Impact of Plant Secondary Metabolites on the Soil Microbiota

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Vadim Schütz

geboren in

Nowokrasnoje (Russland)

Bonn, 2022

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät
der Rheinischen Friedrich-Wilhelms-Universität Bonn

Gutachter: Prof. Dr. Peter Dörmann
Gutachterin: PD Dr. Margot Schulz

Tag der Promotion: 12.08.2022
Erscheinungsjahr: 2022

Für meine Oma Frieda**.**

Table of Contents

Figures

Tables

Abbreviations

% (v/v)	Percent volume per volume (ml per 100 ml)
% (w/v)	Percent weight per volume (g per 100 ml)
3-N-AAP	N-(2-hydroxy-3-nitrophenyl) acetamide
5-N-AAP	N-(2-hydroxy-5-nitrophenyl) acetamide
AAP	2-Acetamidophenol
AP	2-Aminophenol
APO	2-Aminophenoxazinone
ASV	Amplicon sequence variant
BOA	2-Benzoxazolinone
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
ddH_2O	Double deionized water
dH_2O	Deionized water
DAD	Diode-array detection
DGDG	Digalactosyldiacylglycerol
DIBOA	2,4-Dihydroxy-1,4-benzoxazin-3-one
DIMBOA	2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
EtOAc	Ethyl acetate
FAME	Fatty acid methyl ester
forw.	Forward
GC	Gas chromatography
gDNA	Genomic DNA
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
I3A	Indole-3-carboxaldehyde
I3C	Indole-3-carboxylic acid
kb	Kilobase pairs
LC	Liquid chromatography
MBOA	6-Methoxybenzoxazolin-2(3*H*)-one
MGDG	Monogalactosyldiacylglycerol
MS	Mass spectrometry
m/z	Mass-to-charge
NCBI	National Center for Biotechnology Information
NMR	Nuclear magnetic resonance
OD_{600}	Optical density measured at a wavelength of 600 nm
oHPMA	N-(2-hydroxyphenyl)malonamic acid
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PCA	Principal component analysis
PCR	Polymerase chain reaction
PGA	Peptone-glucose agar
PLFA	Phospholipid fatty acids

qPCR	Quantitative polymerase chain reaction
Q-TOF	Quadrupole time-of-flight
rev.	Reverse
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SD	Standard deviation
rpm	Revolutions per minute
SPE	Solid phase extraction
TCA cycle	Tricarboxylic acid cycle
TLC	Thin layer chromatography
Tris	Tris(hydroxymethyl)aminomethane
TSB	Tryptic Soy Broth
TSM	Thornton's standardized medium
UHPLC	Ultra high-performance liquid chromatography
UV	Ultraviolet
WHC	Water holding capacity
x g	Standard gravity ($g = 9.81\ m\ s^{-2}$)
YEP medium	Yeast extract-peptone medium
YPD medium	Yeast extract-peptone-dextrose medium

Fatty acids were abbreviated as X:Y, where X indicates the number of carbon atoms and Y of the double bonds in the acyl chain. Double bond positions are indicated with Δ. The position of the cyclopropane ring in the acyl chain is depicted by cyclo. The position of a methyl branch is indicated with iso (penultimate carbon, n-1) or anteiso (n-2 carbon).

1. Introduction

The pedosphere, commonly referred to as soil, describes one of the most diverse ecosystems on Earth (Gans et al., 2005) and can be divided into two phases. Organic matter and minerals are the basis of the first phase which is called the solid phase. The second phase, containing water, microorganisms, and gases is known as the porous phase. This building block forms a very interactive habitat providing the basis for up to 98.8% of the food consumed by mankind (Kopittke et al., 2019). Being essential for life on earth, the functional purpose of soil is not restricted only to food production. It is also required for filtering nutrients and contaminants, carbon storage, greenhouse gas regulation, and detoxification of waste (Dominati et al., 2014). Many of these aspects are based on the action of microorganisms. The term ‘soil microorganisms' covers all organisms that are invisible to the naked eye. The members of the soil microbiota are not just bacteria, but also fungi, oomycetes, actinomycetes, archaea, algae, viruses, and even protists. Significant variations in soil moisture, nutrient composition like carbon, nitrogen, phosphate content and pH are responsible for the striking differences in soil microbiota. Other key players shaping and modulating the composition of microorganisms in soil are humans, animals as well as plants. Especially plants depend on a well-established microbiota and actively shape the community of microorganisms surrounding their roots. In a world where the conditions for the soil microbiota change due to human overpopulation and due to the increasing impacts of the climate, the understanding of the influence of abiotic and biotic factors that contribute to a healthy microbiota is the key factor for eventually improving or saving food production.

1.1. The Rhizosphere

Interactions between microorganisms and plant roots have been studied for decades. The site of action has been defined as ‘rhizosphere’ by the German plant physiologist Lorenz Hiltner in 1904 (Hiltner, 1904). Following Hiltner’s definition, the rhizosphere is the microorganism-containing area surrounding plant roots. The rhizosphere can be organized into three characteristic zones, that depend on the diversity of root systems in the plant world, and on the distance to the root, chemical, biological as well as physiological properties. Microbes and cations can be located in the apoplastic space, which contains parts of the endodermis as well as the cortex, and is the first zone called the endorhizosphere (McNear, 2013). The next zone is called rhizoplane. It is defined by the root epidermis and mucilage. The third zone, and

therefore the outer zone, is called the ectorhizosphere. This zone is described as the contact area extension of the rhizoplane with the bulk soil. Already more than 100 years ago, Hiltner posed the hypothesis that the rhizosphere is influenced and shaped by the plant with the release of chemicals (Hiltner, 1904). Over the years, numerous studies have supported this hypothesis and showed that the basis of plant-microbe interactions is the exudation of a large number of compounds, representing up to 20% of plant photosynthesis products, via the roots into the soil (Haichar et al., 2008). Furthermore, the exudation of the photosynthesis-derived carbohydrates into the soil is highly beneficial for the plant. Microorganisms attracted by the root-derived carbon in the harsh and competitive environment of the soil may support plant growth by increasing the availability of mineral nutrients, like insoluble phosphate and nitrogen, or even by the production of phytohormones (Bais et al., 2006). Additionally, the microbiota in the rhizosphere shaped by the plant plays an important role in defense mechanisms including the degradation of phytotoxins and the inhibition of soil-born pathogen proliferation (Bais et al., 2006). In fact, up to 90% of all terrestrial plants benefit from interactions with soil fungi (Magdoff & Van Es, 2009; Hoorman, 2019). On top of being an essential hotspot for nutrient exchange, the rhizosphere covers functions in the communication between plants and microorganisms by the release of a variety of substances (Brendsen et al., 2012).

1.2. Root Exudates

The release of root exudates is influenced by a number of factors including the plant species and plant age, but also temperature, insect herbivory, presence of nutrients and possible toxins as well as the chemical, biological and physiological properties of the soil. Continuously produced compounds that are actively released, are called secretions, and passively released compounds are called diffusates (Bais et al., 2001; Koo et al., 2005; McNear, 2013).

Root exudate compounds are generally classified by their molecular weight. Substances with high molecular weight are not easily metabolized by microorganisms in the soil but are the most abundant substances. Mucilage and cellulose belong to the group of high molecular weight substances and are the main carbon source released from the root (Bais et al., 2006; McNear, 2013). However, low molecular weight substances are present with high structural variety and with better known or predicted functions. Amino acids, organic acids, sugars, phenolic compounds, and more secondary metabolites belong to the group of low molecular weight root exudates (Bais et al., 2006). Plant waste materials are released by a gradient-dependent process via the root, while the second root exudation process is described by the release of compounds

with known functions (Bais et al., 2004). Generally, the release of exudates is described by two mechanisms. The first potential mechanism is characterized by the transport of substances across the cellular membrane and their release into the soil. In the second mechanism, exudates are released via cells that separate from roots during their growth, also known as root cap border cells (Hawes et al., 2000). In their life cycles, microorganisms in the rhizosphere are therefore exposed to living, as well as decaying root cap border cells that release exudates upon decomposition (Figure 1; Bulgarelli et al., 2013). Those compounds are usually non-nutritive and build the basis for allelopathy.

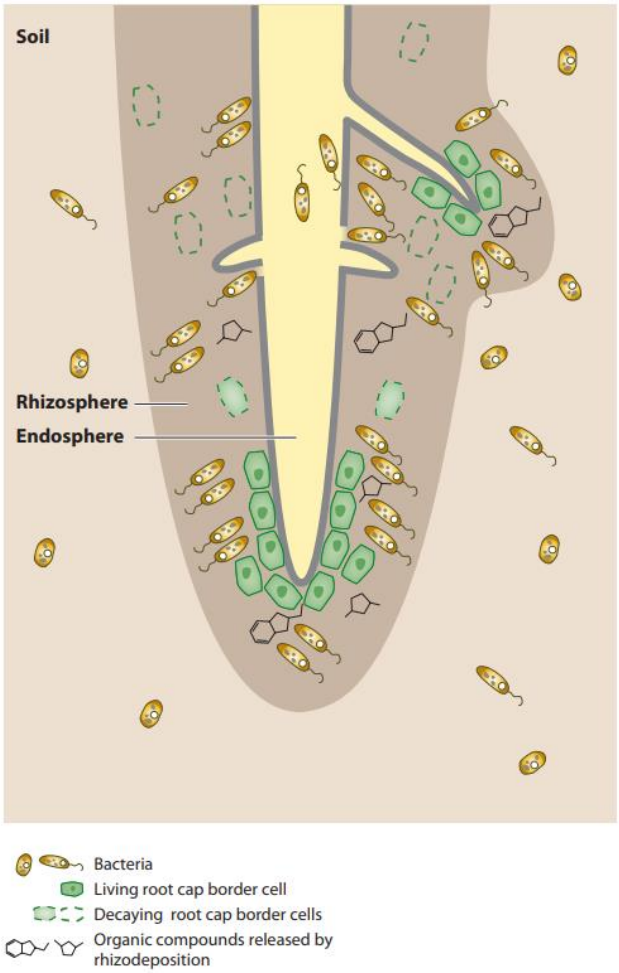


Figure 1: Release of organic compounds via roots into the soil.
Root exudates can be released via living or even decaying root cap border cells into the rhizosphere or the soil, where they impact other organisms (Bulgarelli et al., 2013).

1.3. Allelopathy

Theophrastus described the phenomenon of plant-plant interactions already 300 b.c. by noticing a negative effect of cabbage on vine plant growth. These observations were the basis for a novel field, now called allelopathy. The term ‘allelopathy’ originates from the Greek words ‘allelon’ which is translated to ‘of each other’, and from ‘pathós’ which means ‘to suffer’. It was not until 1937 when Molish picked up the term ‘allelopathy’ again by describing the direct and indirect effects of plants on other plants by the secretion of biochemical substances (Molish, 1937). Almost 50 years later, the term allelopathy was newly defined by Rice in 1984. The new definition now included beneficial and harmful effects from one plant on another, but also on other organisms, like bacteria, fungi, algae, and others (Rice, 1984). These effects are caused by substances termed ‘allelochemicals’, that rarely serve as a nutrient source and are mainly produced in the secondary metabolism or result from decomposition (Rice, 1974; Albuquerque et al., 2010).

Since the mid-1990s, allelopathy is a trending topic in horticulture, agriculture, soil science, and other related fields. Throughout the years, the functions of several secondary metabolites with regard to their influence on the soil microbiota have been studied, including the application of isothiocyanates-containing rapeseed extracts to bulk soil (Siebers et al., 2018). Siebers et al. showed that the addition of rapeseed extract was sufficient to change the microorganism composition in the soil. Interestingly, cultivable survivors of the isothiocyanate exposure had already been described in the literature as strains with possible growth promotion capabilities for some plants (Siebers et al., 2018). Lack of iron in the soil results in the exudation of redox-active molecules by certain plants, which support plant growth and survival by chelation and reduction of minerals. Compounds in *Arabidopsis thaliana* known to be exudated during iron deficiency are iron-mobilizing fraxetin, sideretin, esculetin, and coumarin (Rajniak et al., 2018; Schmid et al., 2014). Coumarin also plays an important role in the shaping of the soil microbial community. Utilizing the *A. thaliana* mutant *f6’h1*, which does not produce coumarin, it was demonstrated that the bacterial community surrounding the roots is changed by the inhibition of an abundant *Pseudomonas* species if coumarins are exuded into the soil (Voges et al., 2019). Some root exudates are important defense compounds of plants. Brassicaceae produce phytoalexins which deter and inhibit the growth of pathogenic fungi, resulting in improved plant growth and fitness (Bednarek et al., 2009; Hiruma et al., 2016). Other classes of allelochemicals that have negative effects on other organisms include benzoxazinoids (Reberg-Horton et al., 2005; Tabaglio et al., 2008; Schulz et al., 2013), the indole alkaloid gramine (Maver et al., 2020) and the flavonoid quercetin (Szwed et al., 2019).

While the metabolites described above are mainly exclusive root exudates, plant-derived allelochemicals can be introduced in different ways into the soil. Next to root exudation, three main pathways of the release of allelochemicals are depicted in Figure 2 (Albuquerque et al., 2010). Exudates produced in plant leaves will eventually be deposited on the leaf surface. From here, they can be washed off by rain and therefore be transported into the soil (Figure 2 (1), Albuquerque et al., 2010). Volatile compounds produced in leaves, stems, flowers or pollen are actively released by plants and do not require outside forces for introduction into the environment (Figure 2 (2), Albuquerque et al., 2010; Bertin et al., 2007; Kruse et al., 2000). Another major part of allelochemicals released into the soil is covered by decaying plant material (Figure 2 (3), Albuquerque et al., 2010). As for products of the primary metabolism, the production of allelochemicals in plants depends on abiotic and biotic factors including nutrient availability, diseases, neighboring plants, pathogens or even insects, drought, and temperature (Csekse and Kaufman, 2006; Einheillig, 1996).

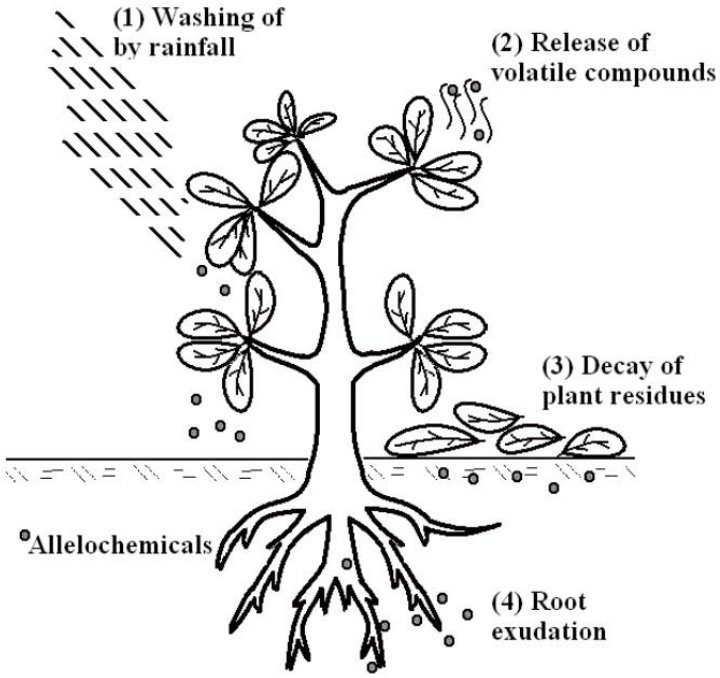


Figure 2: Schematic overview of the possible release of allelochemicals.
Allelochemicals can be released by four possible major pathways into the environment. (1) Release by rainfall from the leaf surface; (2) Release of volatile compounds from green plant organs; (3) Release of allelochemicals from decaying plant material; (4) Exudation of allelochemicals by roots (Albuquerque et al., 2010).

1.4. Benzoxazinoids as Allelochemicals

The plant family of Poaceae harbors several members that are able to produce the well-studied defense secondary metabolites benzoxazinoids (Niemeyer, 2009). This group of allelochemicals can be divided into the two main substance classes benzoxazinones and benzoxazolines, which are produced in important crops like *Zea mays*, *Hordeum lechleri*, *Hordeum brachyntherum*, *Secale cereale*, and *Triticum aestivum* (Sicker et al., 2000; Grün et al., 2005; Dick et al., 2012). The most active substances of the group of compounds with a 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one core structure are referred to as hydroxamic acids. A hydroxyl group bound to heterocyclic nitrogen is characteristic for these compounds (Niemeyer, 2009). Throughout the years, different studies highlighted the role of the indole-derived benzoxazinoid hydroxamic acids, in defense against insects, as well as their antimicrobial, phytotoxic, and regulatory activities for other defense mechanisms (Niemeyer, 2009; Wouters et al., 2016, Maag et al., 2015). Due to their phytotoxicity, benzoxazinoid hydroxamic acids are stored as inactive glucosides in vacuoles. Upon wounding, β-glucosidases hydrolyze the compounds which results in higher bioactivity of the molecules (Frey et al., 2009). The benzoxazinones resulting in this process are unstable compounds that are subsequently degraded to benzoxazolinone. Most of the allelochemical activities of benzoxazinoids can be assigned to these two groups (Sicker and Schulz, 2002; Niemeyer 2009). In soil, benzoxazinoids like 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) and 4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA) are commonly converted by the opening of the heterocyclic ring yielding 6-methoxy-2-benzoxatolinone (MBOA) and benzoxazolin-2(3H)-one (BOA) (Figure 3; Schulz et al., 2013) which are then detectable for up to 12 weeks (Sicker and Schulz, 2002; Fomsgaard et al., 2004; Macías et al., 2005). The fact that they are stable in soil for several months, highlights the importance of BOA and other benzoxazinoids in interactions with the soil microbiota.

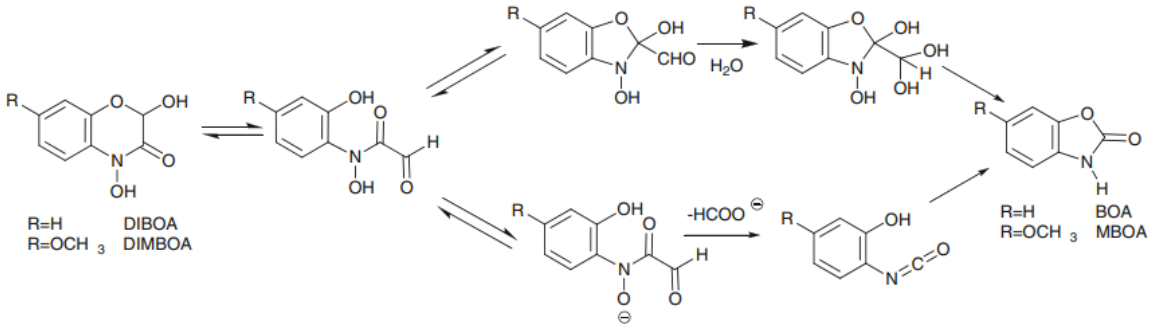


Figure 3: Conversion of DIBOA/DIMBOA to BOA/MBOA.
Suggested pathways for the transformation of DIBOA to BOA and DIMBOA to MBOA in soil (Schulz et al., 2013).

1.4.1. Biosynthesis of Benzoxazinoids

The biosynthesis of benzoxazinoid hydroxamic acids has been studied since the early 1960s. First approaches involved isotope experiments in maize followed by later characterization and isolation of enzymes involved in the pathway (Reimann and Byerrum, 1964; Woodward et al., 1979; Frey et al., 1997; Bailey and Larson, 1989; Leighton et al., 1994). Over the years, the biosynthetic pathway has been elucidated in maize, but also other plants, that are capable to produce benzoxazinoids, have been studied intensively (Schullehner et al., 2008; Dick et al., 2012). Wouters et al. summarize important reactions of the pathway as depicted in Figure 4 (Wouters et al., 2016). The biosynthesis of benzoxazinoids is initiated in the chloroplast by the conversion of indole-3-glycerol phosphate via the indole-glycerolphosphate lyase (BX1) resulting in indole (Figure 4; Gierl and Frey, 2001). In the next steps, the indole is converted to DIBOA in the ER by the four monooxygenases BX2, BX3, BX4, and BX5 (Figure 4). The toxic aglucon DIBOA is then converted into the less toxic DIBOA-Glc by glycosylation catalyzed by the UDP-glycosyltransferases (UGTs) BX8 and BX9. Afterwards, DIBOA-Glc is transported to the vacuole for storage, where it is activated upon plant damage via hydrolysis by plastid localized β-glucosidases and utilized for possible defense reactions (Sicker and Schulz, 2002; Frey et a., 2009) (Figure 4).

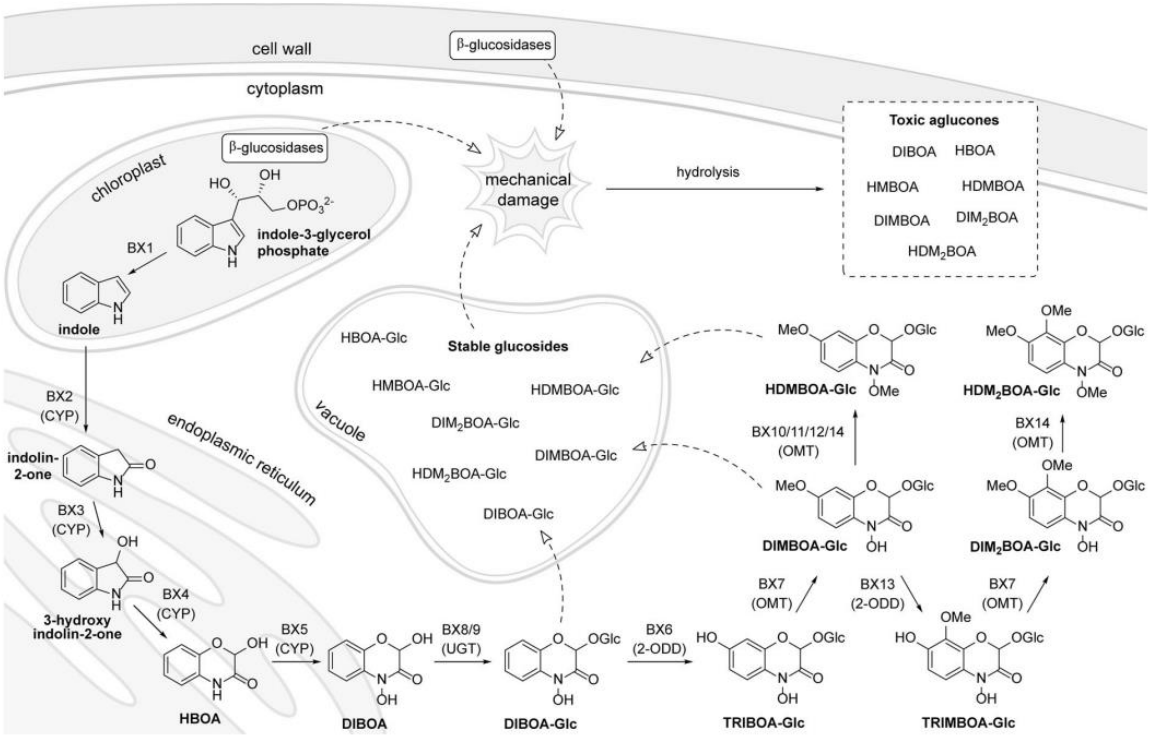


Figure 4: Benzoxazinoid biosynthesis in maize cells.
Biosynthesis of 4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA) in maize from indole-3-glycerolphosphate (Wouters et al., 2016).

1.4.2. Mode of Action and Detoxification of Benzoxazinoids

After the discovery of benzoxazinoids, it became evident, that compounds belonging to this group play an important role in defense against pathogens, insects, and other herbivores, but also in damaging competitive plants, which led to the idea to use the compounds as a basis for the design of new herbicides (Fomsgaard et al., 2004; Macias et al., 2005; Macías et al., 2009; Niculaes et al., 2018). First records showed that BOA was able to inhibit the transport of electrons from NADH-dehydrogenases to ubiquinone and was therefore harmful to mitochondrial electron transport (Niemeyer, 1988). Furthermore, BOA appeared to interfere with the ATP-synthase complex by inhibition of oxidative phosphorylation resulting in damage to the energy metabolism (Niemeyer, 1988). Within the following years, studies highlighted the importance of benzoxazinoids not only as a defense compound but also during bacterial interactions. Growth of plants carrying a mutation in the gene BX1, which encodes the enzyme converting indole-3-glycerolphosphate to the benzoxazinoid precursor indole, changed the bacterial and the fungal species composition in the soil microbiota compared with wild type (Hu et al., 2018). After analysis of plants with mutations in BX1, BX2, or BX6, Cotton et al. (2019) were able to support previous findings by reporting shifts in the bacterial and fungal species composition. While detoxification of benzoxazinoids in plants occurs via glucosylation,

interactions with microorganisms are less known. In fact, within the bacteria, only *Acinetobacter calcoaceticus* was so far reported to be able to degrade BOA, but in the fungal kingdom, on the other hand, *Fusarium* species and the plant pathogen *Gaeumannomyces graminis* were described to detoxify BOA via heterocycle cleavage by γ-lactamase (Friebe et al., 1998; Kettle et al., 2015; Glenn et al., 2016). Schütz et al. (2019) summarized known plant and microorganism-derived benzoxazinoid products which are shown in Figure 5 (green highlights plant-derived products, fungal products are represented by purple lines, and bacterial compounds by grey lines). Degradation of BOA results in the core metabolite 2-aminophenol (AP) (Figure 5, Schütz et al., 2019) which is further converted by bacteria and fungi into 2-acetamidophenol (AAP) or exclusively by fungi to N-(2-hydroxyphenyl)malonamic acid (oHPMA) (Figure 5). Another important benzoxazinoid degradation product is the lipophilic 2-aminophenoxazinone (APO) (Figure 5). APO is a product form oxidative dimerization and can be detected in soil for several months (Zikmudova et al., 2002). Regardless of its stability, APO can be destroyed by Fenton reactions. Venturelli et al. (2016) suggested that APO may function as a histone deacetylase inhibitor, but its full mode of action is yet to be revealed. BOA can also be hydroxylated by plants resulting in the more toxic BOA-6-OH, which is commonly detoxified by forming BOA-6-*O*-glucoside (Hofman et al., 2006) (Figure 5). BOA-glucoside carbamate is another detoxification product mainly found in grasses (Schulz et al., 2013). Several studies have been conducted to determine the impact of benzoxazinoids on the microbiota, in particular, rye mulches containing DIBOA were investigated so far (Schulz et al., 2013). The mode of action and impact of BOA, which is also present in wheat or even dicots, on the soil microbiota yet remains to be revealed (Reberg-Horton et al., 2015; Rice et al., 2005; Understrup et al., 2005).

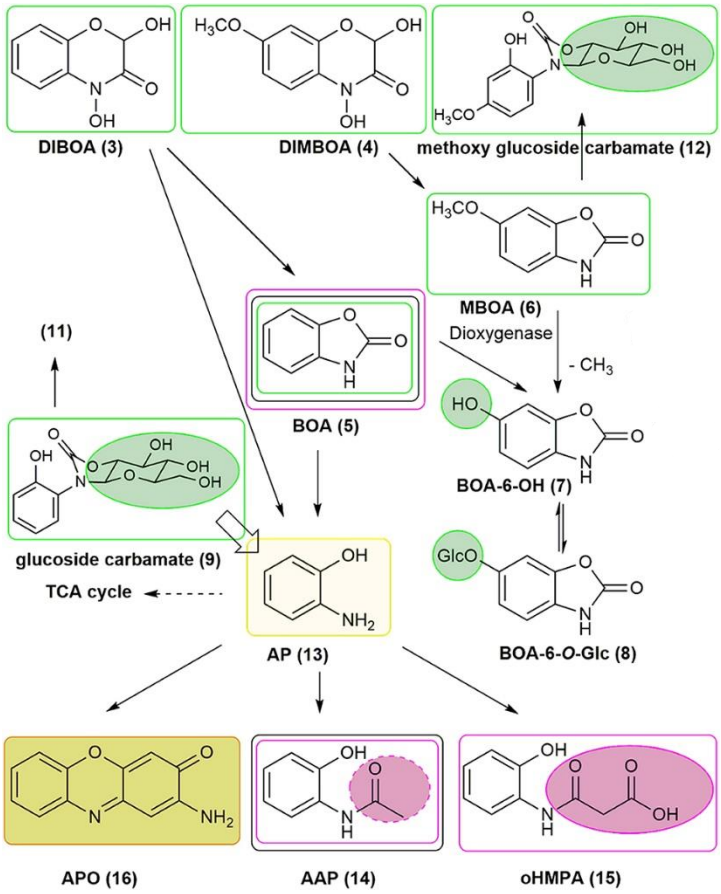


Figure 5: Conversion of benzoxazinoids by microorganisms and plants.
Overview of degradation of benzoxazinoids by microorganisms and plants. Substances highlighted in green are plant-derived, purple indicates compounds derived from fungi, and grey from bacteria (Schütz et al., 2019).

