distillation de graines d'angélique sur un sol non pollué sont 5 à 7 fois supérieurs à ceux obtenus dans nos conditions expérimentales (Jakab et al., 2014). Plusieurs hypothèses peuvent être émises pour expliquer ce résultat : la taille limitée de la parcelle qui ne permet pas d'optimiser la récolte des graines à la moissonneuse batteuse, un stade avancé de maturité des graines qui entraîne des pertes importantes de graines lors de la récolte, ou encore la taille du dispositif de distillation non optimisé pour le volume de graines récoltées dans le cadre de ce projet.

Ces résultats sont néanmoins encourageants et démontrent la tolérance de l'angélique aux ET présents dans le sol. Cependant, les mécanismes de tolérance aux ET de l'angélique n'ont à notre connaissance jamais été étudiés. C'est pourquoi, une étude de la capacité de l'angélique à produire certains peptides tels que le glutathion et les phytochélatines, ou protéines telles que les métallothionéines ou autre polypeptides riches en cystéine et pouvant jouer un rôle important dans la tolérance des plantes au stress métallique pourrait être envisagée (Sterckeman and Thomine, 2020). Un suivi de l'expression des gènes codants pour ces protéines pourrait être envisagé (Jain et al., 2018).

C. Qualité des HE produites sur sols pollués

Synthèse générale et perspectives

1. Les HE produites sur sols pollués présentent la même qualité que les HE commercialisées

Dans le but de vérifier la qualité des HE produites sur le sol pollué, une analyse de la teneur en ET des HE a été réalisée ([Publication 1 - Langrand et al., 2024, à soumettre](#)). Il a ainsi été établi que :

- ⇒ **Les HE d'angélique contiennent des ET en proportions très faibles, souvent inférieures aux limites de quantification de l'appareil. De plus, la teneur en ET de l'HE d'angélique produite sur un sol pollué est équivalente à celle d'une l'HE commerciale produite sur un sol non pollué.**

Une analyse multirésidus de pesticides a également été effectuée dans le but de contrôler la qualité des HE ([Publication 1 - Langrand et al., 2024, à soumettre](#)). Ces résultats montrent que :

- ⇒ **Les HE d'angélique sont de bonne qualité, seul huit substances actives ont été détectées sur les 480 recherchées. Celles-ci étaient majoritairement présentes à l'état de traces ou en faibles quantités.**

Il est important de signaler que la législation Française ne donne aucune limite maximale de résidus de pesticides ou d'ET dans les HE à usage à alimentaire ou non (Tascone et al., 2014). Ainsi, pour combler ce vide juridique, il est important que cette question soit soulevée et traitée par les juristes du droit de l'environnement dans le futur.

2. Composition chimique des HE produites sur sols pollués similaire à celle des HE commerciales

Nos résultats indiquent que l'HE d'angélique est majoritairement composée de monoterpènes (89%). Parmi ces composés, nous pouvons noter la présence de β -phéllandrène (73 - 80%), α -pinène (5 - 7%), α -phéllandrène (2 - 4%), et de β -myrcène (0.5 - 2%) ([Publication 2 - Langrand et al., 2024, à soumettre](#)). Cette composition est conforme à celle d'une HE d'angélique commerciale, et aux données de la littérature qui rapportent des valeurs sensiblement similaires (Kylin, 2010; Nivinskiene et al., 2007; Nivinskienè et al., 2005). En outre, la composition des HE issues des conditions NI et I restent comparables quelle que soit l'année étudiée. Aucun effet de l'inoculation mycorhizienne n'a été

observé au niveau de la composition chimique des HE produites sur sols pollués, les plantes étant mycorhizées à des taux relativement proches (mycorhization spontanée).

⇒ **Les compositions chimiques des HE d'angélique récoltées en 2020 et 2021 sur les parcelles I et NI sont identiques.**

Ces résultats sont en accord avec les résultats de Raveau *et al.* (2021b) qui ont montré que l'inoculation mycorhizienne de deux autres espèces de PPAM, la coriandre (*C. sativum L.*) et la sauge (*S. sclarea L.*), ne modifiait pas la composition chimique des HE produites. Bien que la littérature mentionne de nombreux facteurs pouvant influencer la composition des HE, tels que le manque d'eau, le type de sol ou l'ensoleillement, les données sont souvent contradictoires (Khalid, 2006; Sowndhararajan *et al.*, 2017). En particulier, il a été suggéré que le stress induit par les ET chez les plantes peut également conduire à des changements de leur métabolisme secondaire (Prasad *et al.*, 2014; Skrypnik *et al.*, 2022). Par exemple, la menthe poivrée (*Mentha × piperita L.*) exposée au Cd a vu sa teneur en menthol diminuer et celles en menthofurane et en pulégone augmenter (Azimychetabi *et al.*, 2021). Il semble que ces modifications varient considérablement en fonction des espèces de PPAM et du type d'ET, de leur capacité à les accumuler, et de leur phytotoxicité (Biswas *et al.*, 2011; Prasad *et al.*, 2014).

D. Les HE d'angélique produites sur sols pollués par les ET présentent un gros potentiel dans les domaines de la protection des cultures et de la santé humaine

Une autre partie de mes travaux de thèse s'est intéressée à l'étude des propriétés biologiques des HE distillées à partir des graines d'angélique produites sur un sol pollué par les ET. En effet, la demande mondiale en HE est en constante augmentation, avec un marché pouvant dépasser les 3 milliards de dollars d'ici 2025 (Yan *et al.*, 2019). Leur utilisation dans des filières non alimentaires telles que les domaines de la santé humaine ou de la protection des cultures pourrait garantir la pertinence de la filière PPAM-HE sur sols pollués.

1. Propriétés biologiques des HE en protection des cultures

Afin d'évaluer le potentiel des HE d'angélique en protection des cultures, ses propriétés antifongiques ont été évaluées *in vitro* sur des champignons ou oomycète phytopathogènes des grandes cultures : *F. culmorum*, *F. graminearum*, *Z. tritici*, *B. graminis*, *P. infestans*, et *in planta* contre l'oïdium du blé, ainsi que ses propriétés herbicides sur le ray-grass et la laitue (Publication 2 - Langrand *et al.*, 2024, à soumettre).