1.5. The Allelochemical Gramine

The family of Poaceae produces a variety of secondary metabolites, including one of the structurally simplest indole alkaloids in nature, 1-(1*H*-indol-3-yl)-*N*,*N*-dimethylmethanamine, also known as gramine. Gramine was described to be synthesized in *Arundo donax*, *Phalaris arundinacea*, *Acer saccharinum*, and the important crop plant *Hordeum vulgare* (Pachter et al., 1959; Corcuera, 1993; Cheeke, 1989). In the genus *Hordeum*, the highest gramine concentrations can be found in seedling and young plant tissue where the concentrations decrease in later stages and gramine becomes undetectable 50 days after germination (Grün et al., 2005; Larsson et al., 2006; Hanson et al., 1983; Lovett et al., 1994). Different from benzoxazinoids, gramine is not released as a root exudate, but it is introduced into the soil by either accumulation on the leaf surface and subsequent washing off by rainfall or by decaying plant material (Hanson et al., 1983). Previous research indicated that gramine functions as a defense compound. Kanehisa et al. (1990) described its negative effect on aphids and therefore highlighted the importance of gramine in defense against insects. Furthermore, several studies suggested that gramine is toxic to mammals, fungi as well as bacteria (Gallagher et al., 1964; Goelz et al., 1980; Wippich and Wink, 1985; Matsuo et al., 2001; Sepulveda and Corcuera, 1990). The allelopathic character of gramine was also highlighted in studies with important crops like oat, rye, and wheat where it revealed negative impacts on the germination of these plants (Bravo et al., 2010).

1.5.1. Biosynthesis of Gramine

The indole alkaloid gramine is a *L*-tryptophan-derived secondary metabolite. The initial step of the biosynthesis involves the transformation of *L*-tryptophan to 3-aminomethylindole (AMI) with subsequent conversion to *N*-methyl-3-aminomethylindole (MAMI) (Gross et al., 1974). While the *N*-methylating enzyme required for the formation of MAMI from AMI was identified and characterized, the conversion of *L*-tryptophane to AMI remains yet to be clarified (Mudd, 1961; Leland and Hanson, 1985). Larsson et al. further analyzed the biosynthesis of gramine and demonstrated that the *N*-methyltransferase (NMT) is not only involved in the formation of MAMI from AMI but also in the conversion of MAMI to gramine as depicted in Figure 6 (Larsson et al., 2006).

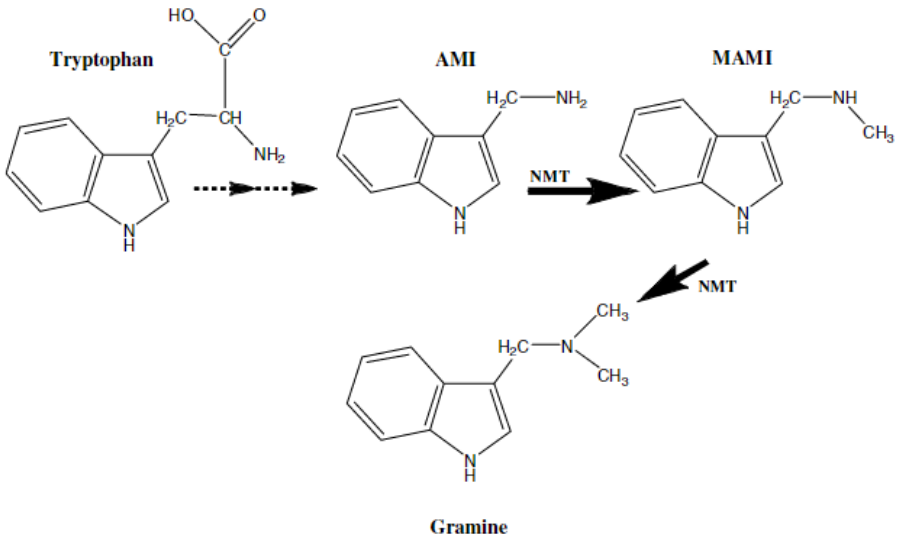


Figure 6: Gramine biosynthesis pathway.
Biosynthesis of gramine starts with the initial conversion of *L*-tryptophan to 3-aminomethylindole (AMI) which is subsequently converted to *N*-methyl-3-aminomethylindole (MAMI) by a *N*-methyltransferase (NMT). In the last step, MAMI is converted to gramine by NMT (Larsson et al., 2006).

1.5.2. Mode of Action and Detoxification of Gramine

Studies on the harmful impact of gramine on mammals, insects, bacteria, fungi as well as different plants, demonstrated the broad spectrum of its functions. Early studies highlighted negative effects on the plant pathogen *Pseudomonas syringae* by increasing the duration of the lag phase during growth and a reduction of the population size at the stationary phase (Sepulveda and Corcuera, 1990). Furthermore, higher concentrations of gramine resulted in an increased respiration rate and inhibited the growth of *P. syringae* (Sepulveda and Corcuera, 1990). Infections of plants with the pathogen *P. syringe* cause the formation of necrotic areas on the leaves. This effect was drastically decreased in leaves that naturally contain gramine, highlighting its importance as a defense compound in certain members of the Poaceae family (Sepulveda and Corcuera, 1990). Exposure of young barley tissue to powdery mildew fungi resulted in increased synthesis of gramine, and damaged leaves showed a higher leakage of gramine (Matsuo et al., 2001). Matsuo et al. (2001) were able to link gramine to the systemic acquired resistance (SAR) by showing a correlation between the increase in gramine synthesis and an increase in resistance to the powdery mildew fungus, making gramine a reliable marker for SAR analysis. The phytotoxic character of gramine was highlighted in cyanobacteria. Growth of *Microcystis aeruginosa* in the presence of gramine was retarded, accompanied by an increase in reactive oxygen species (ROS) and malondialdehyde, a lipid peroxidant product (Hong et al., 2009). The mode of action of gramine in *M. aeruginosa* could be ascribed to the

decrease of superoxide dismutase (SOD) which caused oxidative stress by oxidation of ROS (Hong et al., 2009). While the detoxification of gramine by aphids was reported to involve carboxylesterases and glutathione *S*-transferase, detoxification in bacteria remained unclear (Cai et al., 2004). In the early 1990s, a possible biodegradation pathway of gramine in barley seedlings was proposed by Ghini et al. (1991). Initially, gramine is converted to indole-3-carboxylic acid (Figure 7 (1)) which is further oxidized to *N*-formyl-isatinic acid (Figure 7 (2)) (Ghini et al., 1991). After decarboxylation, the intermediate *N*-formylanthranilic acid (Figure 7 (3)) is formed in the biodegradation pathway of gramine (Ghini et al., 1991). In the last step, *N*-formylanthranilic acid is deformylated to anthranilic acid (Figure 7 (4)) which after the release of CO2 is incorporated into the general metabolism of the plant (Ghini et al., 1991). The impact of gramine on plants and microorganisms suggests possible functions in the shaping of the plant microbiota.

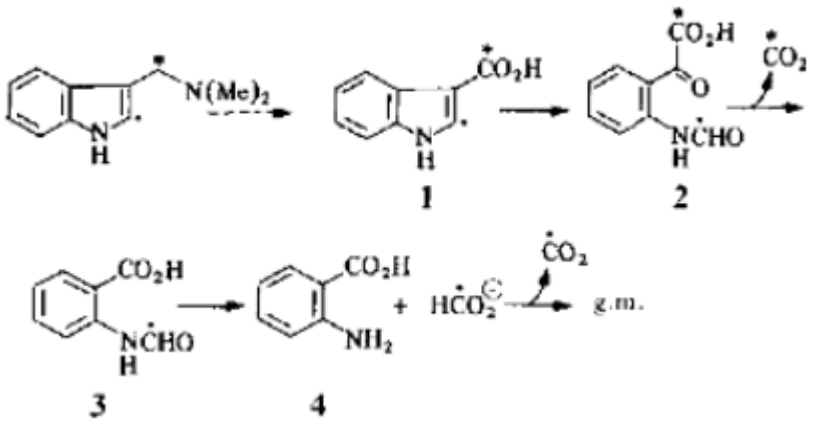


Figure 7: Proposed biodegradation pathway of gramine in barley.
Gramine is converted to indole-3-carboxylic acid (1) which is oxidized to *N*-formyl-isatinic acid (2). Further decarboxylation results in *N*-formylanthranilic acid which is converted to anthranilic acid (4), followed by release of CO_2 and the further degradation in general metabolism (g. m.) (Ghini et al., 1991).

1.6. The Allelochemical Quercetin

3,3',4',5,7-Pentahydroxyflavone, also known as quercetin, belongs to the compound class of flavonoids which are widespread phenolic compounds in plants, carrying at least one hydroxyl group (Singh et al., 2021). The approximately 10,000 substances of the flavonoid class cover a variety of functions and are involved in plant-animal interactions, control of plant development as well as plant-microorganism interactions (Winkel-Shirley, 2001). Due to their important role in the capillary wall and capillary resistance maintenance, flavonoids were described initially as vitamin P (Havsteen, 1983). Until today, flavonoids play an important role in pharmaceutical research. Quercetin was shown to play a role in the prevention of cardiovascular diseases but also functions as an anti-mutagenic, anti-inflammatory and antioxidative, anti-viral, and anti-diabetic substance (Ishizawa et al., 2011; Chang et al., 2012; Lotito et al., 2011; Yoshino et al., 2011). Several studies highlighted the impact of quercetin in the shaping of the gut microbiome (Le et al., 2017; Santangelo et al., 2019; Agarwal et al., 2022). Quercetin is one of the most abundant flavonoids in seeds and root exudates of a variety of plants (Mathesius, 2018). In *Lotus japonicus,* quercetin is the main flavonoid and plays an important role in plant-microbe interactions (Suzuki et al., 2008).

1.6.1. Biosynthesis of Quercetin

The biosynthesis of quercetin involves enzymes that are mainly found at the cytoplasmic face of the ER as characterized in *Zea mays*, *Vitis vinifera,* and *Arabidopsis thaliana* (Boss et al., 1996; Bogs et al., 2006; Castellarin and Di Gaspero, 2007). Some of the enzymes belong to the cytochrome-P450 group and can also be found in other cellular compartments like plastids and vacuoles (Petrussa et al., 2013). In planta, the biosynthesis follows the phenylpropanoid metabolic pathway (Singh et al., 2021). In the first step, the quercetin precursor phenylalanine is converted to cinnamic acid by phenylalanine ammonia lyase (PLA). Afterwards, cinnamic acid is hydroxylated by cinnamate 4-hydroxylase (C4H) resulting in *p*-coumaric acid (Figure 8). Reaction with a CoA ligase (4CL) forms 4-coumaroyl-CoA which is further converted to naringenin chalcone by chalcone synthase (CHS) and the addition of three malonyl-CoA molecules (Figure 8). The heterocyclic C-ring of naringenin is formed in the next step by a chalcone isomerase (CHI) (Figure 8). Hydroxylation of naringenin by 3ß-hydroxylase (F3H) results in dihydrokaempferol (Figure 8). In the final steps, dihydrokaempferol is further hydrolyzed by a flavonol 3'-hydroxylase (F3'H) forming dihydroquercetin, which is converted to quercetin by reactions catalyzed by flavanol synthases (Figure 8) (Nabavi et al., 2020).

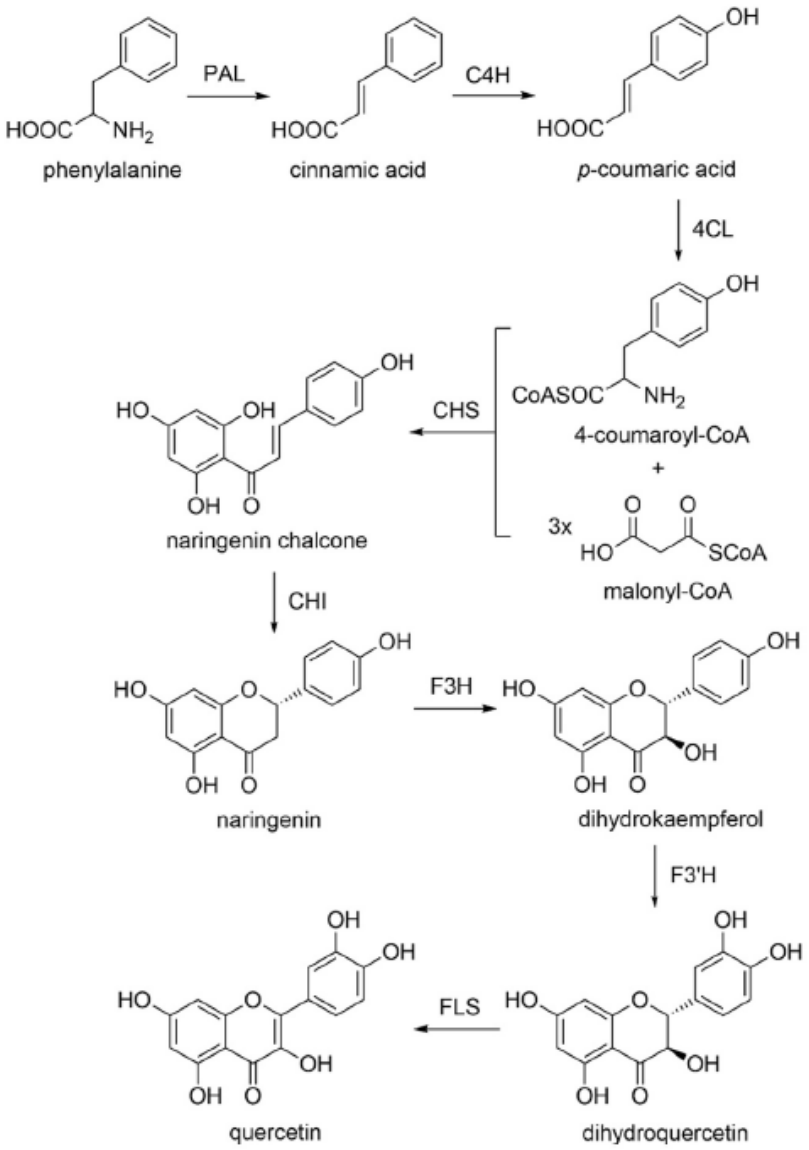


Figure 8: Biosynthesis of quercetin in plants.
Quercetin is synthesized from the precursor phenylalanine via an initial reaction catalyzed by ammonia lyase (PAL) resulting in cinnamic acid. The following reactions include cinnamate 4-hydroxylase (C4H) to form *p*-coumaric acid, CoA ligase (4-Cl) to form 4-coumaroyl-CoA, condensation with three malonyl-CoAs catalyzed by chalcone synthase (CHS) to form naringenin chalcone, chalcone isomerase (CHI) based synthesis of naringenin, 3ß-hydroxylase (F3H) catalyzed formation of dihydrokaempferol, flavonol 3'-hydroxylase (F3'H) based formation of dihydroquercetin, flavonol synthase (FLS) based synthesis of quercetin (Singh et al., 2021).

1.6.2. Mode of Action and Detoxification of Quercetin

Quercetin was described to have a prooxidant character that results in the production of oxygen radicals when activated (Hodnick et al., 1986). This makes quercetin a potential defense substance against potential herbivores and pathogens. To cope with the oxidant activity, organisms feeding on plants containing high concentrations of quercetin would therefore require detoxifying enzymes. Already in the late 1980s, Pritsos et al. showed that the activity of the antioxidant superoxide dismutase (SOD) in *Papilio polyxenes* (black swallowtail) was increased two-fold when the larvae were fed with leaves with a high quercetin content (Pritsos et al., 1988). Compared to other substances that cause oxidative stress, the activation of quercetin appears to be different. While, for example, xanthotoxin is photoactivated, oxygen radicals are produced after the metabolic degradation of quercetin (Musajo and Rodighiero, 1962; Hodnick et al., 1986). Several microorganisms, including *Rhizobia sp*., *Agrobacterium sp*., *Pseudomonas sp*., *Bacillus sp.,* and *Rhodococcus sp.*, are able to metabolize quercetin (Pillai and Swarup, 2002). *Rhizobium loti* degrades quercetin via C-ring fission resulting in phloroglucinol and protocatechuic acid (Rao et al., 1991). Further studies of intestinal bacteria supported the degradation of quercetin with phloroglucinol as a product (Schneider et al., 1999). Nevertheless, only few studies have been conducted to elucidate the fate of quercetin in soil microbe interactions. The plant growth-promoting bacterium *Pseudomonas putida* PML2 is able to metabolize quercetin also resulting in phloroglucinol and protocatechuic acid (Pillai and Swarup, 2002). Later it became evident, that the activation of quercetin to a substance with prooxidant activity, might result from degradation to protocatechuic acid. Allelopathic effects of catechin exuded from *Rhododendron formosanum* appeared to be due to its degradation to protocatechuic acid, supporting the hypothesis of quercetin activation by the formation of protocatechuic acid (Wang et al., 2013). Nevertheless, the impact of quercetin on the soil microbiota yet remains to be revealed.

1.7. Impact of Secondary Metabolites on Bacterial Lipids

Lipids are the backbone of the bilayer membrane, they play important roles in signal transduction, carbon storage, and stress responses in all organisms (Welti et al., 2007). In animals and bacteria, membranes are the first contact with the environment since they are usually missing a cell wall. Therefore, the lipid bilayer is constantly exposed to stresses and interactions with other organisms. The properties of membranes differ in bacterial species and depend on the lipid composition. Polar lipids form membranes and are usually composed of a variable phosphate-containing head group, a glycerol moiety, and two fatty acids (Sohlenkamp and Geiger, 2016). Main polar lipids are phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), cardiolipin, and phosphate-free ornithine lipids (OL) (Raetz and Dowhan, 1990; Sohlenkamp and Geiger, 2016). Some bacterial strains are able to produce phosphate-free glycolipids like monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). Detoxification of secondary metabolites can be limited by the harmful impact of the substances on the bacterial membranes. Escher et al. (1996) demonstrated that the accumulation of phenolic substances can result in the uncoupling of phosphorylation. Furthermore, phenolic substances change the permeability which may result in cell death (Unell et al., 2006). Non-toxic secondary metabolites of Caribbean sponges reduced the colonization of bacteria on their surface by impacting the bacterial membrane (Kelly et al., 2005). Despite harmful impacts, some bacterial strains are able to overcome environmental stresses, changes in temperature, or even the presence of toxic compounds, by adaptation of the membranes (Weber and de Bont, 1996). In the early 1970s, Sinensky (1974) defined the change of the fluidity of the membrane to avoid cell death as homeoviscous adaptation. Most important mechanisms involve changes in the fatty acid composition of the bacterial strains (Heipieper et al., 1994; Sikkema et al., 1995; Ramos et al., 2002). Nevertheless, the impact of the plant secondary metabolites BOA, gramine, and quercetin on the lipid composition of soil bacteria has yet to be unraveled.

1.8. Objectives

Secondary metabolites play a key role in the shaping of the soil microbiota. Evidence accumulated that root exudates can alter the microorganism community in the soil by functioning as an additional carbon source for organisms that can degrade the substances. Other organisms may be repealed due to the toxic character of the secondary metabolites and their degradation products. The interplay of these two mechanisms may result in an accumulation of plant beneficial microorganisms that increase plant competitiveness (Siebers et al., 2018). Understanding the mechanisms of shaping the soil microbiota is important for agriculture since a bacterial community that increases the competitiveness of one plant may be disadvantageous for another. The present study will unravel the impact of three selected secondary metabolites on the soil microbiota. The secondary metabolites BOA and gramine are produced by plants in the Poaceae family and are therefore important for agriculture (Larsson et al., 2006). To assess the specificity of the changes, the flavonoid quercetin will be added as a third secondary metabolite which is not limited to Poaceae. Their impact on the soil microbiota will be revealed in this study by the addition of the substances to native agriculture soil. After the treatment, genomic DNA extracted from soil will be amplified and changes in the microbiome will be determined by metagenomic analysis via next-generation sequencing. Phospholipid fatty acids will be measured to demonstrate the impact of the metabolites on the microbial biomass. Furthermore, cultivable microorganisms will be extracted from soil and the impact of BOA, gramine, and quercetin on isolated strains will be studied. Bacterial degradation products of the secondary metabolites presented here will be investigated and the interaction between plants (*A. thaliana*, *Z. mays*), soil bacteria, secondary metabolites, and their bacterial as well as plant detoxification products will be studied by co-cultivation and gene expression studies. Characterization of bacterial lipids after exposure to BOA, gramine, or quercetin will show possible survival strategies of the organisms during interactions with toxins. The workflow of the project is presented in Figure 9.

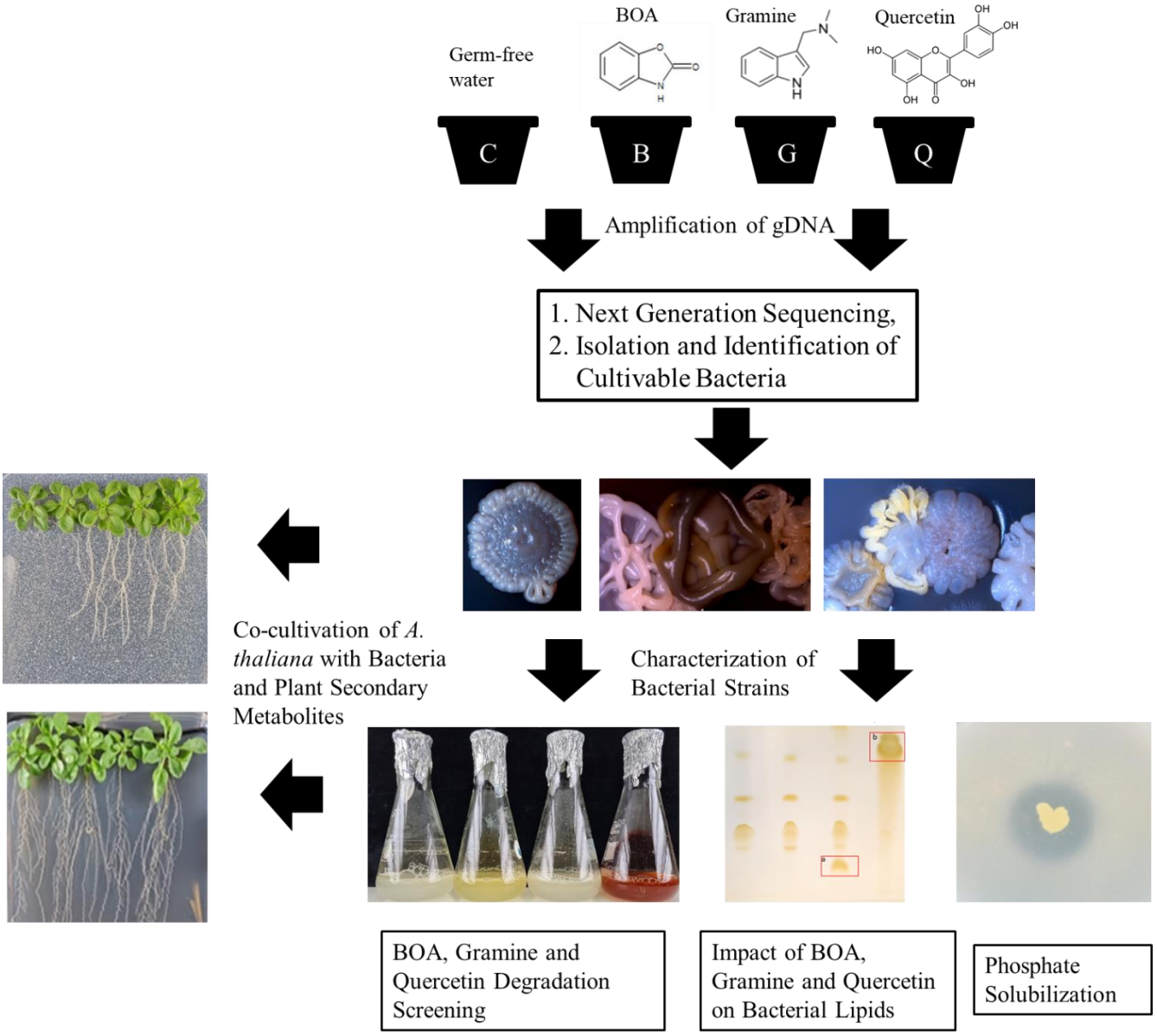


Figure 9: Graphical abstract of project workflow.

2. Materials

2.1. Instruments

6530 Q-TOF MS/MS	Agilent Technologies, Santa Monica (USA)
7300 Real Time PCR System	Applied Biosystems, Darmstadt (D)
7890 Gas chromatography (GC) with mass spectrometry (MS)	Agilent Technologies, Santa Monica (USA)
Autoclave	Tuttnauer Systec, Kirchseeon-Buch (D)
Balance 770	Kern, Balingen-Frommern (D)
Balance PG503-S Delta Range	Mettler Toledo, Gießen (D)
Binocular microscope SZX16	Olympus, Hamburg (D)
Block heater SBH130D/3	Stuart, Bibby Scientific, Staffordshire (USA)
Centrifuge 5810 R	Eppendorf, Hamburg (D)
Centrifuge 5417R	Eppendorf, Hamburg (D)
Chemiluminescence documentation system	Bio-Rad, München (D)
Gel caster, Mighty small II	GE Healthcare Europe GmbH, Freiburg (D)
Growing cabinet, Rumed	Rubarth Apparate GmbH (D)
Heating block	Bioer, Hangzhou (CHN)
Homogenizer Precellys®24	PeQlab, Erlangen (D)
HPLC, SCL-10A VP (System Controller), LC-10AD VP (Pumps A and B)	Shimadzu, Analytic and Measuring Instruments (D)
HPLC-DAD, 1200 Series	Agilent Technologies, Santa Monica (USA)
Incubator, Kelvitron®	Thermo Scientific Heraeus®, Waltham (USA)
Incubation shaker, Multitron 28570	INFORS, Einsbach (D)
pH meter inoLab pH Level 1	WTW GmbH, Weilheim (D)
Pipettes, Research® Plus	Eppendorf AG, Hamburg (D)
Percival plant growth chamber	CLF Laborgeräte GmbH (D)
Quantus Fluorometer	Promega, Madison (USA)
Running chamber for gel electrophoresis	Cti, Idstein (D)
Rotary evaporator LABOROTA4001	Heidolph, Schwabach (D)
Sample concentrator	Techne (Bibby Scientific), Stone (UK)
Sorvall centrifuge RC 5B Plus	Eppendorf AG, Hamburg (D)
Spectrophotometer Nanodrop 1000	PeQlab, Erlangen (D)
Sterile bench model 1.8	Holten Lamin Air, Allerød (DK)

Thermocycler TPersonal	Biometra, Göttingen (D)
Ultracentrifuge Optima L 90K	Beckman Coulter, Krefeld (D)
UV-transilluminator DP-001 T1A	Vilber Lourmat GmbH, Eberhardzell (D)
UV VIS Spectrophotometer, Specord 205	Analytik Jena AG, Jena (D)
Vortex Cetromat® MV	Braun Biotech, Melsungen (D)
Water purification system ELIX® 35	Mercck Millipore, Merck KGaA, Darmstadt (D)

2.2. Consumables

Ceramic beads	Mühlmeier Mahltechnik, Bärnau (D)
Coverslips, 24 mm x 60 mm	Marienfeld, Lauda-Königshofen (D)
Cuvets, semi-micro, PS	Ratiolab GmbH, Dreieich (D)
Frame Star “96 well“ PCR plates, qPCR	Bio-Budget Technologies GmbH, Krefeld (D)
Glass pipettes	Brand, Wertheim (D)
Glass tubes, 8 mL	Fisher Scientific, Schwerte (D)
MicroAmp Optical Adhesive Film, PCR compatible	Thermo Fisher Scientific, Karlsruhe (D)
Microliter pipette tips type 3 series 1700	Labomedic, Bonn (D)
Parafilm M	Sigma-Aldrich, Taufkirchen (D)
Petri dishes, 94 x 16 mm	Greiner bio-One, Frickenhausen (D)
Petri dishes, 145 x 20 mm	Greiner bio-One, Frickenhausen (D)
Pipette tips	Labomedic, Bonn (D)
Reaction tubes, 1.5 mL and 2 mL	Sarstedt, Nümbrecht (D)
Reaction tubes, 15 mL and 50 mL	Greiner bio-One Frickenhausen (D)
SPE silica column	Macherey-Nagel, Düren (D)
Teflon septa for screw camps	Schmidlin, Neuheim (D)
TLC plates Silica 60 Durasil with and without concentration zone	Macherey-Nagel, Düren (D)

2.3. Chemicals

2-Acetamidophenol (AAP)	Sigma-Aldrich, Taufkirchen (D)
2-Aminophenol (AP)	Sigma-Aldrich, Taufkirchen (D)
2-Ammoniumacetat	Sigma-Aldrich, Taufkirchen (D)
2-Benzoxazolinone (BOA)	Fulka Chemika, Neu-Ulm (D)
ß-Mercaptoethanol	AppliChem, Darmstadt (D)
Acetic acid glacial	AppliChem, Darmstadt (D)
Acetone	Prolabo VWR, Darmstadt (D)
Agarose	PeQlab, Erlangen (D)
Ammonium acetate	Sigma-Aldrich, Taufkirchen (D)
Ammonium nitrate	AppliChem, Darmstadt (D)
Ammonium sulfate	Sigma-Aldrich, Taufkirchen (D)
Asparagine	Roth, Karlsruhe (D)
Bacto agar	Duchefa Biochemie, Haarlem (NL)
Bacto peptone	Duchefa Biochemie, Haarlem (NL)
Bicinchoninic acid solution (BSA)	Sigma-Aldrich, Taufkirchen (D)
Boric acid	Grüssing, Filsum (D)
Bovine serum albumin	Sigma-Aldrich, Taufkirchen (D)
Calcium chloride	Merck, Darmstadt (D)
Chloroform	Merck, Darmstadt (D)
Diethylether	Prolabo VWR, Darmstadt (D)
Dimethyl ether sulfoxide	Sigma-Aldrich, Taufkirchen (D)
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich, Taufkirchen (D)
Dipotassium hydrogen phosphate	AppliChem, Darmstadt (D)
Ethanol	Merck, Darmstadt (D)
Ethidium bromide	Serva, Heidelberg (D)
Ethyl acetate	Merck, Darmstadt (D)
Ethylenediaminetetraacetic acid (EDTA)	Roth, Karlsruhe (D)
Formaldehyde	AppliChem, Darmstadt (D)
Formic acid	VWR, Darmstadt (D)
Glucose anhydrous	AppliChem, Darmstadt (D)
Glycerol	AppliChem, Darmstadt (D)
Gramine	Sigma-Aldrich, Taufkirchen (D)

Hexane	Merck, Darmstadt (D)
Hydrochloric acid (37%)	AppliChem, Darmstadt (D)
Iron(III) chloride	Sigma-Aldrich, Taufkirchen (D)
Isopropanol	AppliChem, Darmstadt (D)
Magnesium chloride	AppliChem, Darmstadt (D)
Magnesium sulfate	Duchefa Biochemie, Haarlem (NL)
Maltose monohydrate	USP AG, Basel (CH)
Methanol	J.T. Baker, Phillipsburg (USA)
Midori Green Advance	NIPPON Genetics GmbH, Düren (D)
Morpholinoethanesulfonic acid hydrate (MES)	ChemCruz™ Bio-Connect B.V., Huissen (NL)
Murashie and Skoog basal salts including vitamines	Duchefa Biochemie, Haarlem (NL)
Peptone	Formedium™, Norfolk (UK)
Potassium chloride	Merck, Darmstadt (D)
Potassium dihydrogen phosphate	Merck, Darmstadt (D)
Potassium hydroxide	Merck, Darmstadt (D)
Potassium nitrate	Grüssing, Filsum (D)
Phytoagar	Duchefa Biochemie, Haarlem (NL)
Quercetin dihydrate	ABCR GmbH & Co. KG, Karlruhe (D)
Sodium chloride	Duchefa Biochemie, Haarlem (NL)
Sodium hypochlorite	Roth, Karlsruhe (D)
Sodium nitrate	Roth, Karlsruhe (D)
Sorbitol	Duchefa Biochemie, Haarlem (NL)
Sucrose	Duchefa Biochemie, Haarlem (NL)
Toluene	VWR, Darmstadt (D)
Tridecanoic acid (13:0)	Sigma-Aldrich, Taufkirchen (D)
Tris(hydroxymethyl)aminomethane (Tris)	Duchefa Biochemie, Haarlem (NL)
Tryptic soy broth (TSB)	Sigma-Aldrich, Taufkirchen (D)
Tryptone	AppliChem, Darmstadt (D)
Water, HPLC grade	VWR, Darmstadt (D)
Yeast Extract	Duchefa Biochemie, Haarlem (NL)

Further chemicals were obtained from Merck (Sigma-Aldrich), Applichem, or Carl Roth.

2.4. Kits and Enzymes

Agencourt AMPure XP beads	Beckman Coulter, California (USA)
Antarctic phosphatase	New England BioLabs GmbH, Frankfurt (D)
DNA, RNA, and protein purification kit	Machery-Nagel, Düren (D)
dNTPs	Thermo Fisher Scientific, Karlsruhe (D)
Exonuclease I	New England BioLabs GmbH, Frankfurt (D)
GeneRulerTM 1 kb DNA Ladder	Thermo Fisher Scientific, Karlsruhe (D)
GoTaq G2 DNA polymerase, 2500 U	Promega, Madison (USA)
my-Budget 5x EvaGreen (ROX) qPCR-MixII	Bio-Budget Technologies GmbH, Krefeld (D)
PCR-clean-up-kit	Machery-Nagel, Düren (D)
QuantiFluor ONE dsDNA systems	Promega, Madison (USA)
RevertAid First Stand cDNA Synthesis kit	Thermo Fisher Scientific, Karlsruhe (D)
Taq DNA Polymerase	Fermentas, St. Leon-Rot (D)

2.5. Oligonucleotides

Oligonucleotides used in this study are provided in Table 1. The primers were synthesized by Integrated DNA Technologies (IDT) (Belgium). The oligonucleotides were not integrated into the Bn number system of the institute but instead collected in a separate stock.

Table 1: Oligonucleotides used during this project.

Primer	Description	Sequence
799F	Bacterial 16S rRNA V4-V7 forward	AACMGGATTAGATACCCKG
1192R	Bacterial 16S rRNA V4-V7 reverse	ACGTCATCCCCACCTTCC
ITS1F	Fungal ITS1 region forward	CTTGGTCATTTAGAGGAAGTAA
ITS2	Fungal ITS1 region reverse	GCTGCGTTCTTCATCGATGC
ITS1-O	Oomycetal ITS1 region forward	CGGAAGGATCATTACCAC
5.8s-O-Rev	Oomycetal ITS1 region reverse	AGCCTAGACATCCACTGCTG
B5-F	Bacterial 16S 2nd PCR forward	AATGATACGGCGACCACCGA GATCTACACGACTGCGACTGG CGAACMGGATTAGATACCCKG
Ft-F	Fungal ITS1 2nd PCR forward	AATGATACGGCGACCACCGAGA TCTACACTCACGCGCAGGCTTGG TCATTTAGAGGAAGTAA

Ot-F	Oomycetal ITS1 2nd PCR forward	AATGATACGGCGACCACCGAGAT CTACACAGTTCCAGGCTCATGCG GAAGGATCATTACCAC
B5-R1	Bacterial Illumina sequencing	ACGACTGCGACTGGCGAACMGG ATTAGATACCC
B5-R2	Bacterial Illumina sequencing	CAGCCATTTAGTGTCACGTCATC CCCACCTTCC
B-5Index	Bacterial Illumina sequencing	GGAAGGTGGGGATGACGTGACA CTAAATGGCTG
Ft-R1	Fungal Illumina sequencing	TCACGCGCAGGCTTGGTCATTTA GAGGAAGTAA
Ft-R2	Fungal Illumina sequencing	CGTACTGTGGAGAGCTGCGTTCT TCATCGATGC
Ft-Index	Fungal Illumina sequencing	GCATCGATGAAGAACGCAGCTCT CCACAGTACG
Ot-R1	Oomycetal Illumina sequencing	AGTTCCAGGCTCATGCGGAAGG ATCATTACCAC
Ot-R2	Oomycetal Illumina sequencing	GCCTGGAGTCATAGAGCCTAGAC ATCCACTGCTG
Ot-Index	Oomycetal Illumina sequencing	CAGCAGTGGATGTCTAGGCTCTA TGACTCCAGGC
VTE1 F	qPCR tocopherol cyclase forward	TCCGGACTCCTCACAGTGGGTAA
VTE1 R	qPCR tocopherol cyclase reverse	AAAGTAGTCGTCGAATGGTGAGCC
TPS02 F	qPCR terpene synthase forward	TAAAGAAGAGGTGAGGAAGAC
TPS02 R	qPCR terpene synthase reverse	CTAGAAATAAGTTTAAGTTCT
TPS04 F	qPCR terpene synthase forward	ATGGGAAGGAGAAGAGCTTAA
TPS04 R	qPCR terpene synthase reverse	TTAGTAGAAGCATGGTGCGAAT
TRYPS02 F	qPCR tryptophane synthase forward	GTATCCCAATTCCCAACTTGTGT
TRYPS02 R	qPCR tryptophane synthase reverse	AGCAGACACATGTAAGCAGACC
GLN1.1 F	qPCR glutamine synthase forward	CAATGAGGGAAGAAGGCGGT
GLN1.1 R	qPCR glutamine synthase reverse	CGCAACACCCCAAAGGAAAG
GLN1.2 F	qPCR glutamine synthase forward	CTTTCCTTTGGGGTGTTGCG
GLN1.2 R	qPCR glutamine synthase reverse	AGCTGGCCTCCTATCCTCAA
NIA1 F	qPCR nitrate reductase forward	ACAAAGGCAAAGGCAACTTC
NIA1 R	qPCR nitrate reductase reverse	CCACATACATCTCGGTTTCGT
NIA2 F	qPCR nitrate reductase forward	GCGTGGTGTCCCTCTCTG

NIA2 R	qPCR nitrate reductase reverse	TGATGCTCGTTCCGTATTTG
Cat1 F	qPCR catalase forward	ACCTGAAGCCGAGCATGTAAGGAT
Cat1 R	qPCR catalase reverse	ATAATCGACCACCGACCATCAGCA
Cat3 F	qPCR catalase forward	CCGGCTCAACATGAAGGCAAACAT
Cat3 R	qPCR catalase reverse	TTCTCTTGTTCCTGGCGACGACAT
FAD2.1 F	qPCR oleyl desaturase forward	ACCACCTCTTCTCCACCATGCC
FAD2.1 R	qPCR oleyl desaturase reverse	CCTTGCGGTTGCGGTTCTCAG
FAD2.2 F	qPCR oleyl desaturase forward	TGCCGCTGCTGATCGTGAAC
FAD2.2 R	qPCR oleyl desaturase reverse	TGCGTGTCCGTGATGTTGTGG
NPR1 F	qPCR pathogen response forward	CTCCAGAGGGGCACAGCCGA
NPR1 R	qPCR pathogen response reverse	CGAGAACACGCTGCCCTCCG
PR1 F	qPCR pathogen response forward	TCAGTCATGCCGTTCAGCTT
PR1 R	qPCR pathogen response reverse	TTGTCCGCGTCCAGGAA
PR4 F	qPCR pathogen response forward	TGATGGATAGATGGCGATTGC
PR4 R	qPCR pathogen response reverse	AGAATTGACACCGCCAAACC
SOD2 F	qPCR superoxide dismutase 2 forward	CACCAACGGCTGCATGTC
SOD2 R	qPCR superoxide dismutase 2 reverse	ATGCTCCTTGCCAACAGGAT
ACT1 F	qPCR actin forward	GGGATTGCCGATCGTATGAG
ACT1 R	qPCR actin reverse	GAGCCACCGATCCAGACACT

2.6. Plants

Arabidopsis thaliana wild type Columbia 0 was purchased from ABRC (Columbus, USA). *Zea mays* cultivar Cassila seeds were purchased from KWS (Einbeck, Germany).

2.7. Microorganisms

Additionally, to strains isolated in this project, bacterial strains *Arthrobacter sp.* MPI761, MPI762, MPI763, MPI764 and *Pseudomonas sp.* MPI9 were provided by the Max-Planck Institute for Plant Breeding Research in Cologne (Germany).

2.8. Soil

Cologne agriculture soil, harvested from a local site in Cologne (Bei et al., 2015), was provided by the Max-Planck Institute for Plant Breeding Research in Cologne (Germany). The soil was stored at 4°C, then the soil was processed through a 5 mm sieve followed by a second sieve with a mesh size of 2 mm to remove plant material, rocks, and other unwanted materials. Analysis of the soil composition, shown in Table 2, was performed by Raiffeisen Laborservice (Ormont, Germany).

Table 2: Cologne agriculture soil main nutrient composition

Description	
Soil type	clay
Nitrogen	0.047%
Carbon (total)	0.279%
Sulfur	0.003%

3. Methods

3.1. Working with Soil

3.1.1. Determination of Soil Moisture Content

The soil moisture content was determined by drying a defined amount of soil. Initially, exactly 50 g of soil, which was stored at 4°C, was filled into a beaker. After drying at 110°C for 24 h, the weight of the soil in the beaker was documented and the following equation was used to calculate the initial soil moisture content:

$$\text{Moisture content [\%]} = \frac{(\text{weight of moist soil [g]}) - (\text{weight of dry soil[g]})}{(\text{weight of dry soil[g]})} \cdot 100$$

Using the equation shown above, a water content of 12 ± 1.2% was determined for the Cologne agriculture soil.

3.1.2. Determination of Water-Holding Capacity

The water-holding capacity was determined according to the Hilgard's method. Initially, soil was dried for 24 h at 110°C and afterwards transferred to pots. The empty pots (10 cm diameter, 375 mL volume) with holes in the bottom were prepared by placing a filter paper inside and weighing. After filling the pots with soil, the weight was once again determined, and the pots were placed in a pan containing water. The soil was saturated with water overnight and on the following day, the pots were removed from the pan and then weighed again. In the next step, the soil was prepared for drainage by covering the pots with a plastic wrap. Small holes were poked into the wrap to avoid evaporation of water and to maintain the atmospheric pressure. After the drainage at room temperature, the pots were weighed again to determine the total uptake of water, and the following equation was used to calculate the water-holding capacity:

$$\text{Water} - \text{holding capacity [\%]} = \frac{\text{Amount of water in saturated soil [g]}}{\text{Amount of dry soil [g]}} \cdot 100$$

Following the procedure described above, the water-holding capacity of Cologne agriculture was calculated to be 40.8 ± 0.4%.

3.1.3. Incubation of Soil with Plant Secondary Metabolites

300 g Cologne agriculture soil previously stored at 4°C was sieved again through a 2 mm sieve into a pot (10 cm diameter, 375 mL volume) which was placed in a Petri dish. The secondary metabolites BOA, gramine, or quercetin were mixed in solid form with the soil because of their insolubility in water. 10 µmol of BOA (1.4 mg), gramine (1.8 mg), and quercetin (3.4 mg) were added every other day over a period of 28 days to the pots by mixing with a sterile spatula. Initially, 50 mL of germ-free tap water were added to each pot to reach a water content of ~24.5%. The water content of ~24.5% is equivalent to a maximal water holding capacity of 60% (based on the total calculated 40.8%). Within the course of the 28-day incubation with secondary metabolites, the water content in the soil was ensured to be maintained at ~24.5% by weighing and watering the pots every other day. Pots were placed in trays and covered with plastic covers with holes and incubated under a 16 h light/8 h dark cycle with 160 µmol/m^2/s of light, while the temperature in the chamber was at 21°C and the humidity at 55%. Four replicate pots for each treatment were prepared. Every seven days, soil samples for DNA extraction (0.5 g) were taken. Soil samples for FAME analysis (5 g) were taken after 28 days of incubation, and samples of 300 g of soil (whole pot) for metabolite extraction and HPLC analysis were taken after 2 days in three replicates.

3.2 Microbiology Methods

3.2.1. Cultivation Media

Bacterial strains were cultivated using yeast extract-peptone-dextrose (YPD) medium, malt medium, peptone-glucose agar (PGA), Tryptic Soy Broth (TSB), Thornton's Standardized Medium (TSM) (Thornton, 1922) and Czapek medium.

YPD Medium	
Yeast extract	10 g/L
Bacto peptone	20 g/L
Glucose	20 g/L
Bacto agar	20 g/L

Malt Medium	
Malt extract	15 g/L
Yeast extract	8 g/L
Glucose	5 g/L
Fructose	5 g/L
Bacto agar	10 g/L

PGA Medium	
Bacto peptone	10 g/L
Glucose	20 g/L
Bactor agar	20 g/L

TSB Medium	
Casein peptone (pancreatic)	17 g/L
Soya peptone (papain digest)	3 g/L
NaCl	5 g/L
K_2HPO_4	2.5 g/L
Glucose	2.5 g/L
Bacto agar	15 g/L

Czapek Medium	
$NaNO_3$	2 g/L
$MgSO_4$	0.5 g/L
K_2HPO_4	1 g/L
KCl	0.5 g/L
$FeSO_4$	0.01 g/L
Sucrose	30 g/L
Bacto agar	13 g/L

TSM	
K_2HPO_4	1 g/L
$MgSO_4 \times 7\ H_2O$	0.2 g/L
$CaCl_2$	0.1 g/L
NaCl	0.1 g/L
$FeCl_3$	0.002 g/L
KNO_3	0.5 g/L
Asparagine	0.5 g/L
Glucose	1 g/L
Bacto agar	15 g/L

3.2.2. Cultivation of Bacterial Strains from Cologne Agriculture Soil

After 28 days of treatment with either BOA, gramine, quercetin, or water as control, bacterial strains were isolated from the Cologne agricultural soil by serial dilution and plating on selective media. The scheme in Figure 10 shows the process of bacterial isolation and cultivation from soil. Suspensions were prepared by mixing soil and a germ-free 10 mM $MgCl_2$ solution in a 1:1 ratio (15 g/ 15 g). After vortexing, the samples were centrifuged for 10 min at 1000 x g to separate soil and other particles from the $MgCl_2$ solution. In the next step, serial dilutions (1:100, 1:1000, and 1:10,000) of the supernatant were prepared and 15 µL of each dilution plated on malt medium, TSB, YPD, TSM, Czapek medium, and PGA. The plates were incubated at 28°C and monitored frequently to stop bacterial growth once the colonies were large enough for further separation on fresh plates and colony PCR.

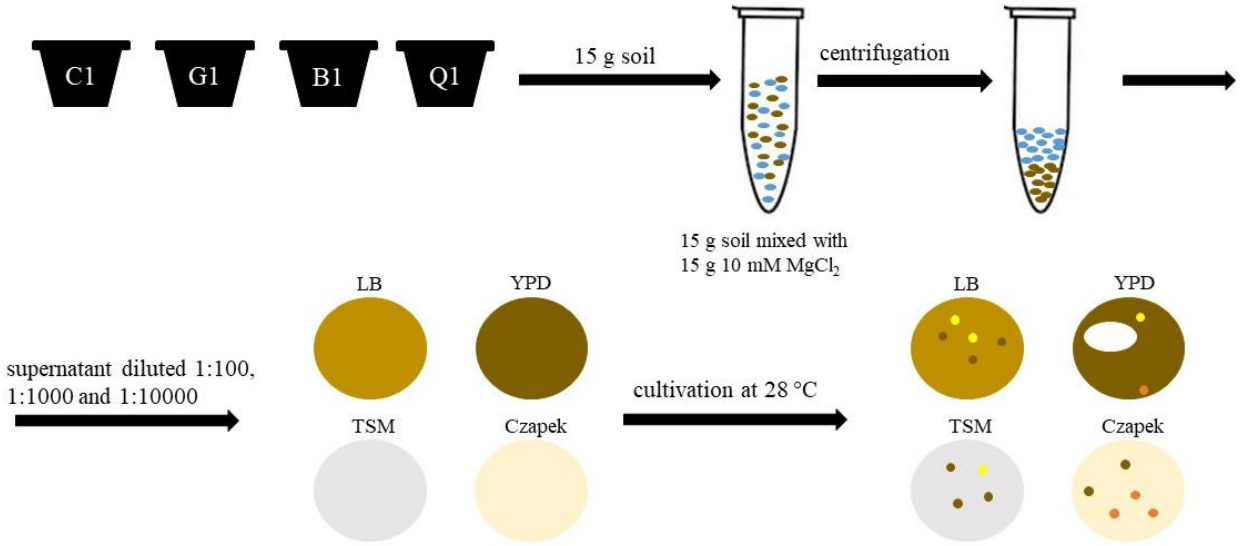


Figure 10: Schematic overview of isolation of bacteria from soil.

3.2.3. Identification of Soil Bacteria

Bacterial strains cultivated on agar plates were identified via colony PCR amplification of the bacterial 16S rRNA gene using the 799F – 1192R primer (Table 1) (Agler et al., 2016). Colonies were picked under sterile conditions using germ-free pipette tips and directly mixed into the PCR reaction. Once amplified, the expected band at 500 bp on a 1.2% agarose gel was purified and prepared for sequencing. Sequencing samples contained 5 µL of 20 – 80 ng/µL of the purified bacterial PCR product and 5 µL of 5 µM (5 pmol/µL) primer (799F – 1192R) in a

1.5 mL reaction tube. Sanger sequencing (GATC) was performed by Eurofins Genomics (Ebersberg, Germany) and the sequences were blasted using the databank of the National Center for Biotechnology Information (NCBI).

The 16S rRNA sequences of identified bacterial strains were quality checked and aligned by Dr. Katharina Frindte (INRES, AG Prof. Claudia Knief, University of Bonn) using the SINA aligner v1.2.11 (Pruesse et al., 2012) software. The phylogenetic tree, based on the maximum-likelihood algorithm PhyML in ARB (Ludwig et a., 2014) was calculated by Dr. Katharina Frindte (University of Bonn).

3.2.4. Preparation of Bacterial Microbanks

For long-term storage of bacteria, single colonies of the strains were picked from agar plates and grown in 5 mL 50% TSB medium at 28°C and 180 rpm overnight. Afterwards, 250 µL of the overnight culture were transferred to microbanks containing beads and a cryopreservative (Pro-Lab Diagnostics, USA). Upon inverting the microbank five times, the mixture was incubated for 2 min at room temperature allowing the bacteria to bind to the beads. Next, as much liquid as possible was removed by pipetting and the microbanks, containing now beads with the bacterial strains, were stored at -80°C.

3.2.5. Bacterial Growth Experiments

The impact of plant secondary metabolites on bacterial strains was tested in the minimal medium TSM. Pre-cultures of the bacterial strains were grown in liquid TSB medium at 28°C and 180 rpm overnight. The next day, cells were harvested by centrifugation at 1000 x g for 15 min and washed two times. The bacterial cells were washed by resuspending the pellet in 10 mM $MgCl_2$ followed by centrifugation at 1000 x g for 15 min. Finally, the cells were resuspended in 10 mL 10 mM $MgCl_2$ and used for the inoculation of the main cultures. Main cultures were employed to determine bacterial growth and screening for bacterial degradation products of plant secondary metabolites. The cultures were prepared in 250 mL flasks containing 100 mL TSM. Cultures for lipid extraction contained 400 mL TSM in 1 L flasks.

The minimal medium TSM was supplemented with 500 µM of either BOA, gramine, or quercetin. Controls contained 0.5% DMSO. Upon inoculation with an initial OD_{600} of 0.1, the cultures were grown over a period of 72 h hours at 28°C and 180 rpm. Samples for determination of growth via OD_{600} measurements and screening for bacterial degradation

products via HPLC with diode array detector (DAD) were taken after 0 h, 2 h, 4 h, 6 h, 24 h, 48 h, and 72 h. Cells for lipid extraction and protein extraction were harvested in their exponential phase after 12 h of growth. The growth rate was calculated using OD_{600} values with x2 being the latest measurement (t2 in h) and x1 the earliest (t1 in h) in the exponential phase of the bacterial culture. The generation time G was also calculated based on OD_{600} values and their corresponding time (in minutes) in the exponential phase. Formulas for calculation of the inoculation, growth rate μ, and generation time G are presented below.

$$\text{Inoculum [mL]} = \frac{0.1 \cdot 100\ \text{mL}}{\text{Pre} - \text{culture OD}_{600}}$$

$$\text{Growth rate } \mu\ [{}^{1}/_{\text{h}}] = \frac{\log \text{x2} - \log \text{x1}}{\text{t2[h]} - \text{t1[h]}}$$

$$\text{Generation time G [min]} = \frac{\text{t2 [min]} - \text{t1[min]}}{3.3 \cdot \log \frac{\text{x2}}{\text{x1}}}$$

3.2.6. Phosphate Solubilization Assay

The ability of the soil bacteria to solubilize phosphate was tested utilizing modified Pikovskayas agar (Pikovskaya, 1948). For better visualization of the phosphate solubilization zone, the initial amount of tricalcium phosphate in the medium was reduced from 5 g to 1 g. A bacterial colony was placed in the center of a Pikovskayas agar plate and incubated at 28°C for 5 – 10 days. If the bacterial strain was able to solubilize the tricalcium phosphate, the colony on the milky medium was surrounded by a clear halo, which can only be formed if the tricalcium phosphate is degraded.

Modified Pikovskayas Medium

Yeast extract	0.5 g/L
Glucose	10 g/L
$Ca_3(PO_4)_2$	1 g/L
$(NH_4)_2SO_4$	0.5 g/L
KCl	0.2 g/L
$MgSO_4$	0.1 g/L
$MnSO_4$	0.0001 g/L
$FeSO_4$	0.0001 g/L
Bacto agar	15 g/L

3.3. Working with Plants

3.3.1. Seed Surface Sterilization

A. thaliana (Col-0) plants were cultivated for all experiments under sterile conditions. Contaminations were avoided by surface sterilization of the seeds. Therefore, *A. thaliana* seeds were collected in 1.5 mL reaction tubes. Then 1 mL of sterilization solution was added to the seeds and the reaction tubes were shaken for 10 min vigorously at room temperature. The following steps were carried out under sterile conditions. The sterilization solution was removed after a brief spin down, and the seeds were washed five times with 96% ethanol and the ethanol was also discarded. To remove residual ethanol, the seeds were washed five times with germ-free ddH_2O. Once dried, the sterile seeds were stored at 4°C until further use.

Sterilization Solution

Sodium hypochlorite 12%	4 mL
Ethanol 96% (v/v)	25 mL
ddH_2O	21 mL

3.3.2. Cultivation of *A. thaliana*

Surface-sterilized *A. thaliana* seeds were distributed on Murashige-Skoog (MS) medium plates which were sealed with a gas-permeable tape. The seeds were stratified for 2 days in the dark and at 4°C. Plants were then grown for 21 days at 21°C, under a 16 h light/ 8 h dark regime, 55% humidity, and 120 µmol m^{-2} s^{-1} light intensity.

Co-cultivation of *A. thaliana* with plant secondary metabolites or soil bacteria was performed on square plates by distributing six surface-sterilized seeds in a row close to the top of the plate. To determine the root length of the plants, square plates were grown vertically. For treatment with plant secondary metabolites, the MS medium was supplemented after autoclaving and cooling with 100 µM or 500 µM of either BOA, gramine or quercetin. Since the three metabolites were dissolved in DMSO, control media was supplemented with the same amount of DMSO. After 2 days of stratification, the plants were grown under the conditions described above.

Co-cultivation of *A. thaliana* with soil bacteria was performed by the addition of bacteria directly to the MS medium after autoclaving and cooling. The bacterial pre-cultures were washed two times and resuspended in 10 mL of 10 mM $MgCl_2$ solution. The cell density of the cultures was determined and diluted to OD_{600} 0.5. The initial OD_{600} of the bacterial cultures was diluted 1:10000 in the MS medium. Prior to harvesting, the plants were grown for 21 days under the conditions described above. The impact of plant secondary metabolites and soil bacteria on *A. thaliana* was measured by the determination of plant fresh weight (without the roots) and root length.

MS Medium

Murashige-Skoog (MS) medium including vitamins	4.41 g/L
2-(N-Morpholino) ethanesulfonic acid (MES) (10 mM)	2.13 g/L
Sucrose	10 g/L
Phyto-agar	8 g/L
pH (adjusted with KOH)	6.0

3.3.3. Incubation of *A. thaliana* with 5-N-AAP

A. thaliana plants were cultivated for 21 days under sterile conditions on MS medium. Afterwards, the plants were collected from the plates and incubated with 1 mM N-(2-hydroxy-5-nitrophenyl) acetamide (5-N-AAP, synthesized and provided by Sergey Girel and Prof Dr. Laurent Bigler (University of Zurich Switzerland)) or without 5-N-AAP as control. During the course of the incubation, plants were harvested after 0 h, 30 min, 1 h, 3 h, 5 h, and 24 h, placed in liquid nitrogen, and stored at -80°C until RNA extraction and gene expression studies.