Avec des CI_{50} allant de 21 pour *Z. tritici* à 221 $mg.L^{-1}$ pour *B. graminis*, l'HE d'angélique possède des propriétés antifongiques intéressantes. En effet, dans tous les cas étudiés, les CI_{50} de l'HE d'angélique sont significativement plus élevés que ceux du fongicide conventionnel, Aviator, utilisé comme contrôle positif, avec des valeurs comprises entre $3,24.10^{-2}$ pour *F. graminearum* à $3,54.10^{-1} mg.L^{-1}$ pour *F. culmorum*. Après l'observation de ces inhibitions de croissance en présence d'HE, des repiquages sur des milieux exempts d'HE ont permis de démontrer une activité fongistatique de l'HE d'angélique sur les champignons pathogènes étudiés.

⇒ **L'HE d'angélique présente une activité fongistatique contre plusieurs champignons phytopathogènes et oomycètes : *F. culmorum*, *F. graminearum*, *Z. tritici* et *P. infestans*.**

En outre, les HE sont connus pour être moins nocives que les fongicides conventionnels pour l'environnement, la santé humaine et les organismes non cibles, en raison de leur faible persistance dans les sols et de leur volatilité (De Clerck et al., 2020). De plus, contrairement aux pesticides chimiques, elles n'entraînent pas le développement de résistances chez les espèces nuisibles ciblées (Acheuk et al., 2022; Khursheed et al., 2022). Cela peut s'expliquer par la complexité, la diversité et le grand nombre de composants des HE, et/ou par la faible fréquence de leur utilisation.

Des tests de protection du blé contre l'oïdium par des traitements préventifs par pulvérisation d'HE d'angélique ont été réalisés *in planta*. L'application de 5 % d'HE d'angélique a entraîné une diminution significative, estimée à 35 %, du nombre de colonies par plante par rapport au témoin de blé non traité.

⇒ **L'activité biologique de l'HE d'angélique contre l'oïdium du blé diminue de plus d'un tiers la pression infectieuse.**

L'étude des propriétés herbicides de l'HE d'angélique a été réalisée sur la laitue et le ray-grass qui sont couramment employées dans les tests de phytotoxicité (Park et al., 2016). Les CI_{50} de l'HE d'angélique étaient respectivement de 144 et 5390 $mg.L^{-1}$ pour le ray-grass et la laitue. Quant aux CI_{50} du glyphosate utilisé comme témoin, ces dernières étaient respectivement de 1,47 et $4,65.10^{-1} mg.L^{-1}$ pour le ray-grass et la laitue. Bien que nettement supérieure à celle du glyphosate, la CI_{50} de l'HE d'angélique contre le ray-grass n'est pas significativement différente du témoin conventionnel.

⇒ **L'HE d'angélique possède un potentiel herbicide contre le ray-grass.**

Les produits à base d'HE peuvent représenter une alternative naturelle pour remplacer les fongicides et les herbicides synthétiques et constituer des biopesticides potentiels (Pavela and Benelli, 2016). D'ailleurs, un certain nombre de produits contenant des HE sont actuellement commercialisés en tant que fongicide antigerminatifs ou herbicides (Raveau et al., 2020). Certaines HE (orange douce, menthe verte, citronnelle ou encore clou de girofle) sont d'ailleurs homologuées et peuvent être utilisées à ces égards, en France ou en Europe (E-phy, <https://ephy.anses.fr> ; EU pesticide database, https://ec.europa.eu/food/plant/pesticides/eu-pesticides-db_en). La plus part des études disponibles dans la littérature décrivent des modes d'action par contact direct des HE, bien qu'une activité fongicide par volatilité soit possible ainsi qu'une stimulation des réactions des défenses des plantes (El-Alam et al., 2020; Nazzaro et al., 2017; Soudani et al., 2022).

2. Propriétés biologiques des HE et santé humaine

Les activités antioxydantes et anti-inflammatoires de l'HE d'angélique ont été évaluées en vue d'une potentielle application dans le domaine de la santé humaine ou animale.

Concernant l'activité antioxydante, l'efficacité de l'HE d'angélique a été comparée à celle d'un antioxydant standard bien connu, le Trolox[®] (analogue de la vitamine E), et exprimée en termes de TEAC, qui est inversement proportionnelle aux valeurs CE₅₀ obtenues. Ces valeurs sont <1 µmol.eq.Trolox[®].g⁻¹ HE à 1H et proches de 2 après 5H. Bien que la comparaison d'activités antioxydantes issues de différentes expérimentations pourrait être biaisée, la littérature rapporte des valeurs globalement supérieures, notamment dans le cas de l'HE de *Pimenta racemosa* qui contient d'avantage de phénols que l'HE d'angélique (Kfoury et al., 2015). Cependant, l'activité antioxydante de l'HE d'angélique sont voisines de celles de la sauge sclarée et de la coriandre et peut être qualifiée de modérée par rapport à d'autres HE comme celle de jasmin (Raveau et al., 2021b; Wei and Shibamoto, 2007).

⇒ **L'HE d'angélique possède activité antioxydante modérée.**

D'autre part, l'activité anti-inflammatoire de l'HE d'angélique a été évaluée en dosant la concentration d'interleukine 8 (IL-8) sécrétée dans le milieu de culture par des cellules pulmonaires inflammatoires.

L'ajout de l'HE d'angélique a divisé par 6 la concentration d'IL-8 par rapport au témoin, et par conséquent,

⇒ **L'HE d'angélique possède un réel potentiel anti-inflammatoire.**

Les résultats obtenus lors de ces travaux démontrent la grande diversité des activités biologiques de l'HE d'angélique produite sur sols pollués. Il est important de rappeler que l'HE utilisée pour ces tests in vitro et in planta n'était pas formulée et qu'un travail de formulation pourrait nettement améliorer son potentiel. Les HE étant par nature volatiles et hydrophobes, l'utilisation d'émulsifiants et de systèmes d'encapsulation tels que les cyclodextrines pourrait permettre de prolonger leur libération et accroître leur efficacité (Kfoury et al., 2018). D'un point de vue plus fondamental, les HE sont des mélanges complexes de molécules susceptibles d'agir seules ou en synergie avec d'autres composés, et les mécanismes déterminant leur mode d'action ne sont pas totalement élucidés (Dalcin et al., 2022; Osorio et al., 2021). C'est pourquoi une étude de l'activité de certaines molécules isolées de l'HE d'angélique, permettrait d'identifier certains principes actifs et d'en rechercher les modes d'action. Par exemple, contrairement à l' α -pinène, l' α -phéllandrène, et le β -myrcène, connus pour leurs propriétés biologiques, notamment antifongique, le β -phéllandrène (composé majoritaire de l'HE d'angélique) est peu décrit dans la littérature et mériterait une attention particulière.