3.3.4. Cultivation and Incubation of *Z. mays* with BOA-OH Isomers

Z. mays Cassila cultivars were grown for 7 days under hydroponic conditions following Schulz et al. (2016). Afterwards, the seedlings were incubated with 500 µM BOA-OH isomers in tap water for 0 h, 0.5 h, 1 h, 6 h, and 24 h. Additionally to BOA-6-OH, the isomers BOA-4-OH, BOA-5-OH, and BOA-7-OH utilized in this study, were synthesized and provided by Prof. Dr. Dieter Sicker (Institute for Organic Chemistry, University of Leipzig). Once treated with BOA-OH isomers, roots of maize plants were harvested, frozen in liquid nitrogen, and stored at -80°C until RNA extraction and gene expression studies.

3.4. Methods in Molecular Biology

3.4.1. Isolation of Genomic DNA from Soil

Samples were taken after 7 days, 14 days, 21 days, and 28 days of Cologne agriculture soil treatment with 10 µmol of either BOA, gramine, quercetin, or germ-free water as control were used for genomic DNA (gDNA) extraction following the manufacturer's instructions of the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, USA). After the addition of phosphate buffer and MT buffer, soil samples were homogenized in the Lysing Matrix E tubes in a Precellys tissue homogenizer (Bertin, Germany) two times for 30 sec at 6000 rpm. In the end, the gDNA was eluted in 60 µL nuclease-free H_20. Prior to further amplification, the extracted gDNA was purified twice with Agencourt MPure XP beads (Beckman-Coulter, Germany) following the manufacturer's instructions. Purified gDNA was stored at -20°C until further use.

3.4.2. RNA Isolation from Plant Material

A. thaliana leaf material was used for RNA extraction following the manufacturer's instructions of the NucleoSpin® RNA Plant Kit (Macherey-Nagel, Germany). First, frozen leaf samples were homogenized using the Precellys tissue homogenizer (Bertin, Germany) two times for 20 sec at 6000 rpm. Due to increased temperatures samples were cooled on ice between the two homogenization cycles. The first digestion of gDNA was performed as described in the manufacturer's instructions. Once extracted, the RNA was eluted in 50 µL RNase-free H_2O and a second gDNA digestion was performed to reduce the gDNA content as much as possible since already minor concentrations might impact further downstream processes like qPCR. The second gDNA digestion was carried out as described in the manufacturer's instructions of the NucleoSpin® RNA Plant Kit (Macherey-Nagel, Germany) utilizing rDNase and purification of RNA samples with ethanol and 3 M sodium acetate. RNA was stored at -80°C until further use.

3.4.3. Nucleic Acid Quantification

The quantity of double-stranded DNA was determined by fluorometry using the QuantiFlour ONE Dye and a Quantus Fluorometer (Promega, Germany). The RNA concentration and quality were measured spectrophotometrically using a Nanodrop (Thermo Fisher Scientific, USA). Pure RNA was expected to result in a ratio of ~2.0 for the absorption at 260 and 280 nm. Additionally, the quality of RNA was determined by gel electrophoresis.

3.4.4. cDNA Synthesis

Quality-checked RNA was used for the synthesis of cDNA following the manufacturer's instructions of the First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). For each reaction, 1 µg total RNA was incubated at 42°C for 1 h. The reaction was terminated by increasing the temperature to 70°C for 20 min, and the synthesized cDNA was stored at -20°C till further use.

3.4.5. Polymerase Chain Reaction Techniques

Different polymerase chain reaction (PCR) techniques were utilized to either amplify DNA fragments of desired bacterial, fungal, or oomycetal regions (Standard PCR) or to study gene expression using cDNA (qPCR). Nevertheless, all PCR experiments were based on the three

characteristic PCR steps which are initiated by denaturation, followed by annealing of the primers, and the elongation of the sequence of interest by a heat-stable polymerase. The melting temperature T_m was determined by the addition of 4°C for each G and C nucleotide and 2°C for each A and T nucleotide. Based on the T_m, the annealing temperature was chosen to be 4°C lower than the T_m. The standard PCR was mainly used for the identification of bacterial strains isolated from soil with 16S rRNA primer of the V4 – V7 region (799F – 1992R) (Table 1). Bacterial colonies were picked and directly used for the PCR reaction mixture. PCR products were afterwards visualized via gel electrophoresis.

Standard PCR Reaction Mixture

Template DNA	10 – 100 ng or colony
Forward primer 10 pmol/µL	5 µL
Reverse primer 10 pmol/µL	5 µL
10 x reaction buffer B	5 µL
50 mM $MgCl_2$	5 µL
DNA polymerase 5 U/µL	0.5 µL
10 mM dNTP – mixture	0.7 µL
ddH_2O	ad 50 µL

Standard PCR Program

Temperature	**Time [min]**	**Cycle step**	**Cycle Number**
95°C	5	Initial Denaturation	1
94°C	0:45	Denaturation	
T_m – 4°C	0:45	Annealing	30
72°C	1/kb (Taq)	Elongation	
72°C	5	Final extension	1
4 – 10°C	∞	Hold	-

3.4.6. Microbial Community Profiling by Amplicon Sequencing

Bacterial, fungal, and oomycetal sequences of the purified gDNA extracted from Cologne agriculture soil treatment with either BOA, gramine, quercetin, or germ-free water as control were amplified in a two-step PCR (Agler et al., 2016). In the first PCR, 4 ng of soil gDNA were used to amplify bacterial 16S rRNA V4 – V7 region (799F – 1992R), fungal ITS1 region

(ITS1F – ITS2), and oomycetal ITS1 region (ITS1-O – 5.8s-Rev-O) (Agler et al., 2016) (Table 1). Each amplification was prepared in triplicates in 25 µL reactions. Different concentrations of bovine serum albumin (BSA) and dimethyl sulfoxide (DMSO) were used to improve the PCR from the soil gDNA. G2 DNA polymerase from Promega (Germany) was chosen for the microbial community profiling. The annealing temperature for all primers was set to 55°C. After the first amplification, single-stranded DNA and proteins were digested using Antarctic Phosphatase and Exonuclease. Bacterial PCR products were further purified after agarose gel electrophoresis by cutting the expected band at 500 bps. The amplified fragments were purified from the gel using the Macherey-Nagel NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, Germany) according to the manufacturer's instructions and the DNA was eluted in 20 µL nuclease-free H_2O. The PCR product of the first amplification was now used in a second PCR with the barcoded primers (B5-F – B5-1 to B5-64; Ft – Ft-1 to Ft-64; Ot – Ot-1 to Ot-64) (Durán et al., 2018) and Illumina adapters (Supplementary Table 1). The reaction mixture was the same as described for the first PCR. In the second PCR, the cycles were reduced from 25 – 30 down to 15, and the products of the technical replicates were combined and purified by agarose gel electrophoresis and Agencourt MPure XP beads. Bacterial, fungal, and oomycetal libraries were prepared by combining 30 ng of each barcoded sample of the different microbial groups. After re-concentration and purification with Agencourt MPure XP beads 100 ng of each library were pooled and prepared for paired-end Illumina sequencing at the Max Planck Institute of Plant Breeding Research in Cologne (Germany).

PCR Reaction Mixture

Template gDNA	4 ng
Forward primer 10 pmol/µL	2 µL
Reverse primer 10 pmol/µL	2 µL
GoTaq reaction buffer	2 µL
25 mM MgCl2	2 µL
GoTaq G2 DNA polymerase 1.25 U	0.2 µL
10 mM dNTP – mixture	0.3 µL
Bovine serum albumin (BSA)	0.3 – 2%
Dimethyl sulfoxide (DMSO)	2 – 10%
Nuclease free H_2O	ad 25 µL

PCR Program

Temperature	Time [min]	Cycle step	Cycle Number
94°C	2	Initial denaturation	1
94°C	0:15	Denaturation	25 – 30
55°C	0:25	Annealing	
72°C	0:45	Elongation	
72°C	5	Final extension	1
4 – 10°C	∞	Hold	-

3.4.7. Quantitative Real-Time PCR

Relative gene expression levels of selected genes (Table 1) were studied using quantitative real-time PCR (qPCR) performed in a 7500 Fast Real-Time PCR cycler (Thermo Fisher Scientific, USA). Synthesized cDNA was diluted 1:10 prior to addition to the qPCR reaction mixture. Each biological replicate was analyzed in three technical replicates and after each qPCR run, the dissociation curve was analyzed to ensure specific amplification. For normalization, the housekeeping gene actin was utilized, and the data was evaluated according to the ΔΔCt method (Livak & Schmittgen, 2001). Gene expression of the primers listed in Table 1 was also normalized to control conditions.

qPCR Reaction Mixture

Template cDNA (1:10)	5 µL
Forward primer 10 pmol/µL	0.5 µL
Reverse primer 10 pmol/µL	0.5 µL
EvaGreen qPCR – Mix II (5x)	4 µL
Nuclease-free H_20	ad 20 µL

PCR Program

Temperature	Time [min]	Cycle step	Cycle Number
50°C	2	Polymerase activation	1
95°C	12	Initial denaturation	1
95°C	0:15	Denaturation	40
60°C	0:20	Annealing	
72°C	0:30	Elongation	
95°C	0:15	Dissociation curve	1
60°C	1		
95°C	0:15		
60°C	1		

3.4.8. Agarose Gel Electrophoresis

DNA and RNA samples were separated and visualized via agarose gel electrophoresis. 1.2% (w/v) agarose gels were developed in 1 x Tris-acetate-EDTA (TAE) buffer. DNA or RNA bands were visualized using the MidoriGreen dye (Nippon Genetics, Düren) and green LED light as described in the manufacturer's protocol. Prior to loading agarose gels, the samples were mixed with 6 x loading dye. A 1 kb DNA ladder (Thermo Fisher Scientific, USA) was used for the estimation of the fragment sizes.

6 x Gel – Loading Dye

Tris-HCl, pH 7.6	10 mM
Glycerol	60% (v/v)
EDTA, pH 8	60 mM
Bromophenol blue	0.03% (w/v)

5 x TAE Buffer

Tris base	2 M
Glacial acetic acid	1 M
EDTA, pH 8	50 mM

3.4.9. Paired-end Illumina Sequencing

Next-generation sequencing of 100 ng of each amplified bacterial, fungal, and oomycetal library of PCR products was carried out using 2 x 300 bp paired-end Illumina sequencing with a MiSeq sequencer (Illumina, Germany). Custom sequencing primers were utilized for this approach (Table 1, Durán et al., 2018). The raw sequencing data was pre-processed and demultiplexed by Pengfan Zhang (Max Planck Institute of Plant Breeding Research Cologne, Germany). Briefly, bioinformatics tools used here included Flash2 to merge, and filter reads that were shorter than 300 bp (Magoé and Salzberg, 2011). Amplicon sequence variant (ASV) files were created with QIIME2 (Version 2019.4) (Bolyen et al., 2019). Denoising and quality check, including removal of ambiguous bases, chimera, and singletons, were carried out with DADA2. Furthermore, chimeras were removed with Uchime once more. The Silva database 138, based on the naïve Bayesian algorithm in QIIME2, was used to classify ASVs taxonomically. This allowed the removal of mitochondria and chloroplast annotated sequences.

3.5. Methods in Biochemistry

3.5.1. Extraction of Secondary Metabolites from Soil

Cologne agriculture soil was incubated for 2 days with either 10 µM BOA, gramine, quercetin, or germ-free water as control under the conditions described above. Afterwards, the soil of the whole pot, 300 g soil, was used for extraction with 300 mL acidified methanol (0.1% formic acid). After mixing, the soil slurry was sonicated for 5 min, to increase the solubility of soil-bound substances. The mixture was centrifuged for 10 min at 4000 x g. Afterwards, the supernatant was collected and evaporated using a rotary evaporator. Once dry, the samples were resuspended in 500 µL methanol and analyzed via HPLC-DAD or stored at -20°C until further use.

3.5.2. Extraction of Metabolites from Bacterial Cultivation Media by Two Phase Partitioning

Samples (1 mL) taken after 0 h, 2 h, 4 h, 6 h, 24 h, 48 h, and 72 h bacterial growth in liquid minimal media TSM, were extracted via liquid-liquid extraction. Due to the poor solubility of BOA, gramine, and quercetin in water, ethyl acetate was chosen as the organic extraction solvent. 250 µL ethyl acetate were added to the 1 mL samples and vortexed for 30 sec. After centrifugation at 11 000 x for 5 min, the upper organic phase was collected in a fresh reaction

tube. The extraction was repeated a second time and the ethyl acetate extracts were combined. The samples were stored at -20°C until analysis of the aqueous as well as the organic phase via HPLC-DAD,

3.5.3. Extraction of Lipids from Soil

Treatment of Cologne agriculture soil was performed for 28 days with 10 µM BOA, gramine, quercetin, or germ-free water as the control in three biological replicates as described before. After the incubation, 5 g of soil were collected in a glass vial and 15 mL chloroform/methanol/formic acid (10:10:1, v/v/v) were added to initiate the extraction of lipids, modified from Siebers et al. (2018). Samples were vortexed for 30 sec and incubated under continuous shaking for 1 h at room temperature. After centrifugation at 300 x g for 20 min, the supernatant was collected in a fresh glass vial and evaporated using nitrogen. Meanwhile, 15 mL chloroform/methanol (2:1, v/v) were added again to the soil and the samples were incubated for 1 h at room temperature after 30 sec of vortexing. Once again, the samples were centrifuged at 300 x g for 20 min and the supernatant of the second extraction was combined with the first extraction and dried using nitrogen. To ensure a high yield, a third extraction of the soil samples was performed using 15 mL of chloroform/methanol (1:1, v/v) by repeating above described steps. The supernatant of the third extraction was combined with the dried samples of the first and second extraction and 7.5 mL 1 M KCl/ 0.2 M H_3PO_4 were added to wash the extracts. After the samples were vortexed, the last centrifugation at 300 x g for 15 min was performed and the lower organic phase was harvested in a fresh glass vial. The samples were dried again under a flow of nitrogen and the dried extracts were resuspended in 500 µL chloroform. Finally, the samples were stored at -20°C until further use. To determine the amount of lipids per gram of soil, the soil from the extractions was dried for 24 h at 110°C and the dry weight was determined.

3.5.4. Lipid Extraction from Bacteria

Prior to extracting lipids from bacteria, the cells from liquid cultures were harvested by centrifugation at 4000 x g for 15 min. The cell pellet was resuspended in 4 mL water and transferred to a fresh glass vial. Lipid degradation by lipases was prevented by immediate boiling of cells for 10 min in 95°C hot water. Once cooled, the samples were centrifuged at 400 x g for 20 min and the supernatant was discarded. The extraction of lipids, modified from Bligh and Dyer (1959), was initiated by the addition of 4 mL of chloroform/methanol (1:2, v/v)

to the cell pellet and after 30 sec vortexing, the samples were incubated under continuous shaking for 30 min at room temperature. Afterwards, the samples were centrifuged at 400 x g for 15 min and the supernatant was collected in a fresh glass vial. A second extraction of the cell pellet was performed using chloroform/methanol (2:1, v/v) following the above described steps. While cells of the second extraction were incubated for 30 min at room temperature, the extract of the first round was dried under a constant flow of nitrogen. Next, the supernatant of the second extraction was combined with the dried extract. Upon addition of 1 mL of a 0.9% NaCl solution, the samples were vortexed for 30 sec and centrifuged at 400 x g for 5 min. The lower organic phase was transferred in a fresh glass vial and dried using nitrogen. Finally, the samples were resuspended in 300 µL methanol and stored at -20°C till further use.

3.5.5. Separation of Phospholipids via Solid-Phase Extraction

Lipid extracts were further purified using silica columns. The solid-phase extraction (SPE) was performed to separate the phospholipids from other lipids in the total extracts. Prior to loading the extracts, the columns were equilibrated with 2 x column volumes of chloroform. Afterwards, the lipid extracts dissolved in chloroform were loaded on the columns and the columns were washed with 2 x column volumes of chloroform. Next, the glycolipids from the columns were eluted with 2 x column volume acetone/isopropanol (1:1, v/v) and in the end, the phospholipids were eluted with 2 x column volume methanol. The methanol phase was collected. Using a constant flow of nitrogen, the methanol fraction was concentrated until 500 µL methanol were left. The extracted phospholipids were stored at -20°C until further use.

3.5.6. Thin-Layer Chromatography

Total lipid extracts were separated, due to their different polarities, and visualized using thin-layer chromatography (TLC). First, lipid extracts were loaded on TLC plates and dried. Next, the TLC plates were transferred into sealed containers containing sufficient mobile phase. The mobile phase for separation of lipid extracts consisted of acetone/toluene/H_2O in a ratio of 91:30:8 (v/v/v). After the separation, the plates were dried at room temperature before they were stained using containers with iodine. The iodine vapor visualized the lipids as bands in a dark yellow to brown tone.

Indoles were separated via TLC using toluene/ethyl acetate/formic acid in a 4:5:1 (v/v/v) ratio as the mobile phase and visualization under UV-light (230 nm).

3.5.7. Fatty Acid Methyl Ester Synthesis

Analysis of fatty acids required their conversion into volatile methyl esters (FAMEs). This was achieved following the protocol of Browse et al. (1986). Up to 100 µL of lipid extracts were transferred with 100 µL of tridecanoic acid (13:0) (5 µg/mL), as an internal standard, in a glass vial, and 1 mL 1 N methanolic hydrochloric acid was added to the lipids. The tubes were closed, and the reaction was carried out at 80°C for 20 min. Once cooled, 1 mL 0.9% NaCl and 1 mL hexane were added, and the samples were vortexed for 10 sec. The phases were separated by centrifugation at 400 x g for 5 min and the hexane phase was transferred to a fresh glass vial. Finally, the hexane phase was evaporated under a stream of nitrogen and the FAMEs dissolved in 100 µL hexane. FAMEs were stored at -20°C if not directly measured via GC-MS.

3.5.8. High-Performance Liquid Chromatography

Screening for possible bacterial degradation products of BOA, gramine, and quercetin was performed using two High-Performance Liquid Chromatography (HPLC) instruments. The first HPLC device (LC-10AD VP), equipped with a diode array detector (DAD) (SCL-10A VP) was from Shimadzu. Its software LC-PDA (LabSolutions Lite) allowed the screening of results at all wavelengths from 200 nm to 800 nm. The second HPLC device (1100 Series) from Agilent Technologies was also equipped with a DAD (1100 Series) but its software (ChemStation for LC 3D Rev. B.01.01 [164]) required presetting of five defined detection wavelengths where signals could be expected. Therefore, the first device was used to screen for expected wavelengths of the metabolites and their degradation products and the second device, which was equipped with an autosampler (1100 Series, Agilent Technologies), was used to analyze the aqueous and organic phases of all replicates of the different treatments. Both devices were equipped with a Nucleodur 100-5 C18 ec column (Macherey-Nagel). Elution was performed as described in Schulz et al. (2018) with H_2O containing 0.1% formic acid as eluent A and methanol as eluent B. Analysis on both devices was performed with the following linear gradient: 1 min = 100% A, 2-30 min = 100% B

3.5.9. Gas Chromatography-Mass Spectrometry

Identification and quantification of FAMEs was carried out using a gas chromatography Agilent 7890 device equipped with mass spectrometer 5975 interxL MSD (Agilent) (GC-MS) (Browse and Somerville, 1986). Fatty acids were separated and identified according to their masses with the National Institute or Standard and Technology (NIST) mass spectrum library or by comparison to standards. FAMEs dissolved in hexane were injected following the below described parameters.

GC-MS Parameter	
Columns	Agilent HP-5MS
Length	30 m
Inner diameter	0.25 mm
Film thickness	0.25 µm
Carrier gas	Helium
Flow rate	2 mL/min
Injection volume	1 µL

3.5.10. Quadrupole Time-Of-Flight Mass Spectrometry

Bacterial lipids were identified using direct infusion Quadrupole time-of-flight mass spectrometry (Q-TOF MS/MS) in the positive ionization mode. Substances were analyzed according to Welti et al. with chloroform/methanol/300 mM ammonium acetate in a ratio of 300:665:35 (v/v/v) as the solvent (Welti et al., 2002). Samples were directly infused in an Agilent 1100/1200 system equipped with a HPLC-Chip Cube MS interface. The Chip Cube contained a HPLC-Chip with a nano-electrospray tip which resulted in a fine and charged spray of the samples. Fragmentation of the samples was achieved by adaptation of the collision-induced dissociation according to the analyte. Analysis was performed in positive ion mode of the Agilent 6530 Accurate-Mass Q-TOF LC-MS. Fragmentation of the different lipid classes was evaluated according to either neutral loss scanning or product ion scanning using the Agilent Mass Hunter Qualitative Analysis software (Version B.06.00).

Q-TOF Parameter	
Infusion chip	FIA Chip II flow injection and infusion
Flow rate	1 µL/min
Injection	5 µL
Drying gas	8 L/min nitrogen
Fragmentation voltage	50 – 200V
Gas temperature	300°C
HOLC-Chip V_{cap}	1700 V
Scan rate	1 spectrum/sec

3.5.11. Ultra-High Performance Liquid Chromatography

Identification and separation of chosen unknown samples was carried out with the help of Dr. Diana Hofmann and Dr. Björn Thiele at the Forschungszentrum Jülich (Germany). Upon extraction, samples were dissolved in methanol and shipped to the Forschungszentrum Jülich (Germany) where they were analyzed via ultra-high performance liquid chromatography (UHPLC) equipped with a mass detector for samples identification. Samples were separated with a Nucleodur 100-5 C18 ec column (Macherey-Nagel) with an elution as described in Schulz et al. (2018) with H_2O containing 0.1% formic acid as eluent A and methanol as eluent B. Analysis was performed with the following linear gradient: 1 min = 100% A, 2-30 min = 100% B.

3.5.12. Identification of Nitro-/Nitroso Acetamidophenols by Nuclear Magnetic Resonance and LC-HRMS

The analyses of unknown compounds were performed by Prof Dr. Laurent Bigler and Sergey Girel (University of Zurich, Switzerland). Briefly, after the separation by a Dionex™ UltiMate™ 3000 UHPLC system (Thermo Fisher Scientific, USA), the compounds of interest were identified with an Orbitrap® mass spectrometer (Thermo Fisher Scientific, USA). In order to obtain MS and MS^2 data, the spectrometer was equipped with a heated electrospray ionization (HESI II®) ion source and a higher energy dissociation collision cell with N_2 as collision gas. Using Pierce® LTQ Velos calibration solution according to the manufacturer's instructions the mass accuracy was calibrated below 2 ppm. Data was controlled by the Xcalibur software (v.4.2.28.14, Thermo Scientific, USA) and the ion source tune settings were set to default.

Identification of hitherto unknown compounds was completed by nuclear magnetic resonance (NMR) spectroscopy. Collected samples were shipped to Prof. Dr. Laurent Bigler and Sergey Girel (University of Zurich, Switzerland) and analyzed via 2D NMR with a Bruker AV-600 spectrometer equipped with BOSS-II shim system, AMOS control system, digital lock control unit, DQD unit, BVT3000 / BCU05 cooling unit and Cryo platform.

3.6. Statistical Methods

If not otherwise stated, all experiments were carried out with three to five biological replicates with three technical replicates of each biological replicate. Mean and standard deviation (SD) are based on Microsoft Office Excel and statistical significance was determined with Student's t-test. P-values < 0.05 were defined as significant (*), P-values < 0.01 as very significant (**) and P-values < 0.001 as highly significant (***).

Further calculations and plotting of the raw next-generation sequencing, data provided by Pengfan Zhang (Max-Planck-Institute Cologne, Germany) utilizing bioinformatical software packages, were carried out by Dr. Katharina Frindte (University of Bonn, Germany). For the calculation of the alpha diversity, the number of ASVs was restricted to 7100 reads in order to have an equal number of reads for the soil treatments with either BOA, gramine quercetin, or germ-free water as control. The software QIIME2 (version 2020.11) was used to calculate the different alpha-diversity indices Shannon, Faith PD, and Pilou's evenness. Differences in the treatments were analyzed non-parametrically with the Kruskal-Wallis test for the four treatments and the Friedman test for the four sampling time points (t7, t14, t21, and t28). Furthermore, Dunn tests (package FSA in R) were used to allow individual comparisons of the treatments with either BOA, gramine, quercetin, or germ-free water as control. The differences between the four timepoints were further analyzed utilizing pairwise Friedman tests in SPSS 25, and Bonferroni-Holm corrections were carried out for all multiple comparisons. According to Martino et al., the principal component (PCA) plot was calculated using DEICODE in QIIME2 based on clr normalized Aitchison distance matrices (Martino et al., 2019). Statistical analysis was performed with Permutational ANOVA (ADONIS) based on clr-normalized Aitchison distance matrix.

Individual ASVs that changed characteristically upon the treatments were identified using QIIME2 with ANCOM (Mandal et al., 2015). Most abundant taxa (> 1% relative abundance) were plotted in a heatmap created with R packages pheatmap and dplyr (Kolde, 2019; Wickham

et al., 2020). WPGMA clustering algorithm based on Euclidean distance matrix which resulted from ASV relative abundance was used to calculate Dendrograms. Additionally, for the comparison of medians from ANCOM, the data was analyzed with STAMP (Parks et al., 2014). Kruskal-Wallis test was calculated in STAMP and significant differences on ASV level of the treatments were tested with Tukey-Kramer Post-hoc corrected with the Bonferroni procedure.

4. Results

4.1. Amplification of Bacteria, Fungi, and Oomycetes from Soil gDNA

The quality of the gDNA, extracted from Cologne agriculture soil, was verified by agarose gel electrophoresis. Figure 11 shows a control gel of the gDNA extraction representative for all samples. Since no smear and no additional bands, except one band above 10,000 bp, representing gDNA, could be detected, the quality of the extracted DNA fulfilled the requirements for further amplification of bacterial, fungal, and oomycetal sequences (Figure 11).

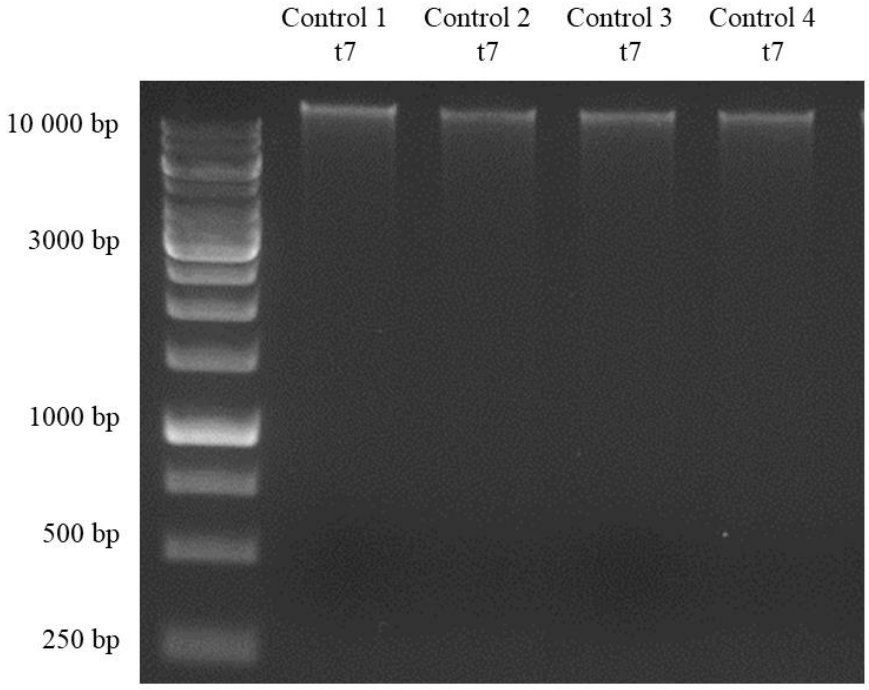


Figure 11: Quality control of gDNA extracted from soil.
1.2% agarose gel electrophoresis of gDNA extracted from untreated control soil after seven days of incubation. Bands above the 10,000 bp marker of the GeneRuler 1kb DNA ladder (Thermo Fisher Scientific) represent gDNA.

4 ng of purified gDNA were used for each amplification with primers for bacterial 16S V4-V7 region (799F – 1192R), fungal ITS 1 region (ITS1F – ITS2), or oomycetal ITS 1 region (ITS1-O – 5.8s-O-Rev) (Table 1) (Agler et al., 2016).

The expected size of the bacterial 16S rRNA gene (V4-V7 region) using 799F – 1192R primers was approximately 500 bps (Agler et al., 2016). Figure 12 shows the agarose gel with products of the first PCR with gDNA of the four control replicates harvested after seven days as templates. The patterns were identical for all samples (data not shown). Bands of 500 bps confirmed the successful amplification of the desired bacterial 16S rRNA gene (Figure 12). An additional, unspecific, band at 1000 bps was detected (Figure 12). Therefore, the band at 500 bps was cut for all samples and purified prior to the second PCR.

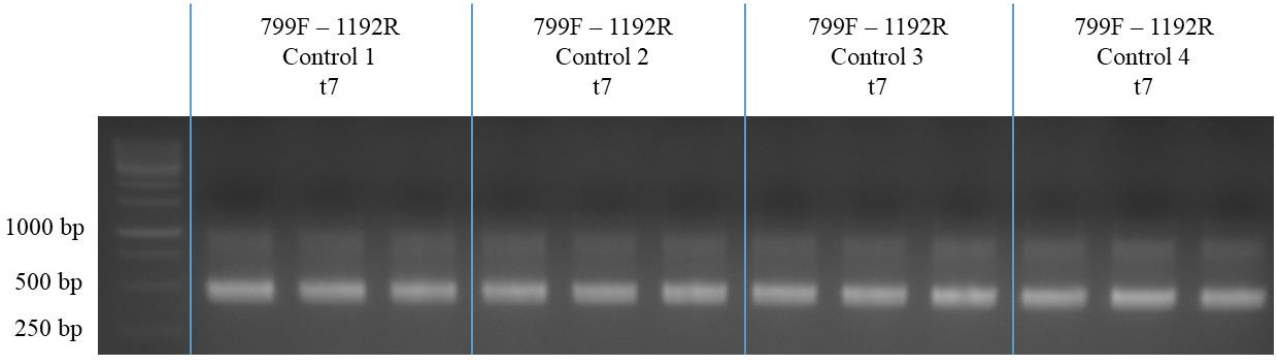


Figure 12: Amplification of the bacterial 16S rRNA V4-V7 region.
1.2% agarose gel electrophoresis of amplified bacterial 16S rRNA gene of soil control gDNA extracted after seven days of treatment. Expected bands, using 799F – 1192R primers, at ~500 bp of the GeneRuler 1kb DNA ladder (Thermo Fisher Scientific) represent the 16S rRNA gene.

In a second PCR of the purified product, barcoded primers B5-F and B5-x (x = each replicate with its individual barcode labeled from 1 to 64) were used to label the samples, and Illumina adaptors were added (Supplementary Table 1) (Durán et al, 2018). The expected bands at 500 bps of the four control replicates are shown in Figure 13, which are representative for all amplifications of the second PCR of the bacterial 16S rRNA gene. PCR products of the technical replications were combined for purification using magnetic beads (Agencourt AMPure XP), and the purified samples were stored at -20°C until the preparation of the library for next-generation sequencing.

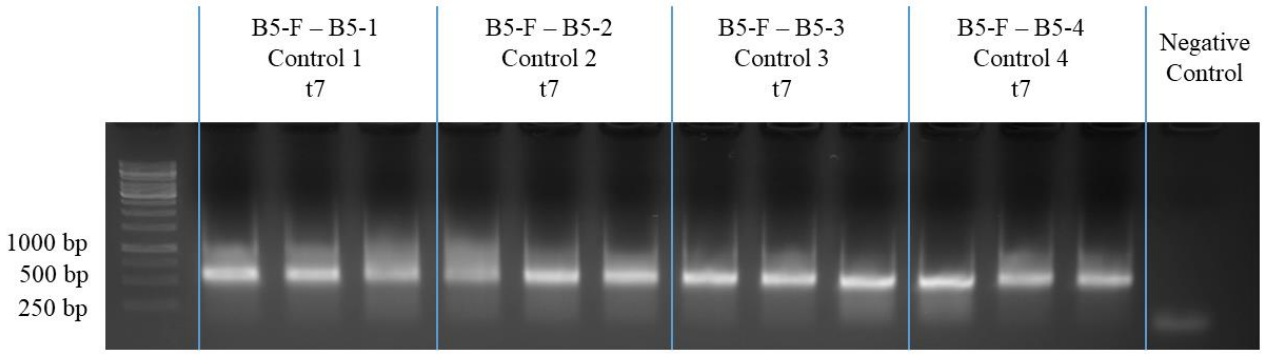


Figure 13: Addition of barcodes and Illumina adaptors to PCR products of bacterial 16S rRNA gene.
1.2% agarose gel electrophoresis of PCR products showing the addition of barcodes and Illumina adaptors to amplified bacterial 16S rRNA gene. Expected bands, using B5F – B5-x primer, at ~500 bp of the GeneRuler 1kb DNA ladder (Thermo Fisher Scientific) represent 16S rRNA gene. x: individual number from 1 – 64 for each sample.

Amplification of the fungal ITS region using ITS1F and ITS2 primes and the oomycetal ITS region using ITS1-O and 5.8s-O-Rev primers was expected to result in a product of approximately 300 bp (Agler et al., 2016) (Table 1). As shown in Figure 14 and Figure 15, for some approaches of the four controls that were harvested after seven days, no bands were detected. Several strategies were used to increase the efficiency of the PCR. Different amounts of DMSO, varying from 2 – 10%, were added to improve the annealing of primers. To decrease the contents of possible PCR inhibiting substances possibly present in gDNA from soil, the BSA concentrations were increased compared to the amplification of the bacterial regions. Furthermore, the annealing temperatures were adjusted, and cycle numbers were increased. None of these attempts did result in positive PCR results. Pure fungal DNA of *T. viride* was used as a positive control. A 300 bps band was always obtained with *T. viride* DNA (data not shown), but not with the gDNA samples from soil (Figure 14 and Figure 15), indicative for the absence or extremely low abundance of fungi and oomycetes in the soil samples. Therefore, the product of the first amplification of the fungal and the oomycetal region was purified using magnetic beads and the samples were used for a second PCR.

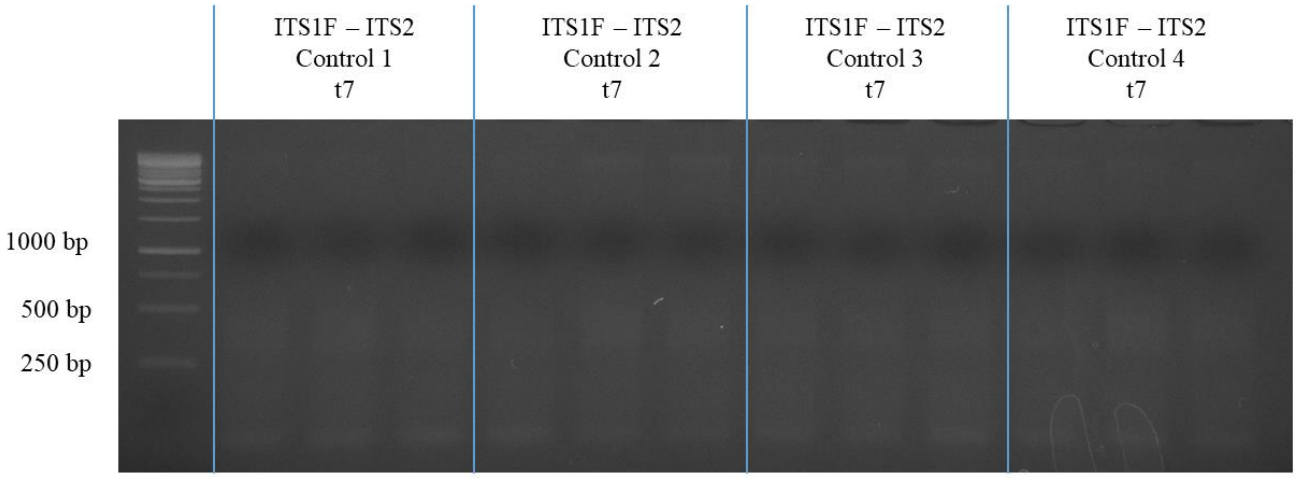


Figure 14: Amplification of fungal ITS1 region by PCR.
1.2% agarose gel electrophoresis of the amplified fungal ITS1 region of soil control gDNA extracted after seven days of treatment. Bands derived from PCR using ITS1F – ITS2 primers are expected at ~300 bp. See GeneRuler 1kb DNA ladder (Thermo Fisher Scientific). However, these bands were not visible indicating the lack of fungi in the soil samples.

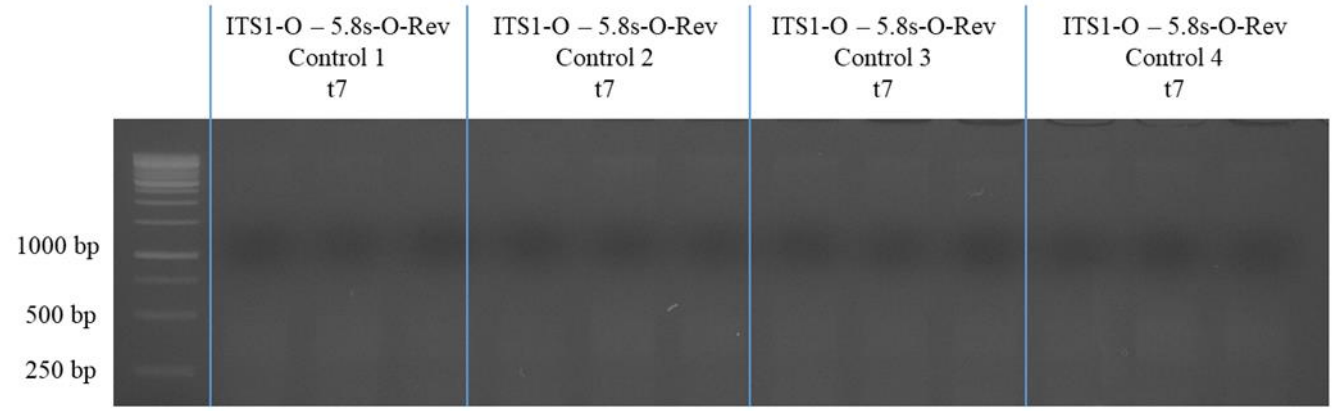


Figure 15: Amplification of oomycetal ITS1 region by PCR.
1.2% agarose gel electrophoresis of the amplified oomycetal ITS1 region of soil control gDNA extracted after seven days of treatment. Bands, using ITS1-O – 5.8s-O-Rev primer, are expected at ~300 bp of the GeneRuler 1kb DNA ladder (Thermo Fisher Scientific), but no bands for the oomycetal ITS1 region were detected.

Using the purified and concentrated PCR products, it was now expected to observe bands at 300 bps after the addition of the barcodes and Illumina adapters in the second PCR (Durán et al., 2018) (Supplementary Table 2 and Supplementary Table 3). As shown in Figure 16 and Figure 17 no bands could be observed in the samples and all replicates. The second PCR was performed on all PCR products resulting from the adaptation of different parameters, described above, but the pattern remained the same for all samples and again no bands could be observed

for fungal (Figure 16) and oomycetal regions (Figure 17), pointing to the absence of fungi and oomycetes in the bulk soil treated with BOA, gramine, quercetin or germ-free water.

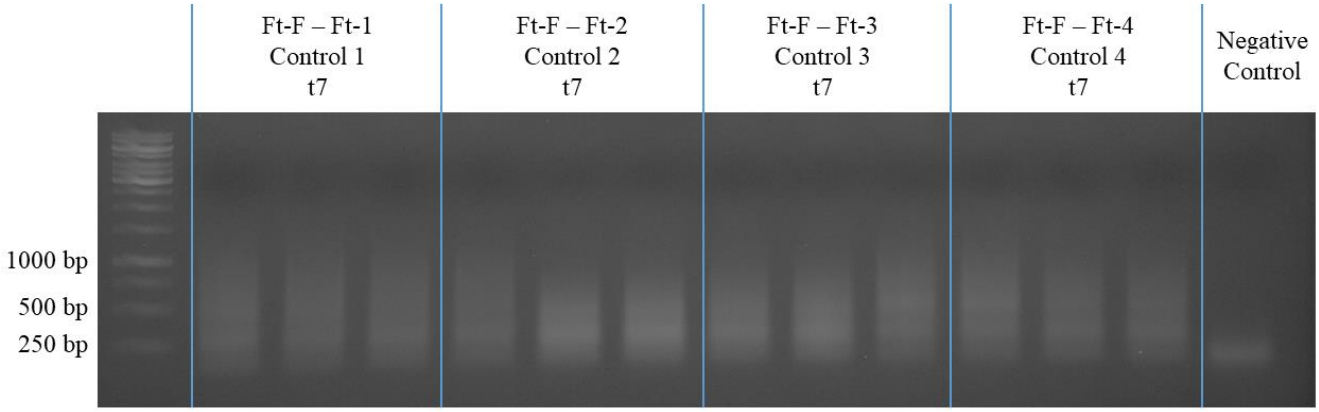


Figure 16: Addition of barcodes and Illumina adaptors to fungal ITS1 region by a second PCR.
1.2% agarose gel electrophoresis of PCR products showing the addition of barcodes and Illumina adaptors to amplified fungal ITS1 region. Bands, using Ft-F – Ft-4 primer, are expected at ~300 bp of the GeneRuler 1kb DNA ladder (Thermo Fisher Scientific). No bands are visible, indicating lack of fungi in soil.

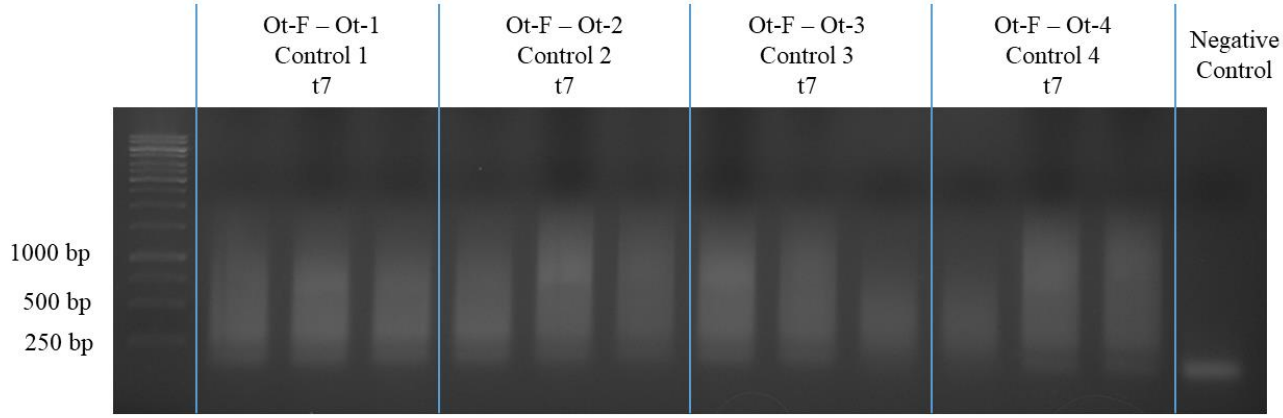


Figure 17: Addition of barcodes and Illumina adaptors to oomycetal ITS1 region by a second PCR.
1.2% agarose gel electrophoresis of addition of barcodes and Illumina adaptors to amplified oomycetal ITS1 region. Bands, using Ot-F – Ot-4 primer, are expected at ~300 bp of the GeneRuler 1kb DNA ladder (Thermo Fisher Scientific). No bands are visible, indicating lack of oomycetes in soil.

In a first approach, a library was prepared using bacterial PCR products. Furthermore, fungal and oomycetal PCR products from the second PCR were included even though no PCR bands were visible in the gel, to cover even low abundant organisms. Since next generation sequencing of the latter samples was not successful, fungal and oomycetal samples were not included in the further measurements. Therefore, next-generation sequencing was restricted to the bacterial library, which was successfully performed at the Max-Planck Institute of Plant Breeding Research (Cologne). The raw bioinformatics data was demultiplexed, filtered and quality checked by Pengfan Zhang (Max-Planck Institute of Plant Breeding Research Cologne, Germany). After the next-generation sequencing data was initially processed, it was used to analyze the impact of the three plant secondary metabolites BOA, gramine, and quercetin on the bacterial community in the soil.

4.2. Influence of BOA, Gramine, and Quercetin on the Soil Microbiome Diversity

During the treatment of the soil with BOA, gramine, quercetin, or germ-free water (control), samples were collected every 7 days over a period of 28 days for gDNA extraction. After purification, bacterial DNA was amplified two times using primers for the V4-V7 region of the 16S rRNA gene and used for next generation sequencing. Demultiplexed and filtered data were used to calculate the alpha diversity indices Shannon, Faith PD, and Pilou's Evenness (Figure 18) by Dr. Katharina Frindte (University of Bonn, Germany). The alpha indices visualize the biodiversity, equal distribution of species within the microbiome, and the phylogenetic diversity based on mean species diversity at local scale. In the left half of Figure 18, alpha indices of all timepoints of a treatment are presented, while the right side of Figure 18 shows the alpha indices for all treatments at the four timepoints. While alpha indices show a less prominent impact of the time on the changes in the microbiome, significant changes after BOA and quercetin treatment were observed (Figure 18). Alpha diversity indices showed partly significant changes at t21 and t28 compared with t14 (Figure 18). Data shown in Figure 18 suggest that changes in biodiversity and phylogenetic diversity were highly significant upon BOA and quercetin treatment, but not dependent on the duration of the exposure.

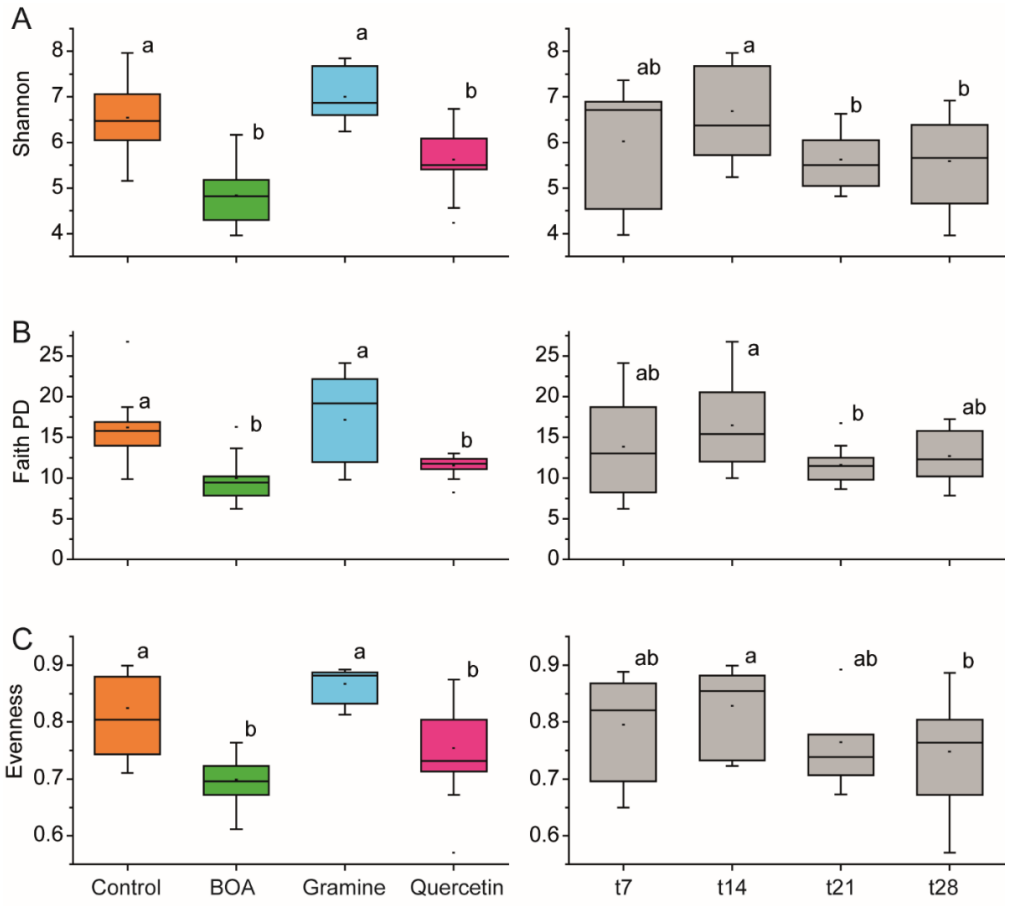


Figure 18: Alpha diversity indices of the bacterial community in soil.
Alpha index box plots after treatment with either BOA, gramine, quercetin, or germ-free water as control and throughout the incubation of 28 days (sampled after 7 days, 14 days, 21 days, and 28 days). A: Shannon index; B: Faith PD index; C: Evenness index. Left column: each treatment for all time points combined. Right column: each time point for all treatments combined. Letters represent significance based on pairwise Mann–Whitney (left column) and Friedman tests (right column). n = 4; each box contains 16 data points. Figure published in Schütz et al., 2021.

Further calculations by Dr. Katharina Frindte (University of Bonn, Germany) using principal component analysis (PCA) revealed the impacts of BOA, gramine, and quercetin on the bacterial community structure (Figure 19). The early timepoints t7 and t14 of the control overlapped with the late timepoints t21 and t28 of gramine treatment, while the late timepoints t21 and t28 of the control overlapped with BOA treatment. Nevertheless, BOA, gramine, and quercetin were always separated from each other in the PCA. Similar to BOA, quercetin treatment did not show any distribution depending on the time since all timepoints were grouped (Figure 19). Gramine treatment was the only approach where the duration of the exposure had a slight effect since the early timepoints t7 and t14 were separated from t21 and t24. ADONIS emphasized that the overall impact of the treatment of soil with BOA, gramine, and quercetin

was less influenced by the time with R^2-values for the treatment at 0.746 (P= 0.001) and R^2 for time at 0.110 (P = 0.001).

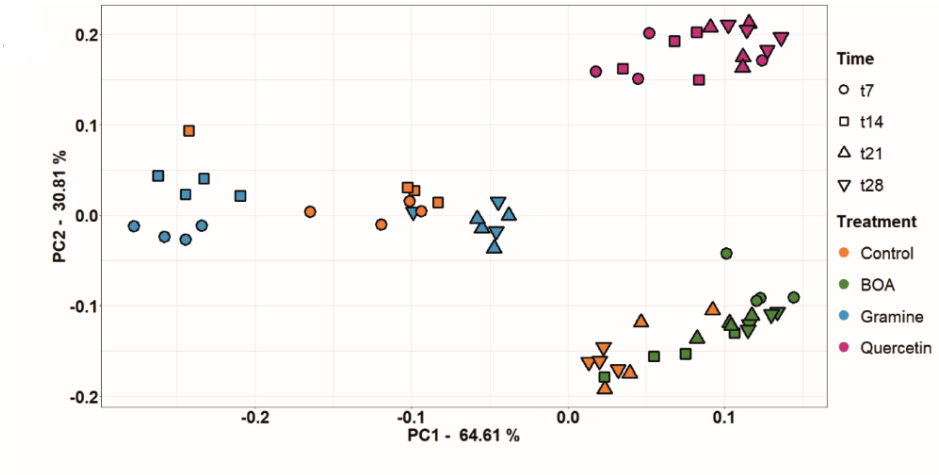


Figure 19: Principal component analysis (PCA) of soil bacteria community.
PCA plots of soil bacterial community after treatment with BOA, gramine, quercetin, or germ-free water. Treatments are indicated with different colors and timepoints with symbols in different shapes. n = 4. Figure published in Schütz et al., 2021.

4.3. Changes of the Microbiome on Phylum Level after BOA, Gramine, and Quercetin Treatment

Incubation of Cologne agriculture soil with the plant secondary metabolites resulted in taxonomic changes on the phylum level. Proteobacteria, Actinobacteriota, and Chloroflexi were the most abundant phyla in control, BOA, gramine, as well as quercetin (Figure 20). In the control soil time-dependent variations of Proteobacteria could be observed, where the phylum dominated especially after 21 days (Figure 20). Upon the last sampling after 28 days, the relative abundance of Proteobacteria was at 46.2%, Actinobacteriota at 31.9%, and Chloroflexi at 12.1%, whereas Acidobacteriota, Bdellovibrionota, Firmicutes, Gemmatimonadota, Nitrospirota, Patescibacteria, unclassified bacteria, and others all together were lower than 2% (Figure 20). Especially Acidobacteriota, Nitrospirota, and Gemmatimonadota were less abundant in the control at t28 compared to t7, while the relative abundance of Patescibacteria increased at t28. Time-dependent variations in the composition after treatment of soil with BOA were less pronounced than in the control. At t7, Proteobacteria was by far the most abundant phylum during BOA exposure. After 28 days, the relative abundance of Proteobacteria in BOA-treated soil was reduced, while Actinobacteriota was increased, and other phyla did not show drastic changes (Figure 20). Compared to the control, especially Proteobacteria and Chloroflexi were decreased by 6.3% and 9.4% at t28 of BOA remaining at a relative abundance of 39.9%

and 2.7%. Additionally, Acidobacteriota and Firmicutes were decreased to 0.2% and 0.6% at BOA treatment on t28. Actinobacteriota on the other hand were increased by 8% resulting in a relative abundance of 54.2% at t28 of BOA treatment. After 28 days, gramine treatment led to the increase of the relative abundance of Proteobacteria by 7.2% resulting in 53.5% compared to the t28 control (Figure 20). The phylum Bdellovibrionota was also increased at gramine t28, resulting in a relative abundance of 0.8%. Only Actinobacteriota showed a considerable reduction by 8.2% down to 23.7% after 28 days of soil exposure to gramine (Figure 20). Within the course of 28 days, soil treated with quercetin was dominated mainly by Proteobacteria (Figure 20). With reaching 76.0%, Proteobacteria was increased at quercetin t28 by striking 29.8%, compared to control t28 (Figure 20). Additionally, Bdellovibrionota was increased in relative abundance resulting in 1.8%, the highest percentage of all treatments. The phyla Actinobacteriota and Chloroflexi were both decreased to a relative abundance of ~15% and 2.2% (Figure 20). The data presented here was calculated and plotted by Dr. Katharina Frindte (University of Bonn, Germany).

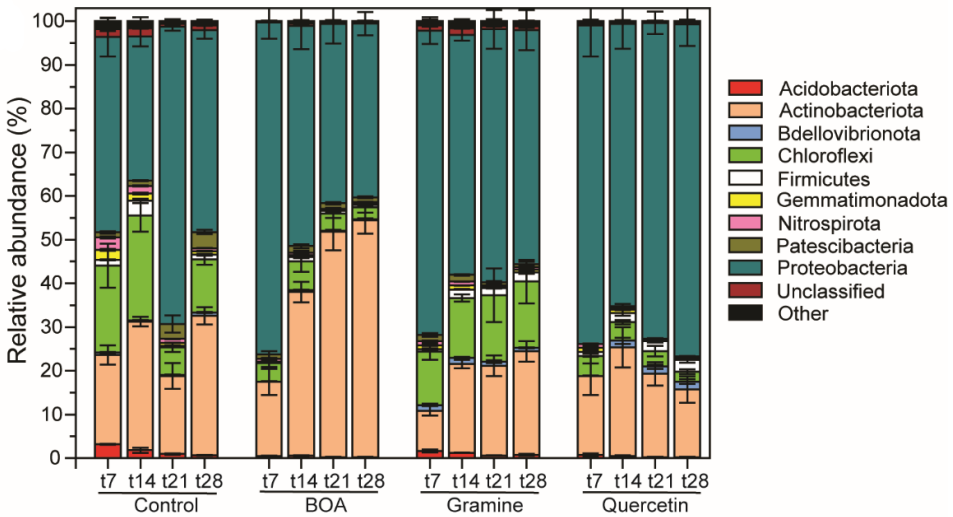


Figure 20: Changes in soil bacterial phyla based on different treatments.
Bars represent the relative abundances of soil bacteria phyla after treatment with BOA, gramine, quercetin or germ-free water as control. Bacterial phyla less abundant than 2% are grouped in "Other". n = 4, mean ± SD. Figure published in Schütz et al., 2021.

4.4. Impacts of BOA, Gramine, and Quercetin on ASV Level

To understand the full impact of the plant secondary metabolites BOA, gramine, and quercetin on the soil microbiome, the next generation sequencing data were further analyzed by Dr. Katharina Frindte (University of Bonn, Germany) on the amplicon sequencing variant (ASV) level. Since ASVs can be used to report changes down to a single nucleotide, they are a precise tool to understand the interaction of the three chosen metabolites with single soil bacterial species. Treatment of soil with BOA, gramine, and quercetin resulted in a significant difference in relative abundances for 96 ASVs (Supplementary Table 2). ASVs with a higher relative abundance than 1% were plotted in the heatmap in Figure 21. The relative abundance is visualized with a color code, where high relative abundance is depicted in blue and lower abundance in yellow/white (Figure 21). As already seen in the PCA plots in Figure 19, the early time points of the control t7 and t14 clustered with gramine treatment, while the late time points t21 and t28 of the control clustered with BOA treatment (Figure 21). The early time points of the control soil did not show any significant accumulations of certain ASVs, except the increase of *Massilia* ASV1 at t7 (Figure 21). *Massilia* ASV1 was also increased at t7 of gramine treatment, but also other time points of the gramine treatment showed a slight increase in *Massilia* ASV1 as well as *Massilia* ASV2 and *Massilia* ASV3 which explains the overlap with the early control time points (Figure 21). The increase of unclassified Oxalobacteraceae ASVs was consistent upon gramine treatment for t7, t14, t21, and t28 (Figure 21). Especially during the first seven days the relative abundance of unclassified Oxalobacteraceae ASV2, unclassified Oxalobacteraceae ASV4, and unclassified Oxalobacteraceae ASV5 were highly increased (Figure 21). During the following days, the relative abundance of those ASVs was decreased but still suggested an accumulation of those bacterial strains. The late control time points t21 and t28 shared a very high increase in the relative abundance of unclassified Micrococcaceae and *Pseudomonas* ASV3 and *Pseudomonas* ASV4 with all BOA time points (Figure 21). Additionally, t21 and t28 of BOA treatment showed an accumulation of *Methylophilaceae* ASV2, which was shared with a slightly lower intensity of t21 control (Figure 21). Quercetin treatment did not show any clustering with other approaches, as already shown with the PCA plots in Figure 19. A very high relative abundance for *Sphingobium* ASV2 was a characteristic feature for all time points of the quercetin treatment, but also *Sphingobium* ASV1 showed an increase (Figure 21). Besides, quercetin treatment also showed an increase of unclassified *Micrococcaceae*, *Pseudomonas* ASV2, and *Pseudomonas* ASV3 during all time points (Figure 21).