E. Bilan économique de la filière PPAM-HE, et acceptabilité sociale du produit issu de cette filière, l'HE

L'un des objectifs de ma thèse était également d'évaluer la viabilité économique de la filière PPAM-HE, notamment celle de l'angélique cultivée sur un sol historiquement pollué par les ET.

Dans nos conditions expérimentales, où la pollution est localisée dans les 40 premiers cm du sol, les coûts des méthodes physico-chimiques étant estimés à 180€.m⁻³ contre 34€.m⁻³ pour des méthodes de phytoremédiation (Wan et al., 2016; Zhang, 2019), son excavation et son traitement par des méthodes physico-chimiques reviendraient à environ 720 000€.ha⁻¹ ([Publication 1 - Langrand et al., 2024, à soumettre](#)).

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Les coûts de production de l'angélique s'élevaient en moyenne à 730 €.ha⁻¹, ce qui est comparable à des cultures conventionnelles telles que le blé, l'orge ou le colza, mais aussi à des cultures de PPAM comme la coriandre (CASDAR, 2015; Raveau et al., 2021a). En 2023, selon les professionnels de la filière HE, le kilogramme d'HE d'angélique se négociait entre 620 et 800€ avec une estimation moyenne aux alentours de 660€. Dans les meilleures conditions (2020 NI), nous avons obtenu un rendement en HE de 1,9 kg.ha⁻¹, équivalent à une marge brute potentielle de 814€.ha⁻¹. Cependant, selon certains travaux, il serait possible d'atteindre des rendements de 8 kg.ha⁻¹ d'HE (Jakab et al., 2014; Kylin, 2012), ce qui donnerait une marge brute de 4 550€.ha⁻¹. Cette marge brute est nettement supérieure à celle d'une culture conventionnelle de blé (environ 1 500€.ha⁻¹) ou celles obtenue dans une précédente étude sur la coriandre (100-200€.ha⁻¹), et équivalente voire supérieure à la sauge sclarée (3 024€.ha⁻¹-Raveau et al., 2021a).

⇒ **La production des HE distillées à partir de graines d'angélique cultivées sur des sols pollués par la TE pourrait constituer une filière de phytomanagement économiquement rentable.**

F. Acceptabilité sociale des HE d'angélique produites sur un sol pollué par les ET

Une des conditions *sine qua non* pour la mise en place d'une nouvelle filière de phytomanagement est l'acceptabilité sociale de celle-ci, et particulièrement celle du produit qui en est issu, en l'occurrence les HE. En effet, la culture de l'angélique sur un sol historiquement pollué par les ET ne peut s'envisager que si les consommateurs finaux acceptent d'utiliser les HE malgré leur origine. Ainsi, dans le cadre du projet DEPHYTOP, une étude d'acceptabilité sociale a donc été réalisée par le Pr H. Flanquart (TVES, ULCO), sur un panel de 504 personnes représentatives de la population, et divisé équitablement en trois sous échantillons : des écologistes membres d'associations de protection de l'environnement, des employés de l'État, et des étudiants en Master du nord de la France ([Publication 1 - Langrand et al., 2024, à soumettre](#)). Les résultats de l'étude ont montré que :

⇒ **Les HE issues de graines d'angélique cultivée sur un sol pollué présente une bonne acceptabilité vis-à-vis du public.**

Ce sont d'ailleurs les étudiants de master, donc une population plutôt jeune et éduquée qui semble avoir l'attitude la plus enthousiaste à l'idée de produire des HE à but non alimentaire sur des sols pollués par les ET.

La filière de production d'HE à partir d'angélique cultivée sur un sol pollué par les ET est une stratégie de gestion offrant des avantages économiques, environnementaux et sociétaux (Cundy et al., 2016). Les potentiels gains économiques ont été évalués (Publication 1 - Langrand et al., 2024, à soumettre), cependant l'évaluation des bénéfices non monétisables est beaucoup plus complexe à mettre en œuvre. En effet, les services écosystémiques rendus par la filière, tels que le stockage du C, le cycle des nutriments, le maintien de la fertilité du sol, la purification et la régulation du débit de l'eau sont autant de paramètres qui pourraient être suivis et évalués pour compléter ce bilan socio-économique (Burgess et al., 2018).

II. Apport des amendements et de la culture de ppam dans le phytomanagement des sols pollués par les et

A. Dispositif expérimental

L'éventuel apport des amendements minéraux (laitiers sidérurgiques) ou biologiques à base de CMA a été étudié en s'appuyant sur un second dispositif expérimental comprenant 20 modalités en microparcelles de 9 m² :végétalisées ou non par la coriandre, amendées ou non par des laitiers sidérurgiques de poche ou de four, utilisés à 1,5 ou 45 t.ha⁻¹et provenant d'une société locale, proche du site d'Evin - Malmaison, ou amendés ou non avec un inoculum mycorhizien (Symbivit® - INOCULUM plus) (Figures 3 & 4).

Les résultats issus du projet PHYTEO (Perlein et al., 2021b), ont mis en évidence le caractère accumulateur dans les parties aériennes de la coriandre vis-à-vis du Cd. Ainsi, l'un des objectifs du projet DEPHYTOP dans lequel s'inscrit ma thèse était d'examiner l'apport d'amendements minéraux dans les capacités phytostabilisatrices de la coriandre, notamment en limitant le transfert d'ET dans les parties aériennes (travail réalisé par l'INERIS) et dans la requalification écologique du sol pollué d'Evin - Malmaison.

En effet, les communautés microbiennes remplissent un rôle fondamental dans les sols, notamment dans les cycles biogéochimiques, et la fertilité des sols mais aussi dans la croissance des végétaux (Adamovic et al., 2015; Qu et al., 2020). Malheureusement, la pollution, d'origine anthropique ou naturelle, affecte généralement les fonctions, l'abondance et la diversité des micro-organismes présents dans ces sols (Jiang et al., 2023). Il est bien établi qu'un sol pollué par les ET peut, voir la

structure des communautés microbiennes bouleversée, et son fonctionnement perturbé (Ding et al., 2017; Epelde et al., 2014; Gutiérrez et al., 2016).