The heatmap pattern of all treatments highlights the importance of the analysis on the ASV level since the plant secondary metabolites seem to interact in a very specific way with single bacterial strains even within a genus.

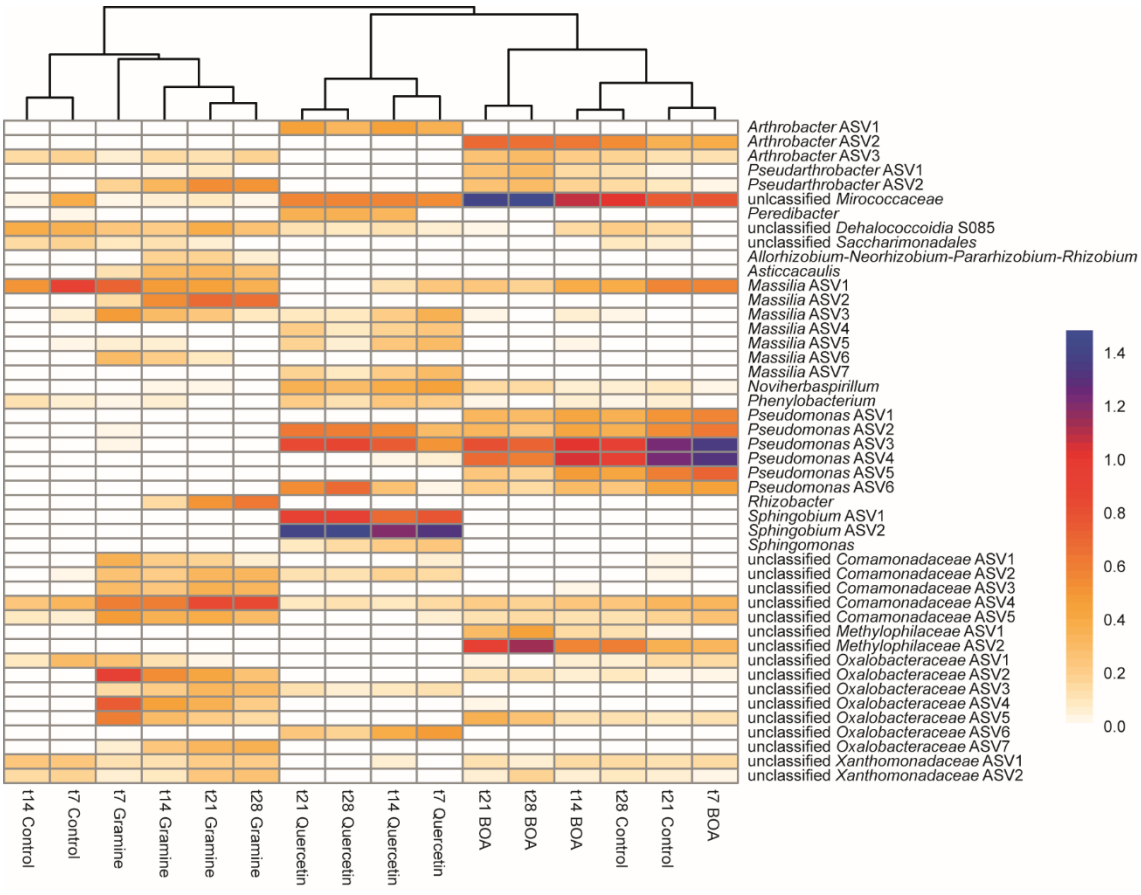


Figure 21: Heatmap of the relative abundances of bacterial ASVs after treatments with secondary metabolites.
Changes of relative abundances (log10-transformed) of bacterial ASVs upon treatment with either BOA, gramine, quercetin, or germ-free water as control. Changes calculated by ANCOM are based on a color scale: blue = high relative abundance; yellow = low relative abundance. ASVs with a relative abundance of 1% or higher are shown. Figure published in Schütz et al., 2021.

Significantly increased ASVs of the three treatments were plotted in a Venn diagram to demonstrate the specific response of bacterial strains to BOA, gramine, and quercetin (Figure 22). Upon BOA treatment, eight ASVs were increased exclusively, of which five belonged to Actinobacteriota and three to Proteobacteria (Figure 22). Additionally, the relative abundance of *Pseudarthrobacter* ASV2 was increased under BOA treatment, but also after exposure of soil to gramine. Another increase of an Actinobacteriota ASV under BOA was shared with quercetin (Figure 22). Gramine treatment increased the accumulation of a total of

34 Proteobacteria ASVs, of which three were also increased after quercetin treatment and two Bdellovibrionota ASVs (Figure 22). Quercetin treatment also resulted with 29 exclusive ASVs in a very high number of Proteobacteria that accumulated (Figure 22). Furthermore, quercetin treatment led to a significant increase of three exclusive Actinobacteriota ASVs and two exclusive Bdellovibrionota ASVs (Figure 22).

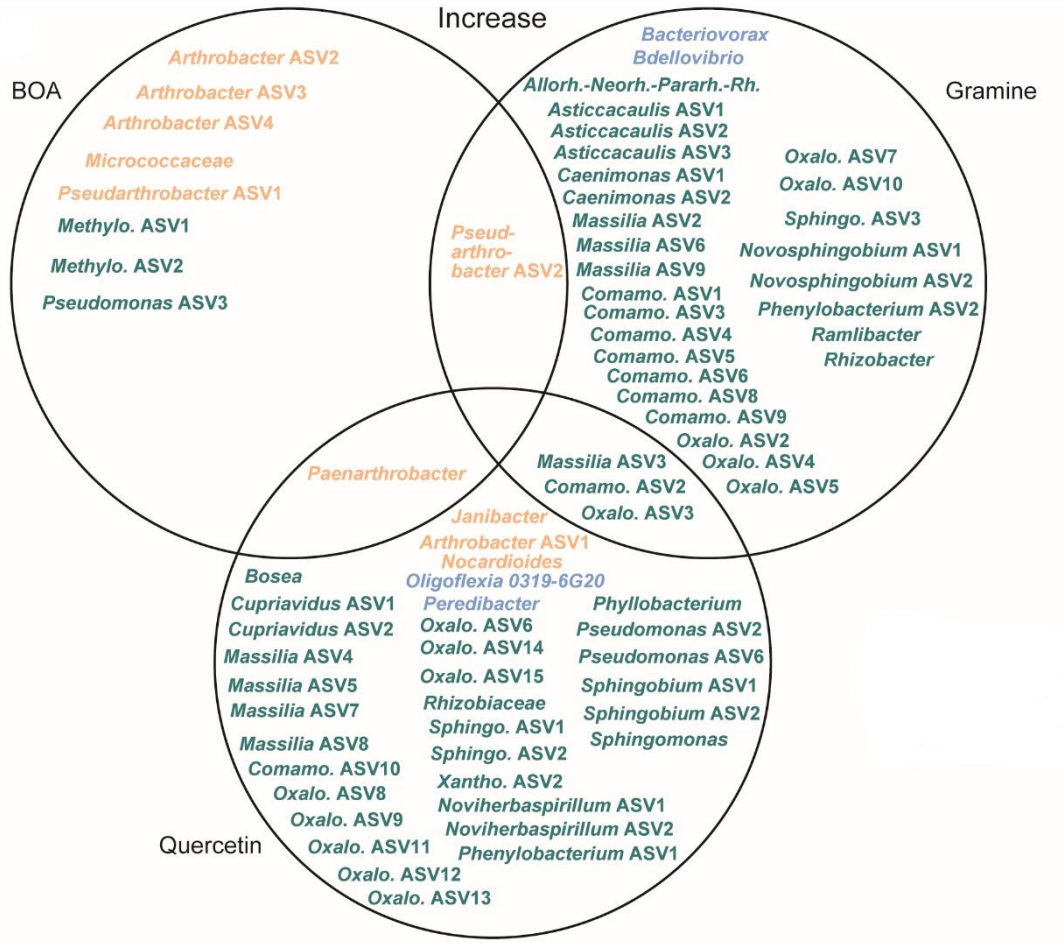


Figure 22: Venn diagram of bacterial ASVs that were increased after treatment with BOA, gramine or quercetin.
The Venn diagram represents the increase of the relative abundance of bacterial ASVs upon treatment with either BOA, gramine, or quercetin. Increase in relative abundance was determined by comparison of a treatment to the control treatment. Figure published in Schütz et al., 2021.

The overall amount of ASVs that were significantly decreased, of which some even dropped below the detection limit, by the plant secondary metabolites was much lower compared to the increased ASVs. Nevertheless, the pattern of the decreased ASVs was characteristic for each substance. After BOA treatment a total of 11 ASVs very decreased (Figure 23). The number of increased and decreased ASVs is with 10 and 11 very similar. Most of the organisms that

decreased after the exposure of soil to BOA also decreased after quercetin treatment. Quercetin and BOA shared a significant decrease of four Chloroflexi ASVs, one Gemmatimonadetes ASV, one Patescibacteria, and two Proteobacteria ASVs (Figure 23). The *Arenimonas* ASV, belonging to Proteobacteria, was the only organism that was decreased by all three substances (Figure 23). Gramine decreased only *Pseudomonas* ASV3 exclusively, while the reduction in the relative abundance of *Pseudomonas* ASV1, *Pseudomonas* ASV4, and *Pseudomonas* ASV5 was shared with quercetin (Figure 23). After treatment of soil with quercetin four Proteobacteria ASVs and one *Actinobacteriota* ASV were exclusively decreased (Figure 23). Especially after gramine and quercetin treatment, much fewer organisms were decreased than increased, indicating their function more as possible attraction substances and less as substances that deter bacteria.

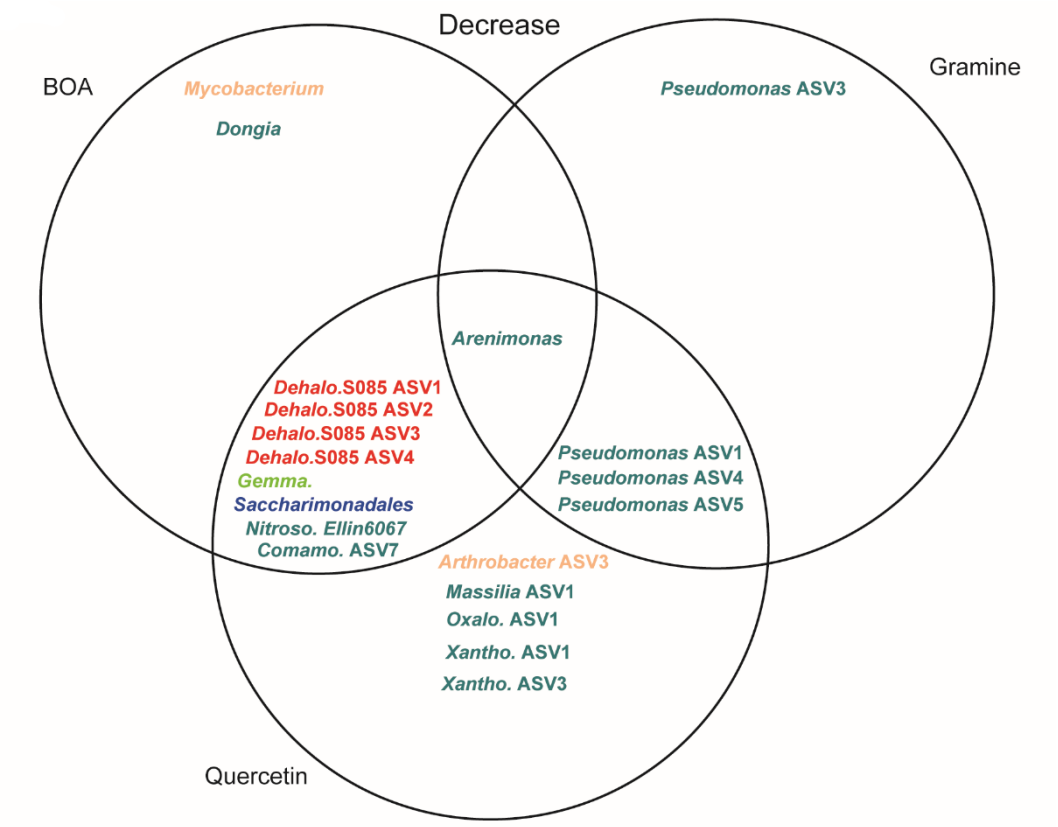


Figure 23: Venn diagram of bacterial ASVs that were decreased after treatment with BOA, gramine or quercetin.
The Venn diagram represents the decrease of relative abundance of bacterial ASVs upon treatment with either BOA, gramine or quercetin. Decrease in relative abundance was determined by comparison of a treatment to the control treatment. Figure published in Schütz et al., 2021.

Gradual changes in the relative abundance of single ASVs upon exposure to the secondary metabolites, as depicted in Figure 24, give further insights into the highly complex interactions within the soil microbiome. Overall, 96 of the significantly changed ASVs were of low abundance and only ten, of which eight were chosen and plotted in Figure 24, showed a higher relative abundance than 2% under one of the treatments or the control (Supplementary Table 2). Part A of Figure 24 shows common dynamics with slight variations in the relative abundance of certain ASVs, that were picked representative, to the four different approaches (including the control) of soil treatment. *Arthrobacter* ASV1 was not detected in the control, BOA, and gramine but showed a response to the quercetin treatment over time (Figure 24 A). The relative abundance increased slightly after 14 days and was at this level until 21 days but dropped to the same level as at t7 after 28 days of soil treatment with quercetin (Figure 24 A). A different pattern was observed for *Arthrobacter* ASV2 (Figure 24 A). While the relative abundance stayed at 0 for gramine and quercetin, the time course in the control and BOA showed a gradual increase of *Arthrobacter* ASV2 from t7 up to t28 (Figure 24 A). Nevertheless, the increase of *Arthrobacter* ASV2 under control conditions indicates that the increase during BOA treatment might not be dependent on the plant secondary metabolite. *Massilia* ASV3 started with a higher relative abundance for gramine and quercetin at t7 which dropped in the course of the 28 days treatment to a similar level as the control and BOA (Figure 24 A). *Pseudarthrobacter* ASV1 showed a similar pattern as *Arthrobacter* ASV2 (Figure 24 A). Upon BOA treatment the relative abundance of *Pseudarthrobacter* ASV1 increased continuously during the course of the treatment. There is also an increase at t21 gramine which dropped again to the t7 level at t28 (Figure 24 A). Under control conditions, *Pseudarthrobacter* ASV1 increased continuously in relative abundance from t14 reaching its peak at t28, which was still drastically lower compared to the increase of the BOA treatment (Figure 24 A).

While the pattern illustrated in Figure 24 A was representative for most ASVs with a low relative abundance, few other bacterial strains showed a relative abundance higher than 2% at one of the given conditions. After 7 days of BOA treatment, *Arthrobacter* ASV5 had a higher relative abundance than 20% (Figure 24 B). During the course of the treatment, the relative abundance of *Arthrobacter* ASV5 dropped at t28 to less than 5% (Figure 24 B). In the soil under control conditions, *Arthrobacter* ASV5 had a relative abundance of 0% until t14. Starting from t14 of the control conditions *Arthrobacter* ASV5 increased drastically peaking at its high at 15% after 21 days and then dropping again to the level of BOA and quercetin at t28 (Figure 24 B). *Pseudomonas* ASV4 showed the same pattern as *Arthrobacter* ASV5, except for not being detected during gramine and quercetin treatment (Figure 24 B). It starts with a very

high relative abundance at ~20% at t7 BOA and drops during the course of the treatment to almost below 5% at t28, while it started to increase at control conditions reaching its peak at ~15% and dropped again at t28 (Figure 24 B). As already depicted in the heatmap in Figure 21, quercetin treatment enhanced especially the abundance of *Sphingobium* ASVs. *Sphingobium* ASV2 for example was at t7 of quercetin treatment already over 20% (Figure 24 B). With a slight drop at t14, the relative abundance increased during the course of 28 days and reach its maximum at about 25% (Figure 24 B). The increase of *Sphingobium* ASV2 was one of the highest relative abundance that could be detected throughout all ASVs of all treatments and timepoints (Supplementary Table 2). Only the increase of unclassified *Micrococcaceae* was higher with a gradual increase up to 30% BOA treatment at t28 (Figure 24 B).

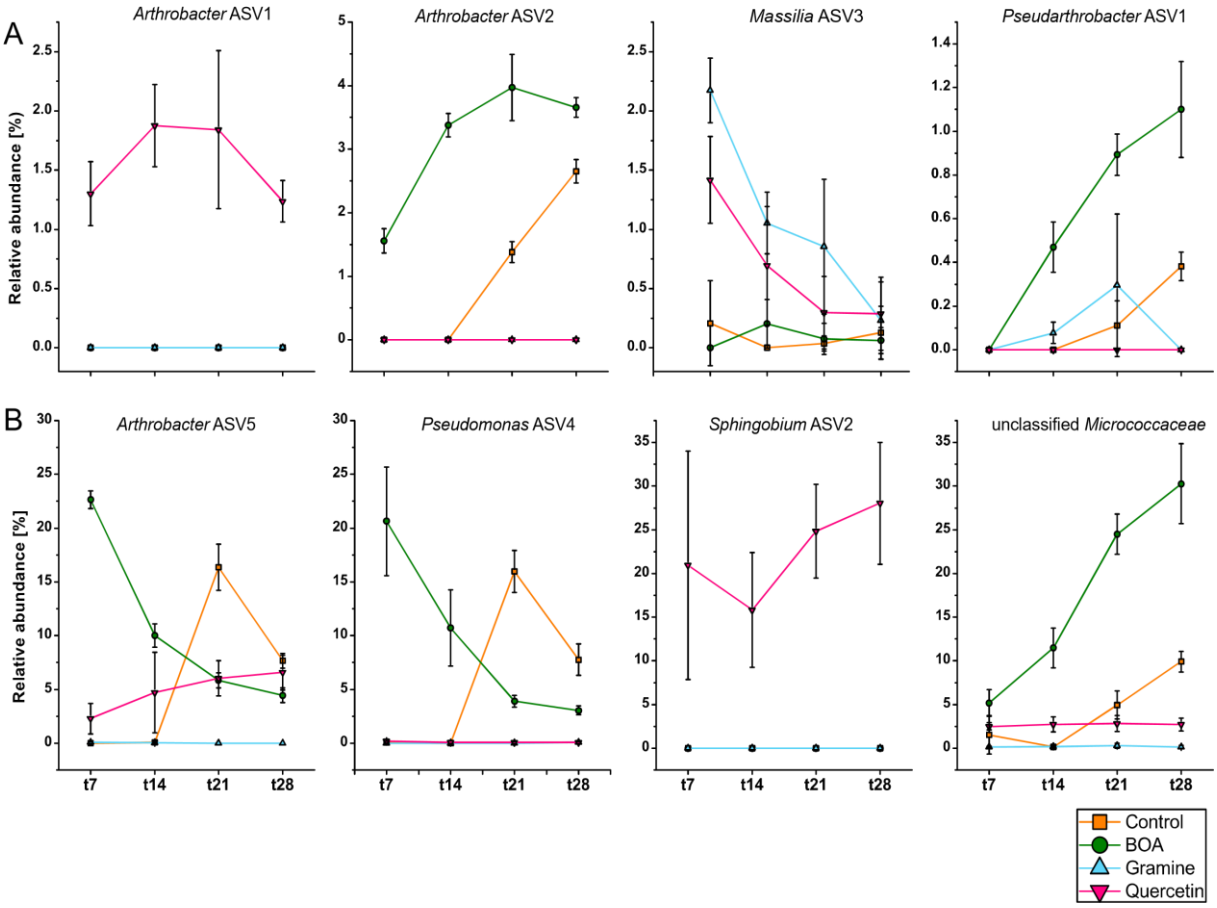


Figure 24: Changes in relative abundances of individual ASVs after treatment with BOA, gramine, or quercetin.
Representative ASVs were chosen to show changes in relative abundance based on amplicon sequencing of soil treated with either BOA, gramine, quercetin, or germ-free water as control. A: The selected ASVs demonstrate the typical trend of changes in relative abundance based on the different treatments. B: ASVs with a very high relative abundance were chosen to demonstrate their changes upon treatment with either of the three metabolites. Figure published in Schütz et al., 2021.

The bioinformatic evaluation of the impact of BOA, gramine, and quercetin at the ASV level highlights the importance of analysis of cultivable organisms regarding their behavior after exposure to one of the secondary metabolites and shed light on the power of plant secondary metabolites to modulate and shape soil microbiomes.

4.5. Identification of Cultivable Bacteria after Soil Treatment with Plant Secondary Metabolites

After treatment of soil for 28 days with either BOA, gramine, quercetin, or germ-free water as control, bacterial strains were isolated with 10 mM $MgCl_2$ and cultivated at 28°C on different media. Separated colonies were used for amplification of the 16S rRNA gene and the PCR products were purified from 1.2% agarose gels. After sequencing the sequences were blasted using the National Center for Biological Information (NCBI) databank. Table 3 contains all 108 bacterial strains that were isolated after the treatment with their annotation, genus, and the media used for their cultivation. The 16S rRNA sequences of the strains are presented in the Supplementary Information.

Table 3: Bacterial strains isolated and identified from treated soil.
Bacterial strains cultivated from soil treated with BOA, gramine, quercetin or germ-free water as control. Isolates and their identified genus and cultivation media are listed.

ISOLATE	GENUS	AGAR USED FOR ISOLATION
BACTERIA ISOLATED FROM CONTROL SOIL (CB)		
CB1	*Pseudomonas*	Malt
CB2	*Pseudomonas*	TSB
CB3	*Pseudomonas*	TSM
CB4	*Arthrobacter*	Czapek
CB5	*Pseudomonas*	PGA
CB6	*Pseudomonas*	PGA
CB7	*Pseudomonas*	PGA
CB8	*Pseudomonas*	PGA
CB9	*Pseudomonas*	TSM
CB10	*Arthrobacter*	Malt
CB11	*Pseudomonas*	TSM
CB12	*Pseudomonas*	TSM
CB14	*Arthrobacter*	TSM
CB15	*Arthrobacter*	TSM
CB16	*Arthrobacter*	Czapek
CB17	*Pseudomonas*	TSM
CB18	*Pseudomonas*	TSM
CB19	*Arthrobacter*	TSM
CB20	*Paenibacillus*	TSM
CB21	*Streptomyces*	TSB

CB22	*Arthrobacter*	TSB
CB23	*Bacillus*	TSM
CB24	*Streptomyces*	TSB
BACTERIA ISOLATED FROM BOA TREATED SOIL (BB)		
BB1	*Pseudomonas*	TSM
BB2	*Pseudarthrobacter*	TSM
BB3	*Paenarthrobacter*	TSM
BB4	*Pseudomonas*	YPD
BB5	*Sphingobium*	YPD
BB6	*Cupriavidus*	TSB
BB7	*Pseudomonas*	TSB
BB9	*Paenarthrobacter*	TSM
BB10	*Pseudomonas*	TSM
BB11	*Paenarthrobacter*	TSM
BB12	*Paenarthrobacter*	TSM
BB13	*Pseudarthrobacter*	TSM
BB15	*Paenarthrobacter*	TSB
BB16	*Pseudomonas*	TSM
BB17	*Streptomyces*	TSM
BB18	*Pseudarthrobacter*	TSM
BB19	*Pseudarthrobacter*	TSM
BB20	*Pseudomonas*	TSM
BB21	*Limnohabitans*	TSM
BB22	*Pseudomonas*	TSM
BB23	*Paenarthrobacter*	Malt
BB24	*Pseudomonas*	Malt
BB25	*Pseudarthrobacter*	YPD
BB26	*Bacillus*	YPD
BB27	*Streptomyces*	YPD
BB28	*Streptomyces*	TSB
BB29	*Pseudomonas*	TSB
BB30	*Pseudarthrobacter*	TSB
BB31	*Paenarthrobacter*	YPD
BB32	*Rhizobium*	YPD
BB33	*Paenarthrobacter*	TSM
BB34	*Pseudarthrobacter*	TSM
BB35	*Paenarthrobacter*	TSM
BB36	*Rhizobium*	Malt
BB37	*Paenarthrobacter*	TSM
BB38	*Nocardioides*	TSM
BB39	*Streptomyces*	YPD
BB40	*Pseudarthrobacter*	TSM
BB41	*Paenarthrobacter*	TSM
BB42	*Massilia*	TSM
BB43	*Mycobacterium*	TSB
BB45	*Phyllobacterium*	TSB
BACTERIA ISOLATED FROM GRAMINE TREATED SOIL (GB)		
GB1	*Arthrobacter*	TSM
GB2	*Pseudomonas*	TSM
GB4	*Pseudomonas*	TSM
GB5	*Arthrobacter*	TSM
GB6	*Pseudomonas*	TSB
GB7	*Pseudarthrobacter*	TSB
GB8	*Pseudomonas*	Malt
GB9	*Arthrobacter*	PGA

GB10	*Pseudomonas*	PGA
GB11	*Pseudarthrobacter*	TSM
GB12	*Pseudomonas*	TSM
GB13	*Pseudomonas*	Czapek
GB14	*Paenarthrobacter*	TSM
GB15	*Pseudomonas*	TSM
GB16	*Streptomyces*	TSM
GB17	*Arthrobacter*	TSM
GB18	*Arthrobacter*	TSB
GB19	*Streptomyces*	Malt
GB20	*Arthrobacter*	PGA
GB21	*Arthrobacter*	TSM
BACTERIA ISOLATED FROM QUERCETIN TREATED SOIL (QB)		
QB1	*Arthrobacter*	TSM
QB2	*Pseudarthrobacter*	TSM
QB3	*Pseudarthrobacter*	TSB
QB4	*Pseudarthrobacter*	PGA
QB5	*Pseudarthrobacter*	TSM
QB6	*Pseudarthrobacter*	Malt
QB7	*Pseudarthrobacter*	Malt
QB8	*Novosphingobium*	Malt
QB10	*Nocardioides*	YPD
QB11	*Novosphingobium*	TSM
QB12	*Pseudarthrobacter*	TSM
QB13	*Pseudarthrobacter*	Czapek
QB14	*Pseudarthrobacter*	TSM
QB15	*Novosphingobium*	TSM
QB16	*Pseudomonas*	Czapek
QB17	*Arthrobacter*	PGA
QB18	*Pseudarthrobacter*	TSM
QB19	*Novosphingobium*	TSM
QB20	*Novosphingobium*	Malt
QB21	*Pseudomonas*	TSB
QB22	*Arthrobacter*	TSM
QB23	*Arthrobacter*	TSM

The isolated bacterial strains in Table 3 belonged to Actinobacteriota, Proteobacteria, or Firmicutes. These three phyla were also the most abundant after next generation sequencing of 16s rRNA sequences presented in Figure 3. To study possible overlaps and relationships of the isolated bacterial strains, a phylogenetic tree containing type strain sequences and the isolated bacterial strains presented in Table 3 was created (Figure 25). While isolates from control and gramine treatment showed overlaps and no distinct groups could be recognized for *Arthrobacter*, *Pseudomonas*, and *Streptomyces*, a treatment-dependent pattern was evident for groups of *Paenarthrobacter* and *Pseudarthrobacter* strains isolated after BOA treatment (Figure 25). *Pseudarthrobacter* strains, resulting from quercetin-treated soil, also clustered in a defined group (Figure 25). Additional BOA-dependent groups were formed for *Sphingobium* as well as for *Rhizobiales,* and a group of *Novosphingobium* aggregated for quercetin isolates (Figure 25). The phylogenetic tree in Figure 25 suggests especially for certain isolates after BOA or quercetin treatment a distinct relation, indicating a specific mode of action on the soil microbiome for the two plant secondary metabolites.

Furthermore, sequences of the isolated strains were aggregated by Dr. Katharina Frindte (University of Bonn, Germany) in a phylogenetic tree, containing ASV sequences from the next-generation sequencing approach (data not shown). Clustering of certain isolates with ASV sequences pointed towards a close relationship of distinct ASV sequences with certain isolate sequences, indicating that the isolated strains might be identical with some of the analyzed ASVs (data not shown).

Figure 25: Phylogenetic tree of isolated bacterial strains and type strain sequences.
Phylogenetic tree containing type strain sequences (black) and all 16S rRNA sequences of bacterial strains isolated after treatment with either BOA (green), gramine (blue), quercetin (pink), or germ-free water (orange) as control. Based on PhyML maximum-likelihood algorithm. Figure published in Schütz et al., 2021.

4.6. Phospholipid Fatty Acid Composition in Soil after Exposure to Plant Secondary Metabolites

Analysis of phospholipid fatty acids (PLFA) is a fast and reliable approach to determine the total amount of microorganisms in the soil, as well as to obtain an overview of the composition of the microorganisms. After 28 days of treatment with the secondary metabolites BOA, gramine, and quercetin, lipids were extracted from the soil, and phospholipids were purified using SPE. After purification, the phospholipids were converted into FAMEs and measured by GC-MS. As depicted in Figure 26, the total amount of PLFAs was ~0.15 µg/g soil after all treatments as well as in the untreated soil (Figure 26). According to this result, the total amount of microorganisms was on the same level after all treatments and no treatment showed a significant increase or decrease in the total number of microorganisms.

The lack of fatty acids like oleic acid 18:1Δ9 and diunsaturated 18:2Δ9,12 indicates the absence of fungal species in all treatments including the control. Additionally, triunsaturated fatty acids 16:3 Δ7, 10, 13 and 18:3 Δ9, 12, 15 could not be detected and therefore it can be concluded that the soil samples did also not contain cyanobacteria or algae. Nevertheless, the composition of PLFAs was changed during the different treatments (Figure 26). Incubation of soil with BOA resulted in an increase of 16:1Δ9, 17:0cyclo, and 18:1 Δ11, while the most abundant fatty acids 16:0 and 18:0 were drastically decreased. Gramine treatment on the other hand resulted in a slightly different PLFA composition pattern than BOA. Additionally to the increase of 16:1Δ9 and 17:0cyclo, 15:0anteiso was increased. Different from the BOA treatment, no decrease in the amount of the abundant 16:0 was detected, but the decrease in the unsaturated 18:0 fatty acid was still significant, even more so, compared to the other treatments (Figure 26). The treatment with quercetin showed a similar pattern as gramine, where no decrease in the abundant fatty acid 16:0, which dominates the overall composition with up to 40%, was detected, but the saturated 18:0 fatty acid was also decreased (Figure 26). Similar to BOA, an increase in 16:1Δ9, 17:0cyclo, and 18:1 Δ11 was detected. The intensity of increase and decrease of different fatty acids suggests possible changes in the microbial composition after the different treatments. The significant increase of 17:0cyclo after exposure of the soil for 28 days to BOA, gramine, and quercetin is worth to be highlighted since the accumulation of cyclopropane fatty acids in bacterial strains is usually related to stress (Figure 26).

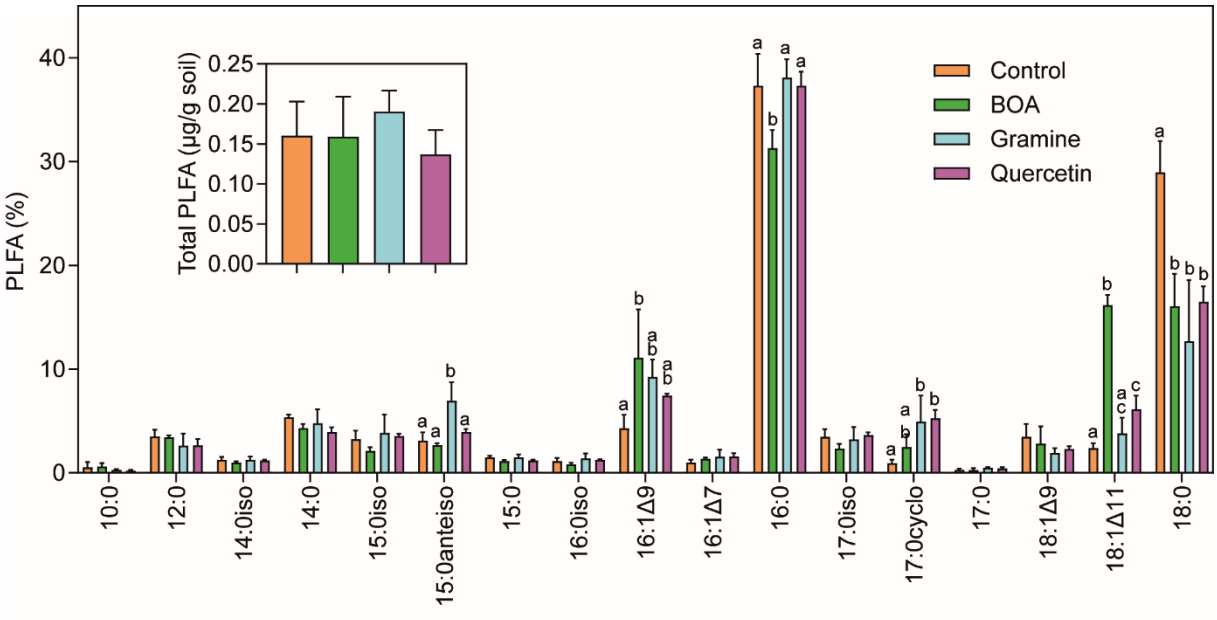


Figure 26: Phospholipid fatty acid (PLFA) composition of soil treatment with plant secondary metabolites.
Phospholipids were extracted from soil treated for 28 days with either BOA, gramine, quercetin or germ-free water as control and their fatty acids were analyzed via GC-MS. The total PLFA content was calculated using tridecanoic acid as an internal standard and the PLFA composition of the different treatments was based on mol%. n = 3, mean ± SD. Figure published in Schütz et al., 2021.

4.7. Impact of BOA, Gramine and Quercetin on Soil Bacteria Growth

Selected bacterial strains, as described in Table 3, were used to analyze the impact of plant secondary metabolites at concentrations of 500 µM and 1 mM on bacterial growth. The four bacterial strains *Arthrobacter sp.* GB1, *Arthrobacter sp.* CB4, *Pseudomonas sp.* CB3 and *Pseudomonas sp.* MPI9 were selected as the focus of the following studies, including the impact of plant secondary metabolites on bacterial growth, bacterial lipids, and to study bacterial degradation of BOA, gramine, or quercetin. While GB1, CB4, and CB3 were bacterial strains as described in Table 3. *Pseudomonas sp.* MPI9 was a strain provided by the Max-Planck Institute of Plant Breeding Research (Cologne), used to determine the influence of the plant secondary metabolites on organisms that are not related to the treatments with BOA, gramine, or quercetin, but resulted from the same soil from previous experiments.

Growth of *Arthrobacter sp*. GB1 was not impaired by any of the three secondary metabolites at 500 µM (Figure 27 and Table 4), nor at 1 mM (data not shown). OD_{600} values revealed that GB1 cells were less abundant at the beginning of incubation with quercetin, resulting from a longer lag phase in the first 2 h compared to control, BOA, or gramine (Figure 27). Nevertheless, the cell density was in a similar range for all metabolites and the control after

24 h, with only gramine treatment showing a decrease with 1.58 ± 0.03 compared to control (1.94 ± 0.23), BOA (1.84 ± 0.07) and quercetin (1.99 ± 0.20) (Figure 27). While OD_{600} values started to drop for control and BOA in the course of 24 h to 48 h, initiating the decline/death phase of bacterial growth, the values for gramine and quercetin incubation increased slightly compared to 24 h, resulting in 2.15 ± 0.04 and 2.15 ± 0.15 (Figure 27). After 48 h, the decline/death phase also started for GB1 exposed to 500 µM gramine and quercetin (Figure 27). The delayed decline/death phase of *Arthrobacter sp.* GB1 during incubation with gramine and quercetin indicates metabolic adaptions necessary for the use of gramine and quercetin as additional carbon sources (Figure 27).

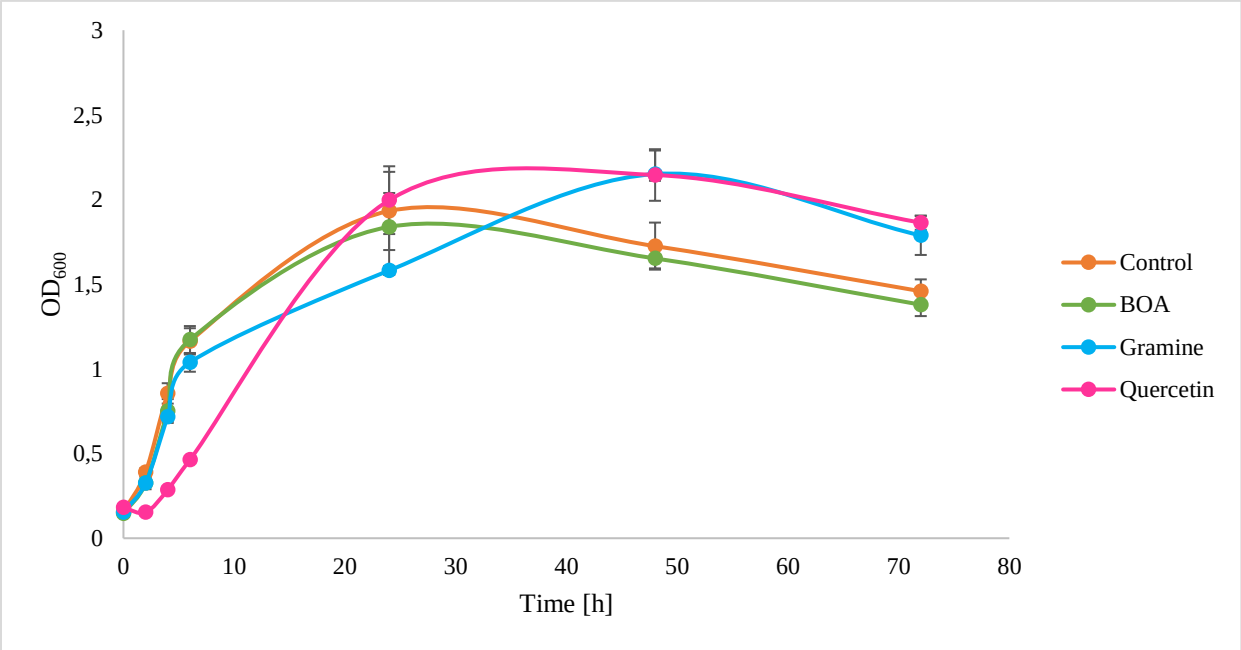


Figure 27: Growth of *Arthrobacter sp.* GB1 in TSM with additional 500 µM BOA, gramine, or quercetin.
Growth of *Arthrobacter sp.* GB1 was determined over a period of 72 h in liquid minimal medium TSM with additional 500 µM BOA, gramine, quercetin or DMSO as control. In the first six hours of growth, OD_{600} was determined every two hours, and afterwards, the cultures were sampled every 24 hours. n = 3, mean ± SD.

Arthrobacter sp. GB1 showed no growth inhibition when exposed to one of the metabolites: The growth rates µ were 0.27 ± 0.019 for the control, 0.32 ± 0.015 for BOA, 0.29 ± 0.007 for gramine, and 0.28 ± 0.005 for quercetin (Table 4). The generation time G was calculated for the evaluation of impacts on bacterial growth. The generation time G is also known as the time necessary for the doubling of the bacterial population. As indicated by a higher growth rate, the generation time was reduced during incubation with BOA to 56.62 ± 2.73 min, highlighting a

possible positive effect of the metabolite on the growth of GB1 (Table 4). Only minor changes in generation time were observed comparing control (67.01 ± 4.48 min), gramine (62.79 ± 1.40 min), and quercetin (65.65 ± 1.25 min), revealing that the impact of gramine and quercetin on the growth of GB1 is negligible (Table 4).

Table 4: Influence of plant secondary metabolites on the growth rate μ and generation rate G of *Arthrobacter* sp. GB1.
Growth rate μ and generation rate G are calculated based on OD_{600} measurements of *Arthrobacter sp.* GB1 in liquid media containing 500 μM of either BOA, gramine, quercetin, or DMSO as control. n = 3, mean ± SD.

Treatment	Growth rate μ [1/h]	Generation rate G [min]
Control	0.27 ± 0.019	67.01 ± 4.48
BOA	0.32 ± 0.015	56.62 ± 2.73
Gramine	0.29 ± 0.007	62.79 ± 1.40
Quercetin	0.28 ± 0.005	65.65 ± 1.25

The *Arthrobacter sp.* CB4 showed a slightly different pattern than *Arthrobacter sp.* GB1 upon exposure to the plant secondary metabolites BOA, gramine, and quercetin. Increasing the concentration from 500 μM to 1 mM did not change the growth of *Arthrobacter sp.* CB4 during exposure with one of the three secondary metabolites, and the growth pattern stayed similar for both approaches (data not shown).

While no differences in cell density could be measured during the first 6 h in the minimal media TSM for the control, 500 μM BOA and 500 μM gramine, OD_{600} values for cells exposed to 500 μM quercetin were continually lower compared to the other treatments (Figure 28). After 6 h growth, the OD_{600} of the control, BOA, and gramine treatments were 1.17 ± 0.001, 1.17 ± 0.02, and 1.16 ± 0.02, and were thus at the same level, whereas the OD_{600} during quercetin treatment was 0.49 ± 0.03 and thus drastically lower (Figure 28). This pattern changed after 24 h of growth. Cells exposed to BOA and gramine showed an OD_{600} of 1.87 ± 0.08 and 1.82 ± 0.13, respectively, which was lower compared to the control, which showed an OD_{600} of 2.09 ± 0.06 (Figure 28). The addition of quercetin resulted in a prominent increase of the OD_{600} to 2.27 ± 0.20, compared to the other metabolites (Figure 28). Similar to *Arthrobacter sp.* GB1, increased OD_{600} values upon addition of 500 μM quercetin into the liquid cultures indicate the capability of *Arthrobacter sp.* CB4 to use quercetin as an additional carbon source. Nevertheless, the decline/death phase was not extended and was initiated for all treatments at

the same time, but the overall cell density stayed after 72 h with 1.92 ± 0.09 the highest for quercetin treatment compared to control (1.46 ± 0.175), BOA (1.29 ± 0.04) or gramine (1.13 ± 0.11) (Figure 28).

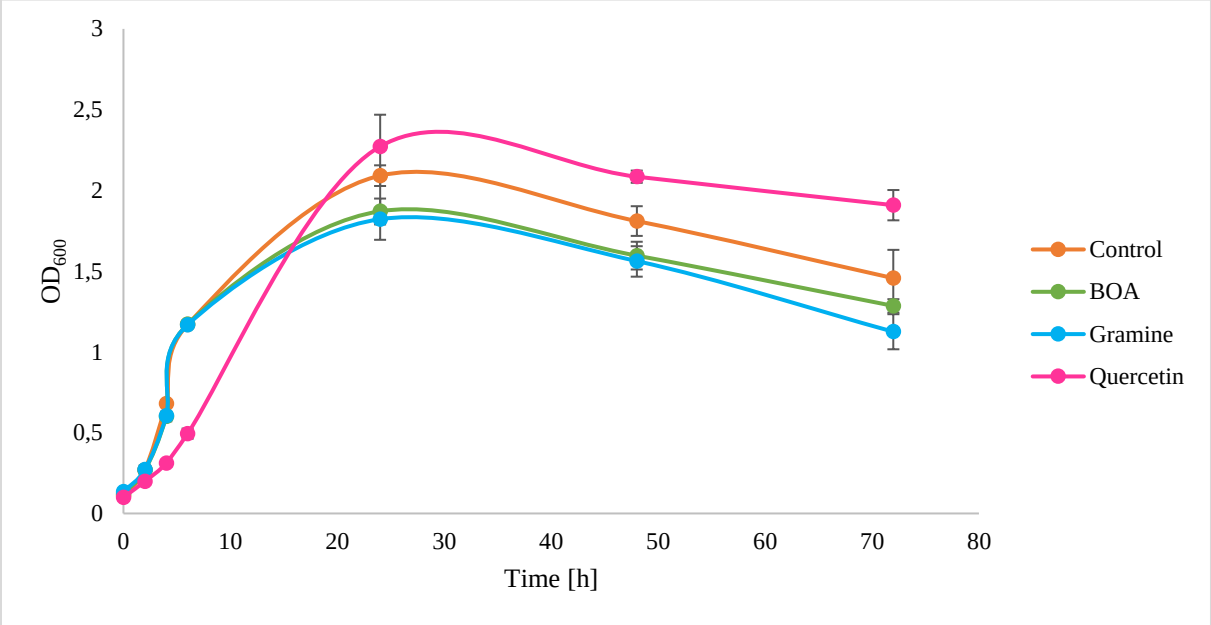


Figure 28: Growth of *Arthrobacter sp.* CB4 in TSM with additional 500 µM BOA, gramine or quercetin.
Growth of *Arthrobacter sp*. CB4 was determined over a period of 72 h in liquid minimal medium (TSM) with additional 500 µM BOA, gramine, quercetin or DMSO as control. In the first six hours of growth, OD_{600} was determined every two hours, and afterwards the cultures were sampled every 24 hours. n = 3, mean ± SD.

Translating OD_{600} values of Figure 28 into growth rate µ revealed that the growth of *Arthrobacter sp.* CB4 was not affected by BOA or gramine with 0.37 ± 0.01 and 0.36 ± 0.01, respectively, compared to 0.37 ± 0.027 of the control experiments (Table 5). With 0.22 ± 0.01, the growth rate was drastically reduced upon exposure of cells to 500 µM quercetin (Table 5). The striking effect of quercetin on bacterial growth can be better visualized by use of the generation rate G. While *Arthrobacter sp.* CB4 cells needed 49.74 ± 3.50 min, 49.14 ± 1.07 min, and 49.92 ± 1.18 min for duplication during growth under control, BOA, or gramine conditions, respectively, the cells needed 79.66 ± 3.35 min upon exposure to quercetin (Table 5). Compared to *Arthrobacter sp.* GB1, CB4 cells did not show an adaptation to quercetin by an increased lag phase, which resulted in a drastic reduction in bacterial growth (Table 4 and Table 5).

Table 5: Influence of plant secondary metabolites on the growth rate µ and generation rate G of *Arthrobacter sp.* CB4.
Growth rate µ and generation rate G are calculated based on OD_{600} measurements of *Arthrobacter sp.* CB4 in liquid media containing 500 µM of either BOA, gramine, quercetin or DMSO as control. n = 3, mean ± SD.

Treatment	Growth rate µ [1/h]	Generation rate G [min]
Control	0.37 ± 0.027	49.74 ± 3.50
BOA	0.37 ± 0.008	49.14 ± 1.07
Gramine	0.36 ± 0.009	49.92 ± 1.18
Quercetin	0.22 ± 0.009	79.66 ± 3.35

Additionally, to the already described *Arthrobacter* strains, the growth of two *Pseudomonas* strains was analyzed upon exposure to BOA, gramine, and quercetin.

OD_{600} of *Pseudomonas sp.* CB3 was not influenced by BOA, gramine, and quercetin at 500 µM, compared to the control, during the incubation period of 72 h (Figure 29). Increasing the concentration of the plant secondary metabolites to 1 mM did not change the growth pattern of the strain CB3 (data not shown). The strain CB3 was able to grow under all tested conditions without any drastic changes in cell density. After 24 h, the OD_{600} value was 1.01 ± 0.08 upon exposure to gramine which was slightly lower compared to control (1.22 ± 0.02), BOA (1.21 ± 0.05), and quercetin treatment (1.27 ± 0.02) (Figure 29).

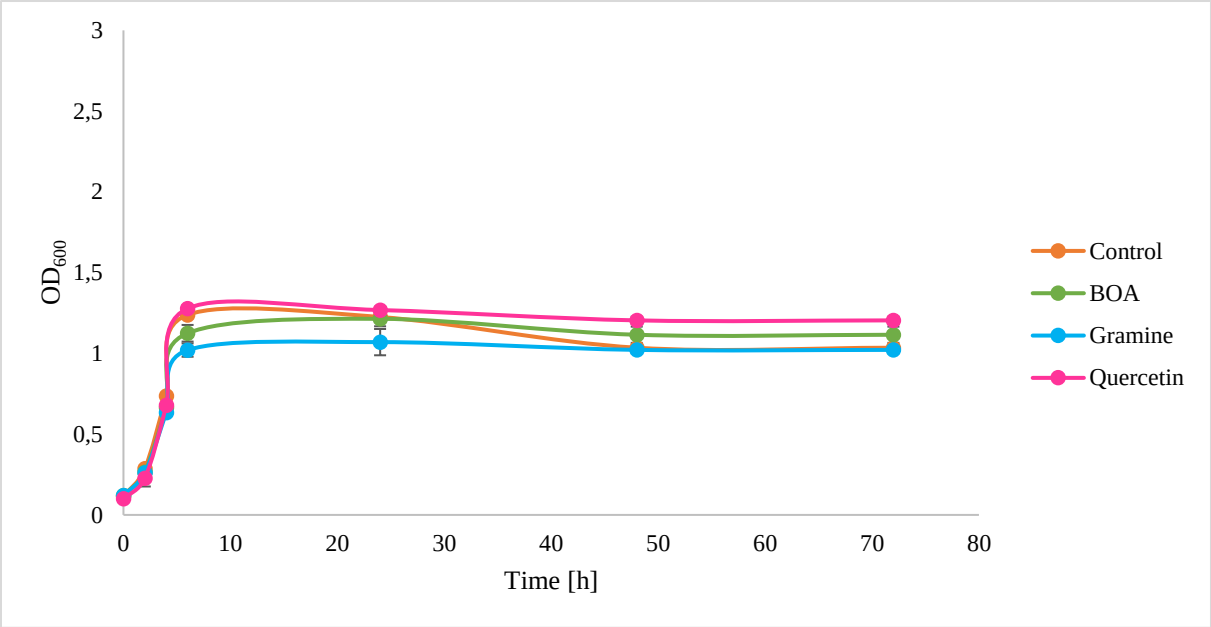


Figure 29: Growth of *Pseudomonas sp.* CB3 in TSM with additional 500 µM BOA, gramine or quercetin.
Growth of *Pseudomonas sp.* CB3 was determined over a period of 72 h in liquid minimal medium TSM with additional 500 µM BOA, gramine, quercetin or DMSO as control. In the first six hours of growth OD_{600} was determined every two hours and afterwards the cultures were sampled every 24 hours. n = 3, mean ± SD.

As already indicated by the less prominent changes in OD_{600} values during the growth of *Pseudomonas sp.* CB3 with BOA, gramine, and quercetin, the growth rate µ was also not impacted. With a growth rate µ of 0.37 ± 0.009 under control conditions, 0.36 ± 0.003 upon exposure to BOA, 0.34 ± 0.007 upon exposure to gramine, and 0.43 ± 0.060 upon exposure to quercetin, the cell growth was not changed (Table 6). Calculations of the generation rate G revealed a duplication time of *Pseudomonas sp.* CB3 for the control of 49.71 ± 1.32 min, growth with BOA of 50.23 ± 0.51 min, growth with gramine of 53.30 ± 1.13, and growth with quercetin of 42.17 ± 5.30 min (Table 6). Only the exposure of the cells to quercetin indicates a slight decrease in duplication time, indicating a possible minor positive effect.

Table 6: Influence of plant secondary metabolites on the growth rate µ and generation rate G of *Pseudomonas sp.* CB3.
Growth rate µ and generation rate G are calculated based on OD_{600} measurements of *Pseudomonas sp*. CB3 in liquid media containing 500 µM of either BOA, gramine, quercetin or DMSO as control. n = 3, mean ± SD.

Treatment	Growth rate µ [1/h]	Generation rate G [min]
Control	0.37 ± 0.009	49.71 ± 1.32
BOA	0.36 ± 0.003	50.23 ± 0.51
Gramine	0.34 ± 0.007	53.30 ± 1.13
Quercetin	0.43 ± 0.060	42.17 ± 5.30

The last presented bacterial strain, *Pseudomonas sp.* MPI9 showed different responses to the plant secondary metabolites compared to *Pseudomonas sp*. CB3 (Figure 30 and Figure 29) but increasing the concentration of the plant secondary metabolites to 1 mM did not change the growth pattern (data not shown).

While within the first 6 h, OD_{600} values were comparable (control: 0.71 ± 0.05, BOA: 0.80 ± 0.01, gramine: 0.78 ± 0.03), the OD_{600} was drastically lowered to 0.29 ± 0.01 in cultures containing 500 µM quercetin (Figure 30). Nevertheless, within 24 h, the OD_{600} was after quercetin treatment at 1.11 ± 0.08 which was similar to control levels (1.07 ± 0.04) (Figure 30). Cells exposed to BOA or gramine, on the other hand, showed an OD_{600} of 1.36 ± 0.07 and 1.32 ± 0.06, respectively, which was slightly increased compared with control (Figure 30).

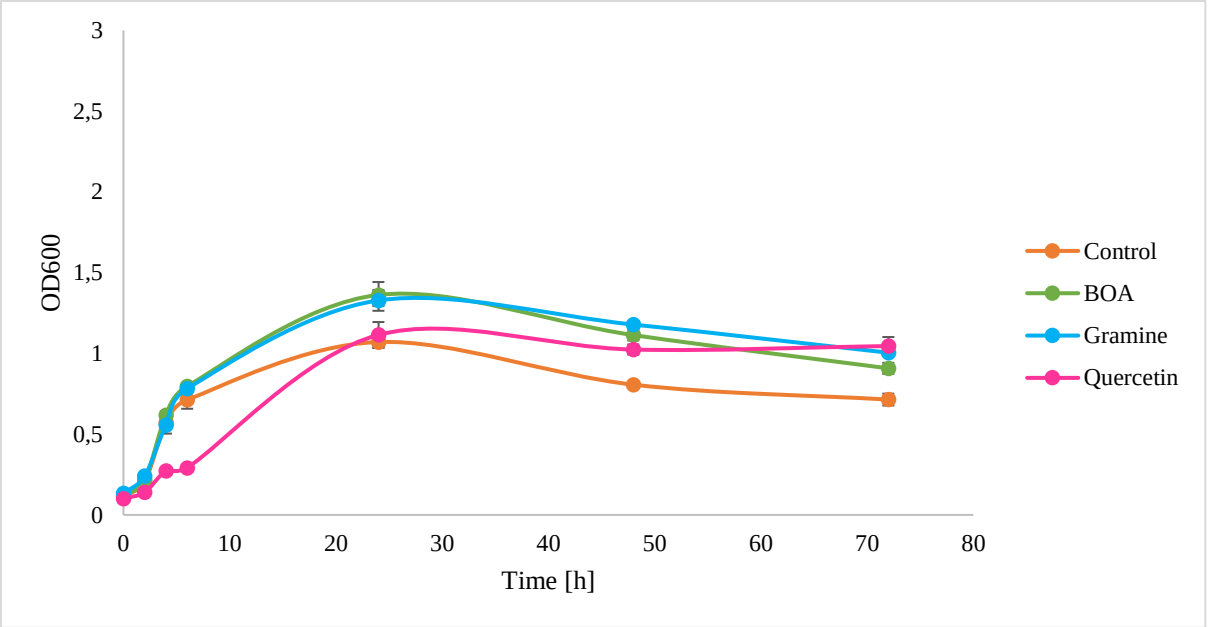


Figure 30: Growth of *Pseudomonas sp*. MPI9 in TSM with additional 500 µM BOA, gramine or quercetin.
Growth of *Pseudomonas sp*. MPI9 was determined over a period of 72 h in liquid minimal medium TSM with additional 500 µM BOA, gramine, quercetin or DMSO as control. In the first six hours of growth OD_{600} was determined every two hours and afterwards the cultures were sampled every 24 hours. n = 3, mean ± SD.

With values at 0.31 ± 0.037, 0.32 ± 0.011, and 0.29 ± 0.022 for the control, BOA, and gramine, respectively, the growth rate µ of *Pseudomonas sp.* MPI9 was in the same range (Table 7). As already indicated in Figure 30, the growth rate of MPI9 cells during exposure to 500 µM quercetin was drastically decreased to 0.18 ± 0.004 (Table 7). While *Pseudomonas* cells were duplicated during 59.40 ± 7.03 min, 56.24 ± 2.02 min, and 61.92 ± 4.79 min under control, BOA or gramine conditions, respectively, cells grown in the presence of quercetin needed 99.74 ± 2.62 min for a duplication (Table 7). These values highlight the need for an adaption of *Pseudomonas sp.* MPI9 to quercetin at the early time points of growth.