L'effet de la végétalisation sur la structure des communautés microbiennes a déjà été suggéré dans de nombreuses études (Burgess et al., 2018; Durand et al., 2018; Garbisu et al., 2020). Cependant, peu de travaux se sont penchés sur l'influence des PPAM sur le microbiote des sols pollués. La dernière partie de cette thèse a donc été consacrée à l'étude de l'influence de la végétalisation du sol avec l'angélique ou la coriandre, mais également celle des amendements minéraux ou biologiques sur les communautés microbiennes du sol historiquement pollué par les ET. En ce qui concerne l'angélique, l'objectif était d'analyser l'apport de la végétalisation et de l'inoculation mycorhizienne après quatre années successives de culture sur la requalification écologique du sol pollué par un suivi de différents indicateurs de fonctionnalité du sol (1) la quantification de la biomasse microbienne via le dosage de marqueurs lipidiques spécifiques (acides gras associés aux phospholipides – AGPL - des différents groupes microbiens du sol : les bactéries à Gram positif et négatif, les champignons saprotrophes et mycorhiziens), (2) la caractérisation de la structure des communautés bactériennes et fongiques du sol (Illumina MiSeq), (3) l'étude de la diversité fonctionnelle microbienne grâce à la détermination des profils métaboliques du sol (test Biolog®, Ecoplates) et enfin (4) l'annotation des taxa fonctionnels, la prédiction de gènes fonctionnels liés aux métabolismes du C, de l'N et du S, et l'établissement de réseaux de co-occurrence. Ces travaux se sont concentrés sur les communautés bactériennes et fongiques du sol ([Publication 3 - Langrand et al., 2024, à soumettre](#), [Publication 4 - Langrand et al., 2023](#)).

B. Apport des amendements minéraux et biologiques sur la croissance de la coriandre

L'effet des laitiers sidérurgiques est controversé dans la littérature, certaines études ont montré que les amendements minéraux contribuent à améliorer les rendements de cultures quand d'autres n'ont montré aucun changement dans les paramètres de croissance (Branca and Colla, 2012; Mench et al., 1994; Ning et al., 2016; O'Connor et al., 2021). Dans notre cas, l'absence d'effet des amendements minéraux pourrait s'expliquer par les faibles besoins en nutriments de la coriandre, mais aussi par la teneur importante en matière organique du sol agricole de la parcelle expérimentale d'Evin - Malmaison (Laribi et al., 2015; Raveau et al., 2021a).

Nous avons montré que la coriandre ou l'angélique étaient mycorhizées par les CMA indigènes présents dans notre sol pollué par les ET. Dans les deux cas, aucun apport de l'inoculation sur la croissance des végétaux n'a été observé. Nous ne pouvons pas exclure que la présence naturelle de

CMA indigènes dans les sols pollués ait masqué la contribution de l'inoculum mycorhizien exogène sur la croissance de la coriandre ou de l'angélique. En revanche, une augmentation des taux de colonisation mycorhizienne de la coriandre a été observée en présence de laitier de four, de même que pour l'angélique dans la condition I 2022. Ces augmentations pourraient être dues à une réduction de la biodisponibilité des ET ou à des modifications du pH ou de la texture du sol (Hu et al., 2019). Ces hypothèses sont en cours de vérification par l'INERIS, en charge de cette partie dans le cadre du projet DEPHYTOP.

C. Impact des PPAM sur le microbiote du sol pollué par les ET

La végétalisation du sol pollué avec l'angélique ainsi que la durée de la culture ont conduit à l'augmentation de la biomasse de bactéries à Gram positif et négatif, tandis que la coriandre a permis l'augmentation de la biomasse de bactéries à Gram négatives uniquement. Les bactéries à Gram positives sont connues pour la grande diversité de leurs capacités métaboliques qui leur permettent de prospérer dans un large éventail d'environnements tels que les sols pollués par les ET. Par rapport aux bactéries à Gram négatives, elles peuvent accumuler des concentrations plus élevées d'ET dans leurs parois cellulaires (Rani and Goel, 2009). Alors que la culture de l'angélique a augmenté de manière significative la diversité bactérienne, aucun effet n'a été observé avec la coriandre. Par ailleurs, l'abondance relative des champignons saprotrophes a augmenté de manière significative avec le temps et la culture de l'angélique.

Il est bien connu que les plantes sécrètent des exsudats racinaires tels que les sucres, les acides aminés et les acides organiques façonnent les structures de la communauté microbienne dans la rhizosphère (Hartmann et al., 2009). De plus, les plantes aromatiques sont également connues pour leur capacité à sécréter plus spécifiquement des métabolites secondaires dans leurs exsudats racinaires (Misra et al., 2019; Raymond et al., 2020; Wu et al., 2015) qui, en raison de leurs propriétés antimicrobiennes, peuvent influencer la richesse et la diversité bactériennes et fongiques (Adamovic et al., 2015; Köberl et al., 2013). C'est par exemple le cas des mono- et sesquiterpènes présents chez la coriandre ou l'angélique (Kylin, 2010; Mandal and Mandal, 2015).

⇒ **La végétalisation du sol par les PPAM (coriandre ou angélique) a augmenté la biomasse microbienne du sol pollué.**

Nos résultats ont mis en évidence des indices de diversité et de richesse bactériennes comparables à ceux obtenus sur des sols non pollués (Gui et al., 2017; Morvan et al., 2020). D'autre part, nos résultats ont montrés que malgré la pollution du sol par les ET, le potentiel métabolique bactérien de ce sol pollué, mesuré avec l'AWCD, s'avère équivalent à des sols non pollués (Feigl et al., 2017; Xie et al., 2016). En effet, le potentiel métabolique bactérien de cinq classes de substrats carbonés, à savoir les hydrates de carbone, les acides carboxyliques et acétiques, les polymères d'acides aminés et les amides ou amines, a été déterminé (Biolog Ecoplates). Les hydrates de carbone ont été les substrats les plus utilisés, avec des valeurs allant de 64 pour l'état initial à 1494 pour l'état I 2020 ([Publication 3 - Langrand et al., 2024, à soumettre](#)). Ces valeurs sont identiques à celles généralement trouvées dans les sols non pollués (Borymski et al., 2018; Gremion et al., 2004; Xie et al., 2016). Par conséquent, la présence de ET dans le sol ne semble pas affecter le potentiel métabolique des communautés bactériennes du sol.

En revanche, la végétalisation par la coriandre ou l'angélique ont modulé l'abondance relative de certains phyla bactériens présents dans le sol pollué. Cependant, alors que l'angélique conduit à une augmentation de l'abondance relative des Gemmatimonadota, Proteobacteria ou Acidobacteria, une diminution de l'abondance relative de ces mêmes phyla a été observée avec la coriandre. Les Actinobacteria, Proteobacteria et Ascomycota étaient les phyla dominants dans le sol avant végétalisation (état initial) et après quatre années de culture de l'angélique ([Publication 3 - Langrand et al., 2024, à soumettre](#)). Les mêmes phyla bactériens ont été retrouvés dans le sol avant et après la culture de la coriandre ([Publication 4 - Langrand et al., 2023](#)). Ces phyla connus pour leur capacité à coloniser les sols à l'échelle mondiale, et posséder un grand nombre de gènes de tolérance aux stress environnementaux tels que ceux liés à la présence d'ET dans le sol (Cui et al., 2018; He et al., 2022; Lin et al., 2019; Sun et al., 2022).

- ⇒ **Les indices de richesse et de diversité bactériennes (coriandre et angélique) et fongiques (angélique) sont comparables à ceux obtenus sur des sols non pollués.**
- ⇒ **La culture de la coriandre et celle de l'angélique ont façonné les communautés microbiennes du sol pollué par les ET.**

D'autre part, la complexité des réseaux d'interactions entre les communautés bactériennes et fongiques, ainsi que le nombre d'interactions positives entre les communautés microbiennes dans le sol pollué par l'ET, et l'abondance relative de gènes liés au métabolisme de C ont augmenté de manière significative avec la durée de la culture de l'angélique. Certaines études rapportent une augmentation du nombre d'interactions positives entre les microorganismes dans les réseaux de co-occurrence

corrélées à la présence d'une culture mais aussi à la durée de la culture (Lan et al., 2023; Luo et al., 2021). Il est probable que cette sélection ait à la fois influencé l'abondance relative des gènes impliqués dans la fermentation et la dégradation des composés aromatiques, et donc l'augmentation du métabolisme du C observée dans nos conditions expérimentales.

- ⇒ **La végétalisation avec l'angélique et l'inoculation ont permis d'augmenter significativement la complexité des interactions entre les différents micro-organismes du sol (Publication 3 - Langrand et al., 2024, à soumettre).**

Cependant, au sein de ces groupes, nous avons observé des variations notables. Par exemple, l'abondance relative du groupe des parasites fongiques a diminué de manière significative avec la culture de l'angélique. À l'inverse, l'abondance relative des sous-groupes de saprotrophes de la litière a augmenté de manière significative après deux années de culture de l'angélique.

L'analyse des principales guildes trophiques fongiques du sol du dispositif angélique (symbiotrophes, saprotrophes et pathotrophes) n'a pas révélé de différences significatives entre les conditions étudiées.

- ⇒ **Les années de culture de l'angélique ont diminué l'abondance relative des champignons parasites et augmenté l'abondance des champignons saprotrophes de la litière.**

Certaines études indiquent que les champignons Ascomycota et Basidiomycota jouent un rôle clé en tant que décomposeurs de la matière organique lignocellulosique dans les sols (Bastian et al., 2009; Ma et al., 2013; Osono and Takeda, 2006; Vandenkoornhuyse et al., 2002; Yelle et al., 2008). Les analyses de sol réalisées au début et à la fin de l'étude ont révélé un enrichissement du sol en matière organique et en C organique. Nous émettons l'hypothèse que :

- ⇒ **Les résidus d'angélique, incorporés au sol chaque année après la récolte, ont probablement contribué à l'augmentation de l'abondance des champignons saprotrophes, à l'amélioration de la qualité du sol et son l'enrichissement en matière organique et en C.**

Cet enrichissement pourrait faciliter l'immobilisation des métaux par chélation, et ainsi améliorer la phytostabilisation des ET dans le sol. En effet, une étude récente a montré que les ET ont tendance à se lier fortement à la matière organique (Palansooriya et al., 2020).

D. Impact des amendements sur le microbiote du sol pollué par les ET

1. Influence de l'inoculation mycorhizienne sur le microbiote du sol pollué

L'étude de la β -diversité microbienne a révélé que les principaux phyla bactériens (Actinobacteria, Proteobacteria, Gemmatimonadota, Acidobacteria) et fongiques (Ascomycota et Basidiomycota) n'ont pas été significativement impactés par l'inoculation mycorhizienne. En revanche, l'analyse de l'abondance des gènes liés aux cycles biogéochimiques a montré une prédominance des gènes impliqués dans le cycle du C, dans les conditions végétalisées par l'angélique et inoculées par rapport à l'état initial.

⇒ **L'inoculation mycorhizienne a augmenté de manière significative l'abondance des gènes impliqués dans le cycle du carbone dans les sols de 2020 et 2021 par rapport à l'état initial, ainsi que dans la condition NI en 2021.**

Pour les gènes impliqués dans le cycle de l'azote, une abondance relative plus élevée a été observée dans la condition I 2020 par rapport à l'état initial du sol et aux conditions NI et I 2021. Cependant, aucune différence significative n'a été observée dans l'abondance relative des gènes impliqués dans le cycle S entre les différents traitements. A l'intérieur des groupes de gènes impliqués dans les cycles (C, N et S), certains gènes spécifiques étaient différentiellement abondants en fonction de la végétalisation ou de l'inoculation mycorhizienne. En effet, les sols I échantillonnés en 2021 étaient caractérisés par une abondance relative plus élevée de gènes impliqués dans la chimio-hétérotrophie aérobie, ce qui signifie un plus grand potentiel de dégradation de la matière organique en présence d'O₂, alors qu'à l'état initial, les sols avaient plus de potentiel pour réaliser ce processus en condition anaérobie, comme le montre l'abondance relative plus élevée de gènes impliqués dans la voie anaérobie de ce processus.

⇒ **L'inoculation a conduit à une abondance relative plus élevée des gènes impliqués dans la fermentation et la dégradation des composés aromatiques en 2020.**

Certaines études indiquent qu'une fois que les hyphes des CMA ont atteint la litière en décomposition, les bactéries, plutôt que les champignons, deviennent les principaux transformateurs du carbone dérivé de la litière, ce qui peut expliquer l'augmentation de l'abondance relative des gènes liés à la fermentation (Herman et al., 2012; Nuccio et al., 2013; Xu et al., 2018). Les champignons mycorhiziens semblent modifier l'environnement physico-chimique des communautés microbiennes en exportant l'azote de la litière en décomposition (Nuccio et al., 2013). Il est probable que cette sélection ait à la fois influencé l'abondance relative des gènes impliqués dans la fermentation et la dégradation des composés aromatiques, et donc l'augmentation du métabolisme du C observée dans nos conditions expérimentales.

En analysant la topologie du réseau microbien, nous avons constaté que la complexité des réseaux a augmenté tout au long des années de culture avec une influence positive de l'inoculation ([Publication 3 - Langrand et al., 2024, à soumettre](#)). En effet, le nombre de nœuds et de liens a augmenté en 2020 et en 2021. La proportion de liens positifs a diminué dans le traitement inoculé en 2020 avant de remonter à sa valeur initiale en 2021. La littérature fait état d'une augmentation de la diversité et de la stabilité des réseaux de co-occurrence corrélée au réchauffement climatique (Yuan et al., 2021). Néanmoins, il apparaît que la présence de plantes, mais aussi la durée de culture, augmente la proportion d'interactions positives entre micro-organismes (Lan et al., 2023; Luo et al., 2021).

2. Influence des amendements minéraux sur le microbiote du sol pollué

L'ajout d'amendements, tels que les laitiers sidérurgiques a modifié la structure de la communauté bactérienne de la rhizosphère de la coriandre sans affecter son potentiel métabolique, qui a été évalué par le paramètre AWCD ([Publication 4 - Langrand et al., 2023](#)). En fait, la plus faible dose d'amendement minéral ($1,5 \text{ t} \cdot \text{ha}^{-1}$) a augmenté la biomasse bactérienne et a provoqué un changement dans la structure de la communauté bactérienne. L'embranchement des Actinobacteria était significativement moins abondant dans les conditions amendées par le laitier, tandis que la proportion relative des Gemmatimonadota augmentait. Ces résultats sont en accord avec ceux de Gremion *et al.*, (2004) sur un sol pollué par les ET. Il a été démontré que la granulométrie des amendements minéraux agit comme un support physique pour la fixation des micro-organismes et des plantes, ainsi que comme une réserve de nutriments, et que l'abondance des Acidobactéries diminue avec l'augmentation du pH (Wang, 2019). À cet égard, et en raison de la nature alcaline des laitiers, le pH et la minéralosphère peuvent être des facteurs importants influençant les communautés microbiennes (Uroz et al., 2015; Wang, 2019).

Synthèse générale et perspectives

Par conséquent, nos résultats suggèrent que les laitiers sidérurgiques, qui contribuent à l'économie circulaire grâce à leur recyclage, en combinaison avec la végétalisation du sol par des plantes aromatiques telles que la coriandre, fournissent potentiellement des avantages écologiques à long terme pour les sols pollués par les ET et devraient être considérés comme un outil fiable pour la remédiation future des sols pollués par les ET. De plus, bien que l'inoculation mycorhizienne n'ait pas d'effet sur la croissance des PPAM dans nos conditions expérimentales, l'utilisation d'inoculum mycorhizien semble influencer positivement les communautés microbiennes du sol pollué par les ET sur le long terme. La végétalisation du sol, la durée de la culture et l'inoculation mycorhizienne, ont influencé positivement les communautés microbiennes du sol pollué par les ET. Son action sur la sélection de certaines guildes trophiques, et l'enrichissement des interactions entre les microorganismes du sol, est susceptible de contribuer à la réhabilitation écologique du sol pollué par les ET.

Conclusion générale

Conclusion générale

L'originalité de ce projet résidait dans le développement d'une filière complète éco-innovante de production non alimentaire d'HE à haute valeur ajoutée, allant de la mise en place des dispositifs expérimentaux, à la culture des PPAM, la distillation des HE, l'étude de la qualité des HE produites (contamination, composition chimique, activités biologiques) et de leurs propriétés biologiques, l'étude de l'impact des PPAM et des amendements (biologiques et minéraux) sur la qualité microbiologique des sols phytomanagés et enfin l'étude socio-économique de la filière.

Nos résultats ont montré que l'angélique représente un bon candidat pour le phytomanagement des sols pollués par les ET. En effet, celle-ci est capable de se développer sur un sol fortement pollué par des ET sans nécessiter d'intrants. L'amélioration des pratiques culturales, et en particulier l'optimisation de la récolte, semble néanmoins être une option appropriée pour améliorer les rendements en HE. L'utilisation d'inoculum mycorhizien ne montre aucun effet sur la croissance de l'angélique. L'HE distillée à partir de graines d'angélique présente une composition et qualité comparables à celle d'une HE commerciale. Seules des traces d'ET et de pesticides, inférieures ou proches des limites de quantification, ont été détectées. La présence d'ET dans le sol, l'inoculation mycorhizienne ou la durée de culture n'ont pas modifié la composition chimique de l'HE d'angélique. Celle-ci est riche en principes actifs (sabinène, d' α -pinène, de β -élémane, de cis- β -ocimène et de β -curcumène) qui sont à l'origine de différentes propriétés biologiques applicables en santé humaine ou animale (antioxydantes, anti-inflammatoires) et dans le domaine de la protection des cultures (antifongiques, herbicides). En outre, la filière angélique-HE semble économiquement viable et bénéficie d'une bonne acceptation auprès du public.

Enfin, cette étude de terrain avec un suivi réalisé sur une durée de quatre années successives a également permis d'étudier l'apport de la végétalisation du sol avec de l'angélique et de l'inoculation mycorhizienne sur la requalification écologique du sol. La culture d'angélique ont permis d'augmenter significativement la biomasse microbienne, la richesse et capacité métabolique bactériennes, ainsi que la complexité des interactions entre les différents micro-organismes du sol (Figure 6). Des résultats similaires ont été obtenu sur une plus courte période (6 mois) avec la coriandre. Parallèlement, une augmentation de l'abondance relative des gènes liés au métabolisme du carbone a été mise en évidence, ainsi que celle des champignons saprotrophes impliqués dans la dégradation de la MO (c'est-à-dire les saprotrophes de la litière). Ce travail fournit de informations nouvelles sur l'évolution du microbiote d'un sol historiquement pollué par des ET après quatre années de végétalisation et sur sa requalification écologique.

Conclusions générales

Microbiote du sol pollué	Inoculum mycorhizien	Laitiers sidérurgiques	Végétalisation	Temps
α -diversité	Augmentation de l'indice de Shannon bactérien	Pas d'influence	Augmentation de l'indice de Shannon bactérien et fongiques	Augmentation de l'indice de Shannon bactérien
β -diversité	Augmentations de certains phyla bactériens	Augmentations de certains phyla bactériens	Augmentations de certains phyla bactériens	Augmentations de certains phyla bactériens
Biomasse microbienne	Pas d'influence	Augmente la biomasse bactérienne	Augmente la biomasse bactérienne et fongique	Augmente la biomasse bactérienne
Capacité métabolique (Biolog)	Pas d'influence	Augmente l'AWCD en combinaison avec la végétalisation	Augmente l'AWCD et la richesse fonctionnelle	Pas d'influence
Réseaux de co-occurrence	Augmentation de la complexité et du nombre d'interactions positives	Non étudié	Augmentation de la complexité et du nombre d'interactions positives	Augmentation de la complexité et du nombre d'interactions positives
Prédiction de gènes fonctionnels	Augmentation de l'abondance relative des gènes liés au métabolisme du C	Non étudié	Augmentation de l'abondance relative des gènes liés au métabolisme du C	Pas d'influence

Figure 8 : Schéma récapitulatif de l'apport la végétalisation du temps et des amendements dans le phytomanagement des sols pollués par les ET

Il reste à compléter ce travail par l'étude de la capacité de phytostabilisation de l'angélique (partie réalisée par le partenaire INERIS dans le cadre du projet DEPHYTOP). Néanmoins, nos résultats ont mis en évidence la pertinence de la filière angélique-HE pour le phytomanagement des sols pollués par les ET aussi bien d'un point de vue économique, écologique que sociétal, et pourrait être exportée sur d'autres sites pollués par les ET ou d'autres types de polluants (organiques par exemple).

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Annexes

Composition des amendements minéraux

Composition Moyenne (Mg/Kg)	Laitier De Poche	Laitier De Four
pH	11,84	11,29
Pb	4,9	20
Ni	51	58
Ba	1446	4583
Mo	17	28
Se	2,8	11
Cd	0,5	2,4
Cr	932	4870
Sb	10	36
As	11	3,9
Cu	155	246
Zn	76	414
Hg	<1,3	<1,3

Annexe 1 : Composition chimique des amendements minéraux

Liste des valorisation de thèse

Publications

J. Langrand, A. Lounès-Hadj Sahraoui, J. Duclercq, R. Raveau , F. Laruelle, V. Bert, N. Facon, B. Tisserant, J. Fontaine. 2023. Coriander (*Coriandrum sativum*) Cultivation Combined with Arbuscular Mycorrhizal Fungi Inoculation and Steel Slag Application Influences Trace Elements-Polluted Soil Bacterial Functioning. *Plants* 12: 618. DOI: 10.3390/plants12030618.

Communications orales

J. Langrand, J. Fontaine, R. Raveau, N. Facon, F. Laruelle, B. Tisserant, A. Lounès-Hadj Sahraoui. Interest of angelica cultivation and mycorrhizal inoculation for the phytomanagement of trace elements-polluted soil: a long-term field study. International Phytotechnology Conference - IPC17, Chicago (US) 23-26 mai 2023.

J. Langrand, A. Lounès-Hadj Sahraoui, R. Raveau, F. Laruelle, B. Tisserant, N. Facon, J. Duclercq, V. Bert, J. Fontaine. Contribution of steel slag and coriander cultivation in the phytomanagement of a soil historically polluted by trace elements. Intersol, Lille (France) 28-30 mars 2023.

Annexes

J. Langrand, J. Fontaine, R. Raveau, F. Laruelle, B. Tisserant, N. Facon, A. Lounès-Hadj Sahraoui. Intérêt de l'inoculation mycorhizienne dans la culture de l'angélique pour le phytomanagement d'un sol pollué par les éléments traces. 6^{èmes} Journées Francophones des Mycorhizes, Dijon (France), 7-9 juin 2022.

J. Langrand, A. Lounès-Hadj Sahraoui, R. Raveau, F. Laruelle, J. Duclercq, J. Fontaine. Culture de la coriandre dans un contexte de phytomanagement : apport de laitiers sidérurgiques dans la refonctionnalisation des sols pollués par les éléments traces métalliques. Journées Jeunes Chercheurs Condorcet (J2C2) 27et 28 Janvier 2022. Format WEBINAIRE.

J. Langrand, A. Lounès-Hadj Sahraoui, R. Raveau, F. Laruelle, J. Duclercq, J. Fontaine. Développement d'une filière éco-innovante de valorisation de la biomasse produite sur des sols agricoles pollués par les ETM dans un contexte de phytomanagement. Workshop Transition Agroécologique, Beauvais, 19 novembre 2021.

J. Langrand, J. Fontaine, A. Lounès-Hadj Sahraoui. Culture de plantes aromatiques pour la production d'huiles essentielles : une nouvelle filière de valorisation de la biomasse produite sur des sols pollués par les éléments traces métalliques. Séminaire Valorisation de la biomasse 10 juin 2021, Dunkerque.

Posters

J. Langrand, P. M. S. Ndour, F. Laruelle, B. Tisserant, N. Facon, J. Duclercq, A. Lounès-Hadj Sahraoui, J. Fontaine. Requalification écologique d'un sol pollué après trois années de culture de l'angélique (*Angelica archangelica*) inoculée avec *Rhizopagus irregularis*. 6^{èmes} Journées Francophones des Mycorhizes, Montpellier (France), 14-16 mai 2024.

R. Raveau, J. Langrand, J. Fontaine, P. Ferrant, A. Lounès-Hadj Sahraoui. Essential oil production from the aromatic plant, clary sage: a revealing non-food valorisation channel for the phytomanagement of aged trace elements-polluted soil. International Phytotechnology Conference, Chicago (USA), 23-26 mai 2023.

A. Verdin, J. Langrand, R. Raveau, L. Mistrulli, J. Fontaine, D. Courcot, S. Fourmentin, A. Lounès-Hadj Sahraoui. Biocide, antioxidant and anti-inflammatory activities of essential oils distilled from aromatic plants grown on trace elements polluted soils. SETAC Europe 33rd Annual Meeting, Dublin (Irlande), 30 avril - 4 mai 2023.

Annexes

J. Langrand, R. Raveau, F. Laruelle, J. Fontaine, A. Lounès-Hadj Sahraoui. Production d'huiles essentielles à activités « biopesticides », à partir de plantes aromatiques cultivées sur sols pollués. PHLOEME, les biennales de l'innovation céréalière, Cité des Sciences et de l'Industrie, Paris, 3 et 4 novembre 2022.

J. Langrand, J. Fontaine, R. Raveau, F. Laruelle, B. Tisserant, N. Facon, A. Lounès-Hadj Sahraoui. In situ cultivation of aromatic plants for the phytomanagement of polluted soil. Journée des doctorants 2022 du pôle MTE, Centre universitaire des Darses ULCO, Dunkerque, 12 juillet 2022.

Résumé

Le phytomanagement correspond à une gestion intégrée des sites et sols pollués, reposant sur la mise en place *in situ* d'un couvert végétal visant à : réduire le risque de toxicité des sols pollués en immobilisant les éléments traces (ET) dans la rhizosphère (phytostabilisation), permettre la réhabilitation écologique des sols pollués en augmentant la biodiversité et l'activité microbienne du sol et, redonner un bénéfice socio-économique aux sites pollués en valorisant la biomasse, via le développement de filières éco-responsables. Dans ce contexte, la culture de plantes aromatiques et médicinales (PAM), destinée à la production d'huiles essentielles (HE) a été récemment présentée dans le cadre du projet DEPHYTOP, financé par l'ADEME, comme une alternative innovante et économiquement viable pour le phytomanagement des sols pollués. Ainsi, ma contribution dans ce projet a permis d'évaluer la pertinence de ce mode de gestion reposant sur la culture *in situ* de PAM, l'angélique et la coriandre et l'emploi d'amendements minéraux (laitiers sidérurgiques) ou biologiques (inoculum mycorhizien) pour le phytomanagement de sols pollués par des ET. À ce titre, différents paramètres ont été suivis : la croissance des plantes, la production de biomasse, la teneur et la qualité des HE ainsi que la biomasse microbienne (acides gras associés aux phospholipides), la composition et la structure des communautés microbiennes (analyse métagénomique - Miseq Illumina) et le potentiel métabolique (Biolog ecoplate) du sol. La coriandre ainsi que l'angélique se sont correctement développées sur le sol pollué, et ont produit une quantité importante de biomasse. Néanmoins, les amendements n'ont pas significativement augmenté la biomasse de la plante, ni les rendements ou la qualité de l'HE d'angélique. En revanche, l'HE d'angélique présente une qualité très satisfaisante, caractérisée par une faible teneur en ET ou en résidus de pesticides (traces). Sa composition chimique, n'est pas modifiée par la présence d'ET dans le sol, ni par l'inoculation mycorhizienne, et est identique à celle d'une HE commerciale. Des propriétés biologiques valorisables dans des filières non-alimentaires, tels que la protection des cultures (effets fongistatiques, et herbicides) ou la santé humaine (effets anti-inflammatoires et antioxydants) ont été mises en évidence. L'analyse technico-économique de la filière PPAM-HE souligne le haut potentiel économique de l'angélique dans une démarche de phytomanagement de sols pollués par les ET. La culture de PPAM et sa durée ont influencé les communautés bactériennes et fongiques du sol pollué, entraînant une augmentation de la biomasse microbienne totale, de l'activité métabolique globale, et du métabolisme du C. En outre, l'inoculation mycorhizienne a amélioré de manière significative la diversité et la biomasse bactérienne après trois années de culture et a permis d'augmenter les interactions positives entre les microorganismes du sol pollué. Enfin, ces résultats combinés à l'étude d'acceptabilité sociale des HE issues de sols pollués, montrent le potentiel de la filière PPAM-HE dans une stratégie de phytomanagement d'un sol pollué par les ET.

Mots clés : Phytomanagement, angélique, coriandre, huiles essentielles, inoculation mycorhizienne

Abstract :

Phytomanagement corresponds to the integrated management of polluted sites and soils, based on the *in situ* installation of a plant cover aimed at: reducing the risk of toxicity of polluted soils by immobilizing trace elements (TE) in the rhizosphere (phytostabilization), enabling the ecological rehabilitation of polluted soils by increasing biodiversity and soil microbial activity and, restoring a socio-economic benefit to polluted sites by valorizing biomass, via the development of eco-responsible channels. In this context, the cultivation of aromatic and medicinal plants (MAP) for the production of essential oils (EO) was recently presented as an innovative and economically viable alternative for the phytomanagement of polluted soils, as part of the DEPHYTOP project funded by ADEME. My contribution to this project has enabled me to assess the relevance of this management method, based on the *in situ* cultivation of MAP, angelica and coriander, and the use of mineral amendments (steel slags) or biological amendment (mycorrhizal inoculum) for the phytomanagement of TE-polluted soil. Various parameters were studied: plant growth, biomass production, HE content and quality, microbial biomass (fatty acids associated with phospholipids), microbial community composition and structure (metagenomic analysis - Miseq Illumina) and soil metabolic potential (Biolog ecoplate). Both coriander and angelica grew well on the soil polluted by the TE, and produced a significant amount of biomass. Nevertheless, the amendments did not significantly increase plant biomass, yield or quality of angelica EO. On the other hand, the quality of angelica EO is very satisfactory, with low levels of TE or pesticide residues (traces). Its chemical composition is not modified by the presence of TE in the soil, nor by mycorrhizal inoculation, and is identical to that of a commercial EO. Biological properties that can be used in non-food applications, such as crop protection (fungistatic and herbicidal effects) and human health (anti-inflammatory and antioxidant effects), have been demonstrated. The technical and economic analysis of the MAP-EO sector highlights the high economic potential of angelica in the phytomanagement of soil polluted by the TE. The cultivation of MAP and its duration influenced the bacterial and fungal communities of the polluted soil, leading to an increase in total microbial biomass, overall metabolic activity and C metabolism. In addition, mycorrhizal inoculation significantly improved bacterial diversity and biomass after three years of cultivation, and increased positive interactions between microorganisms in the soil polluted by the TE. Finally, these results, combined with the study of the social acceptability of EO from polluted soils, demonstrate the potential of the MAP-EO sector in a phytomanagement strategy for soil polluted by the TE.

Key words: Phytomanagement, angelica, coriander, essential oils, mycorrhizal inoculation