Table 7: Influence of plant secondary metabolites on the growth rate µ and generation rate G of *Pseudomonas sp*. MPI9.
The growth rate µ and generation rate G are calculated based on OD_{600} measurements of *Pseudomonas sp*. MPI9 growth in liquid media containing 500 µM of either BOA, gramine, quercetin or DMSO as control. n = 3, mean ± SD.

Treatment	Growth rate µ [1/h]	Generation rate G [min]
Control	0.31 ± 0.037	59.40 ± 7.03
BOA	0.32 ± 0.011	56.24 ± 2.02
Gramine	0.29 ± 0.022	61.92 ± 4.79
Quercetin	0.18 ± 0.004	99.74 ± 2.62

4.8. Degradation of Plant Secondary Metabolites by Soil Bacteria

To understand the direct impact of plant secondary metabolites on microorganisms, previously isolated bacterial strains were cultured in the presence of the compounds. Degradation of the plant secondary metabolites BOA, gramine, and quercetin by soil bacteria was studied in three biological replicates for each organism during growth in the liquid minimal medium TSM containing 500 µM of one of the metabolites. Samples for HPLC analysis were taken at 0 h, 2 h, 4 h, 6 h, 24 h, 48 h, and 72 h. Partitioning with ethyl acetate led to organic and aqueous phases which were analyzed via HPLC-DAD. As concluded from the results of the next generation sequencing and growth experiments, bacterial strains differ specifically in their responses to BOA, gramine, or quercetin. While some are not affected by the metabolites, others might be able to metabolize them, partially or entirely.

4.8.1. Degradation of BOA

BOA is barely soluble in water and can therefore be found in the HPLC chromatograms of the organic phases. The pure BOA standard was eluted at ~20 min (data not shown) showing absorption maxima at 223 and 270 nm when analyzed by HPLC on a C18 column with gradient elution (Figure 31). While shifts in the retention time can occur, the UV spectrum of BOA is a fixed criterium for the identification of this substance. The characteristic UV spectrum of pure BOA is shown in Figure 31.

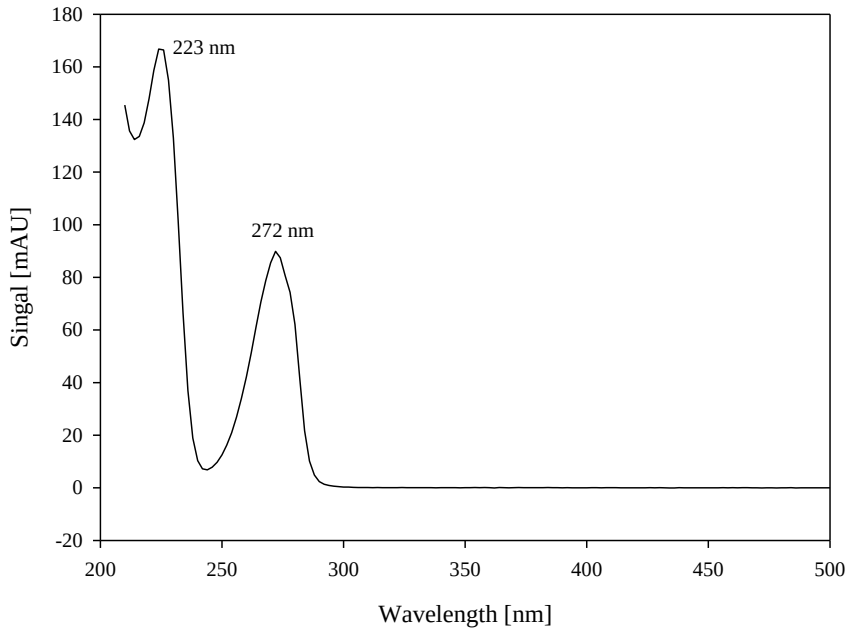


Figure 31: UV spectrum of BOA.
HPLC-DAD UV spectrum of the BOA reference with its characteristic absorption maxima at 223 nm and 272 nm.

Incubation of *Arthrobacter sp*. GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9 with 500 µM BOA revealed that none of the bacterial strains were able to degrade BOA, as shown in Figure 32 which is representative for all bacterial strains. Analysis of all replicates revealed that no degradation product was formed during the entire course of incubation, e.g., as shown for *Arthrobacter sp*. GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9 (Figure 32). BOA eluted in a high peak with a retention time of 20 min as expected (Figure 32). In the organic as well as the aqueous phases (data not shown), prepared from all four bacterial strains, no additional peaks could be detected that might be related to BOA and its possible degradation products (Figure 32). Additional peaks in the chromatograms of the aqueous phase were also present in the controls (Supplementary Figure 1 – 8).

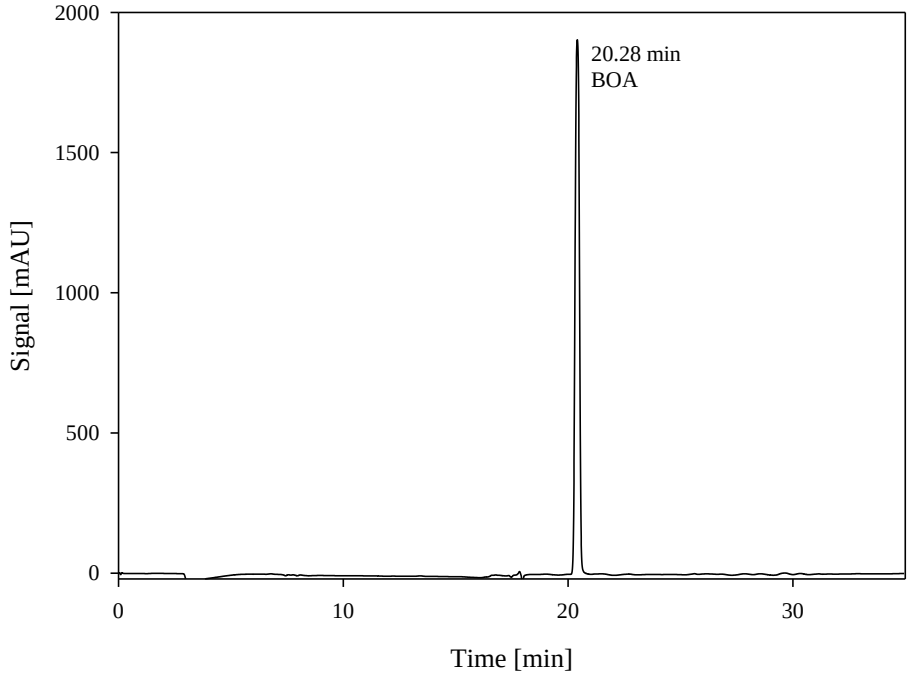


Figure 32: HPLC-DAD chromatogram of an organic extract from an *Arthrobacter sp.* GB1 culture treated with BOA.
HPLC-DAD analysis at 270 nm of an organic extract taken after 72 h of cultivation of *Arthrobacter sp.* GB1 in liquid media containing 500 µM BOA. The peak for BOA could be detected after 20.28 min at 270 nm.

4.8.2. Degradation of Gramine

Addition of 500 µM gramine to the bacterial cultures growing in the liquid medium TSM and analysis of the organic and aqueous phases of the extract revealed that *Arthrobacter sp.* GB1 was the only strain able to degrade gramine. Analysis of organic and aqueous extracts of *Arthrobacter sp.* CB4, *Pseudomonas sp.* CB3 and *Pseudomonas sp.* MPI9 resulted in no additional peaks throughout the 72 h incubation time and the only peak eluting at 270 nm was identified as gramine via its distinct UV spectrum. Gramin shows a characteristic UV spectrum with absorption maxima at 218 nm, 270 nm, and a shoulder at 284 nm (Figure 33).

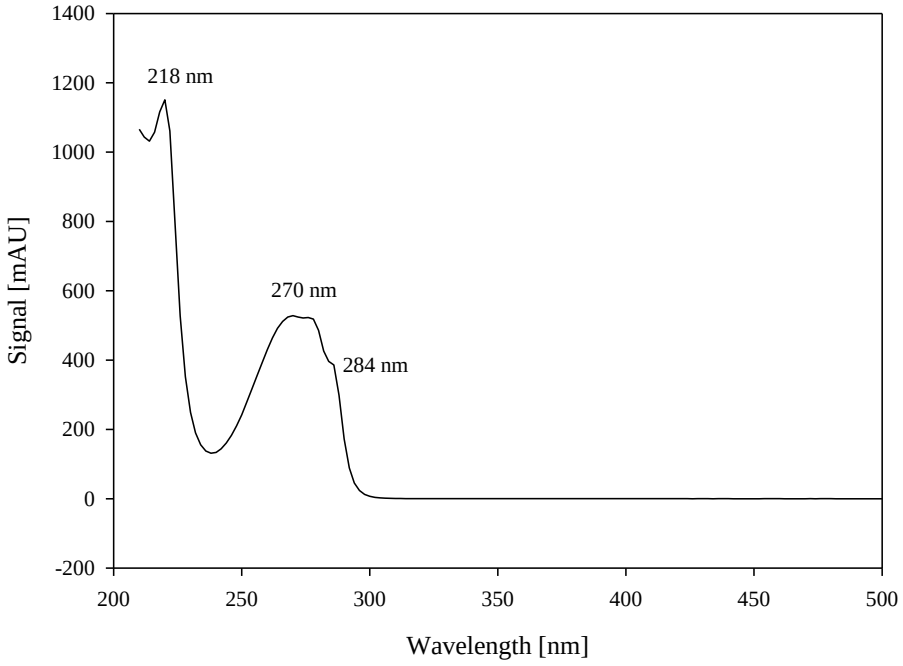


Figure 33: UV spectrum of gramine.
HPLC-DAD UV spectrum of gramine reference compound with its characteristic absorption maxima at 218 nm, 270 nm, and a small shoulder at 284 nm.

Figure 34 and Figure 35 show the HPLC chromatograms of organic and aqueous phases prepared from the 72 h incubation of *Arthrobacter sp.* CB4 and *Pseudomonas sp.* CB3 and MPI9 with gramine. Gramine is partially soluble in water and therefore found in the organic and the water phases. No other compound in addition to gramine was identified in these chromatograms (Figure 34 and Figure 35).

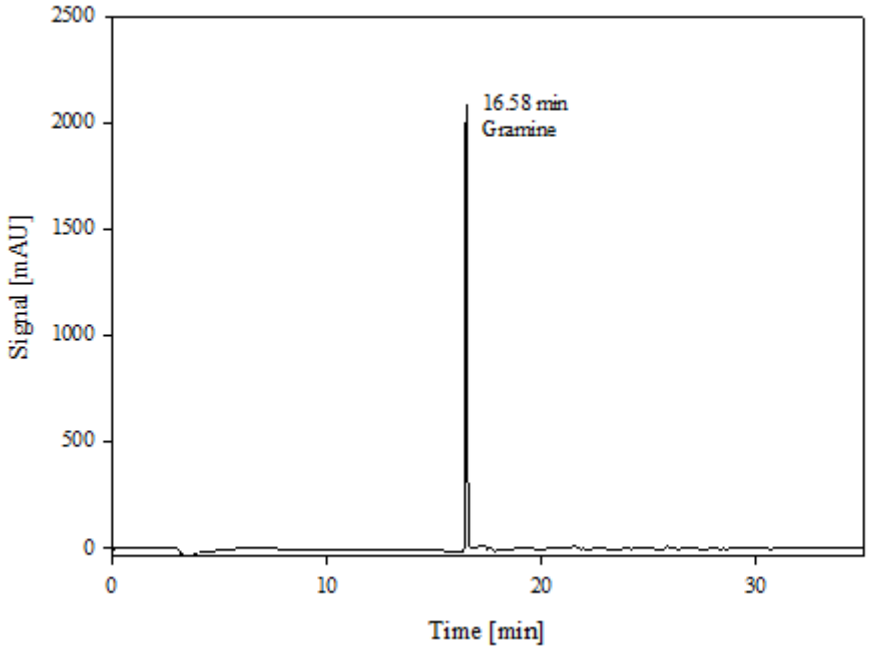


Figure 34: HPLC-DAD chromatogram of an organic extract from an *Arthrobacter sp.* CB4 culture treated with gramine.
HPLC-DAD analysis at 270 nm of an organic extract taken after 72 h of cultivation of *Arthrobacter sp.* CB4 in liquid media containing 500 µM gramine. The peak for gramine could be detected after 16.58 min at 270 nm. The chromatogram shown is representative for all replicates of organic phase extracts.

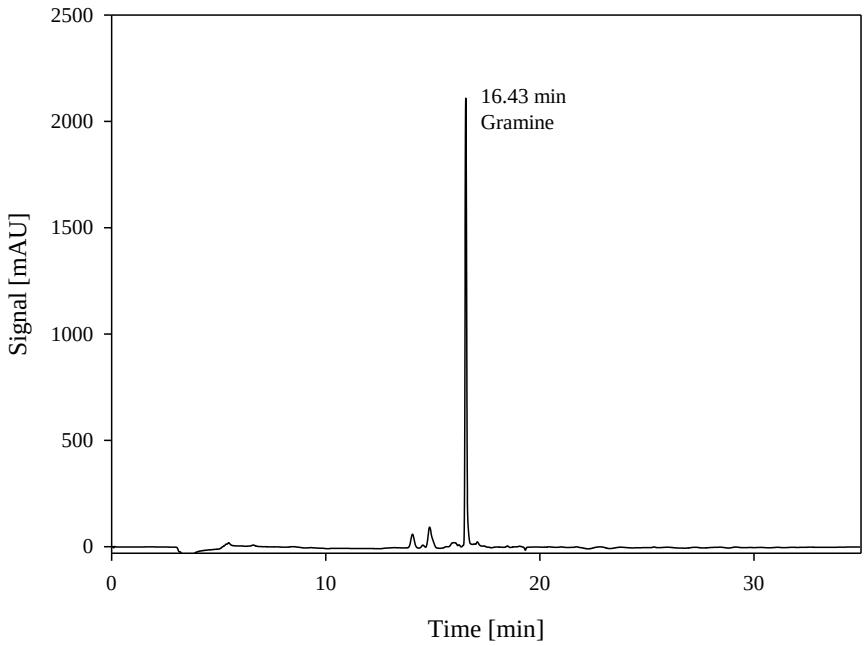


Figure 35: HPLC-DAD chromatogram of an aqueous extract from an *Arthrobacter sp.* CB4 culture treated with gramine.
HPLC-DAD analysis at 270 nm of an organic phase extract taken after 72 h of cultivation of *Arthrobacter sp.* CB4 in liquid media containing 500 µM gramine. The peak for gramine could be detected after 16.43 min at 270 nm. The chromatogram shown is representative for all replicates of aqueous phase extracts.

Arthrobacter sp. GB1 was the only tested organism capable to degrade gramine. The possibility that gramine was degraded by GB1 was already indicated during the course of the incubation by a color change of the minimal media TSM. While controls remained colorless, cultures containing 500 µM gramine turned to a yellowish color after 6 h of incubation (data not shown). This phenomenon was not observed for *Arthrobacter sp*. CB4 exposed to 500 µM gramine. The HPLC-DAD signal at 270 nm revealed already after 6 h an accumulation of an additional peak with a retention time of 23 min (Figure 36). Since this peak was absent from the control, it was most likely a degradation product of gramine (Supplementary Figure 1 and Supplementary Figure 2).

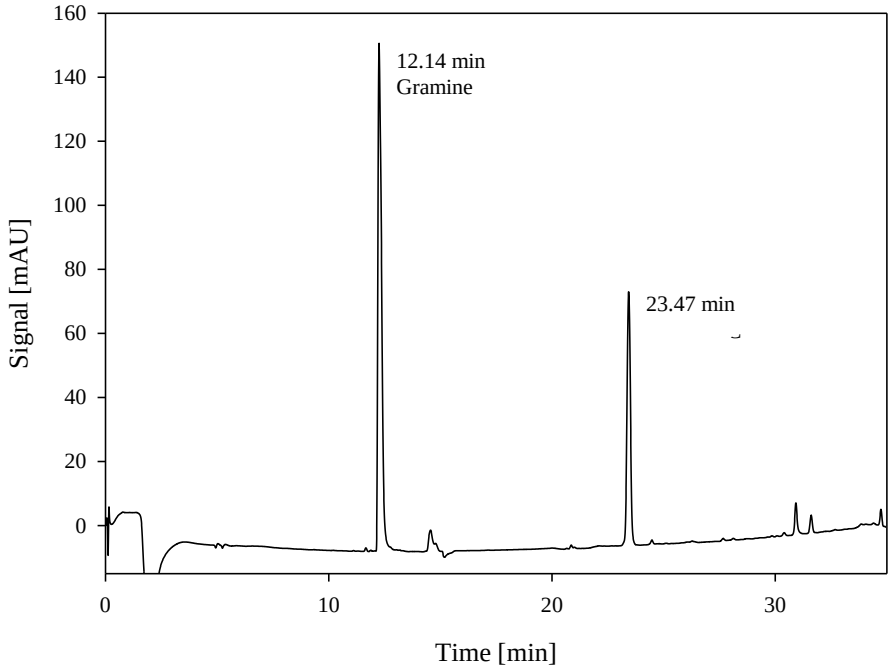


Figure 36: HPLC-DAD chromatogram of an organic extract of an *Arthrobacter sp.* GB1 culture treated with gramine.
HPLC-DAD analysis at 270 nm of an organic extract taken after 72 h of cultivation of *Arthrobacter sp. GB1* in liquid media containing 500 µM gramine. The peak for gramine could be detected after 12.14 min at 270 nm. An additional peak was detected after 23.47 min.

Analysis of the UV spectrum of the peak of the putative gramine degradation product at 23 min revealed a co-migration of two substances with different UV spectra. The first substance of the 23 min peak had an absorption maximum at 280 nm (Figure 37). This product was identified as indole-3-carboxylic acid (I3C), which was already proposed as a possible degradation product of gramine (Ghini et al., 1991). Injection of commercially available I3C revealed a

retention time of 23 min and an identical UV spectrum as presented in Figure 37. Therefore, I3C acid could be identified as one of the degradation products of *Arthrobacter sp.* GB1.

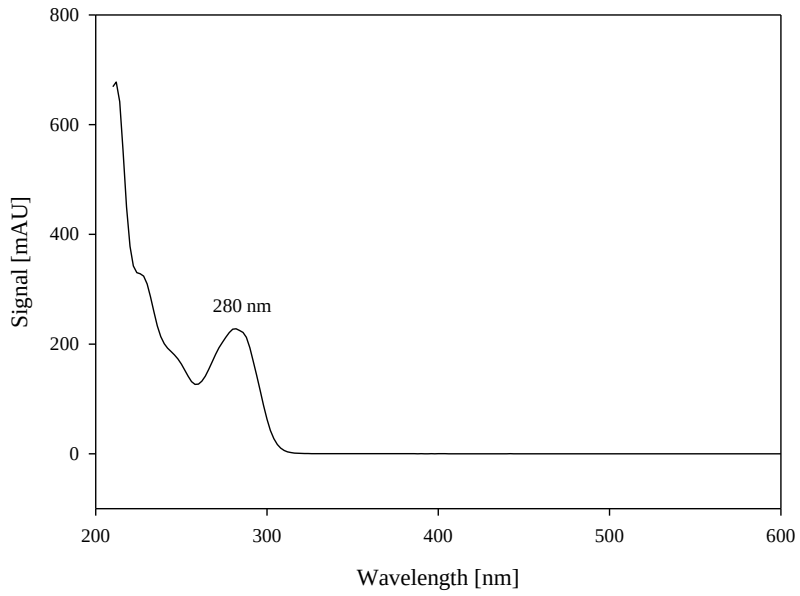


Figure 37: UV spectrum of indole-3-carboxylic acid.
HPLC-DAD UV spectrum of indole-3-carboxylic acid reference with its characteristic absorption maximum at 280 nm.

The second UV spectrum of the peak with almost the same retention time was completely different. Because of the absorption maxima at 240 nm, 260 nm, and 300 nm, I3C could be excluded as a possible candidate (Figure 38).

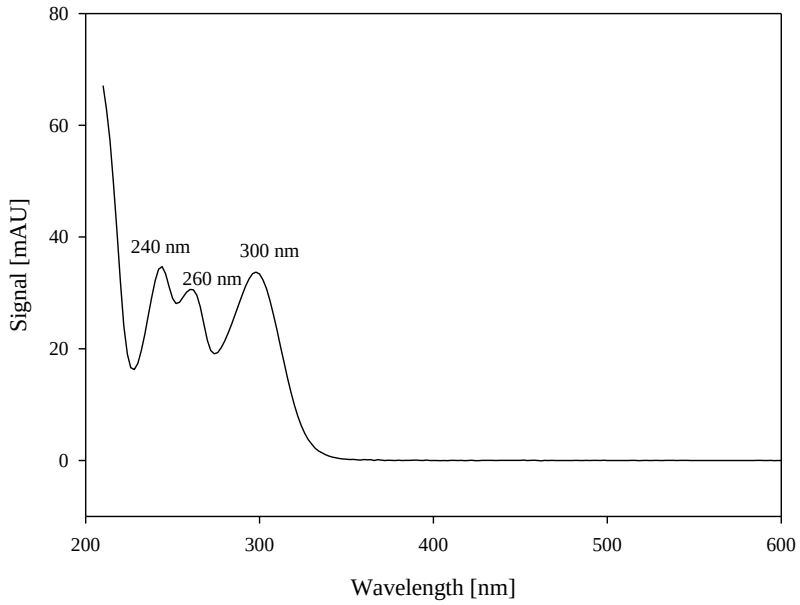


Figure 38: Second UV spectrum of *Arthrobacter sp.* GB1 gramine degradation product.
HPLC-DAD UV spectrum of gramine-derived degradation product eluting at 23.47 min with its characteristic absorption maxima at 240 nm, 260 nm, and 300 nm.

To identify the second gramine degradation product of *Arthrobacter sp*. GB1, mass spectrometry was utilized. Prior to data acquisition via Q-TOF MS/MS, organic phase extractions of *Arthrobacter sp.* GB1 replicates were combined and separated on a TLC plate using solvents for the separation of indoles and UV light for visualization. Upon purification of the separated bands presented on the TLC plate in Figure 39, HPLC-DAD was used to identify the upper band as gramine, and “Substance x” was confirmed to be the substance accumulating at 270 nm and revealing a UV spectrum as depicted in Figure 38.

Figure 39: TLC separation of an organic extract from an *Arthrobacter sp.* GB1 culture treated with gramine.
Organic extracts of *Arthrobacter sp.* GB1 cultures exposed to 500 µM gramine were separated by TLC for indoles. After visualization with UV, the redish band (gramine) and the yellow band (substance x) were isolated, and the compounds injected into LC-MS (Q-TOF) the upper band could be identified as gramine. Substance x represents the gramine degradation product accumulating after 23.47 min with absorption maxima at 240 nm, 260 nm, and 300 nm.

The TLC-purified "Substance x" was analyzed using Q-TOF MS/MS, resulting in a characteristic fragmentation pattern depicted in Figure 40. The protonated parental ion had a mass of 146.05 m/z and resulted in a fragmentation of 118.05 m/z, 91.05 m/z, and 65.03 m/z. Searches of the masses using the databank of massbank.eu revealed that the substance is indole-3-carboxaldehyde (I3A). The fragmentation of protonated I3A ($C_9H_8NO^+$, 146.06 m/z) observed by Q-TOF MS/MS occurs via $C_8H_8N^+$ (118.0651 m/z), $C_8H_7N^+$ (117.0573) and $C_7H_7^+$ (91.0542).

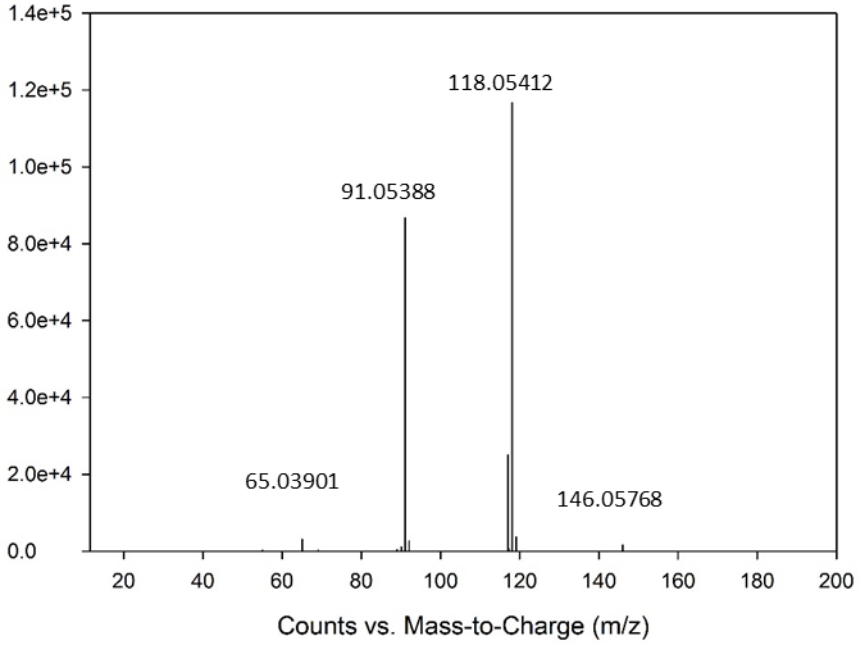


Figure 40: Direct infusion Q-TOF MS/MS spectrum of TLC-purified Substance x.
Fragmentation of TLC-purified 'Substance x' via direct infusion Q-TOF MS/MS resulted in a mass of 146.05768 m/z. Characteristic fragmentation pattern results in masses of 118.05412 m/z, 91.0388 m/z, and 65.03901 m/z.

Commercially obtained I3A was used as a reference compound. HPLC analysis confirmed an identical retention time and UV spectrum, and the second degradation product was identified as I3A.

Arthrobacter sp. GB1 degraded 500 µM gramine within 24h completely. To assess whether the initial conversion of gramine was sufficient for degradation of the compound by other organisms, bacterial strains *Arthrobacter sp.* CB4 and *Psuedomonas sp*.CB3 and MPI9, which were not able to degrade gramine, were incubated with the I3A. *Arthrobacter sp.* CB4 and *Pseudomonas sp.* CB3 and MPI9 were not able to degrade the gramine-derived product I3A (data not shown).

4.8.3. Degradation of Quercetin

The strains *Arthrobacter sp.* GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9 were incubated in liquid minimal medium in the presence of quercetin to study the quercetin degradation. The *Pseudomonas* sp. CB3 and MPI9 were not capable to degrade quercetin within 72 h. HPLC-DAD analysis revealed that no additional compounds accumulated, besides the peak eluting at 33.97 min possessing absorption maxima at 254 nm and 380 nm, characteristic for quercetin (Figure 41). Since no degradation of quercetin by *Pseudomonas sp.* CB3 and MPI9 was detected, the t72 peak of quercetin found in the organic phases of the culture extractions is shown representatively for all replicates and timepoints of both bacterial strains in Figure 42.

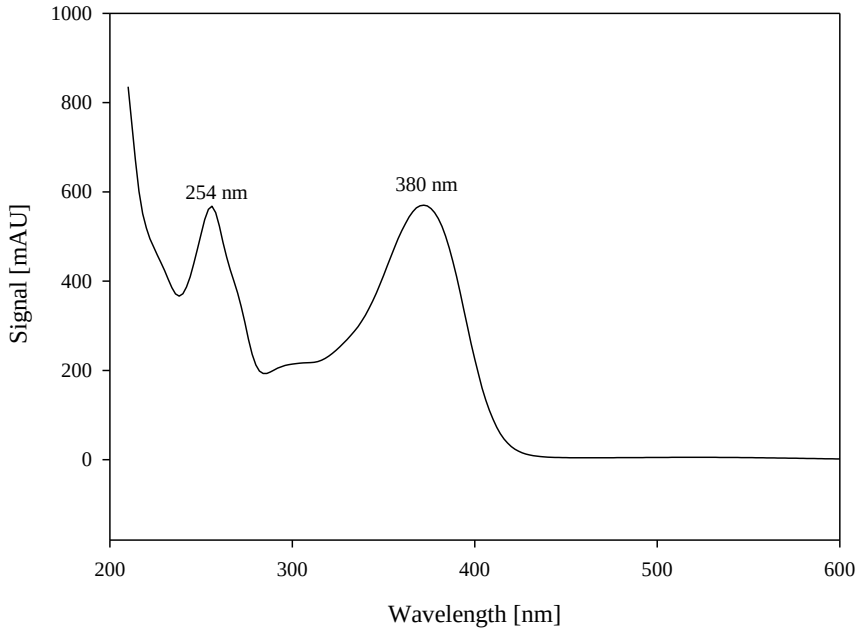


Figure 41: UV spectrum of quercetin.
HPLC-DAD UV spectrum of quercetin reference with its characteristic absorption maxima at 254 nm and 380 nm.

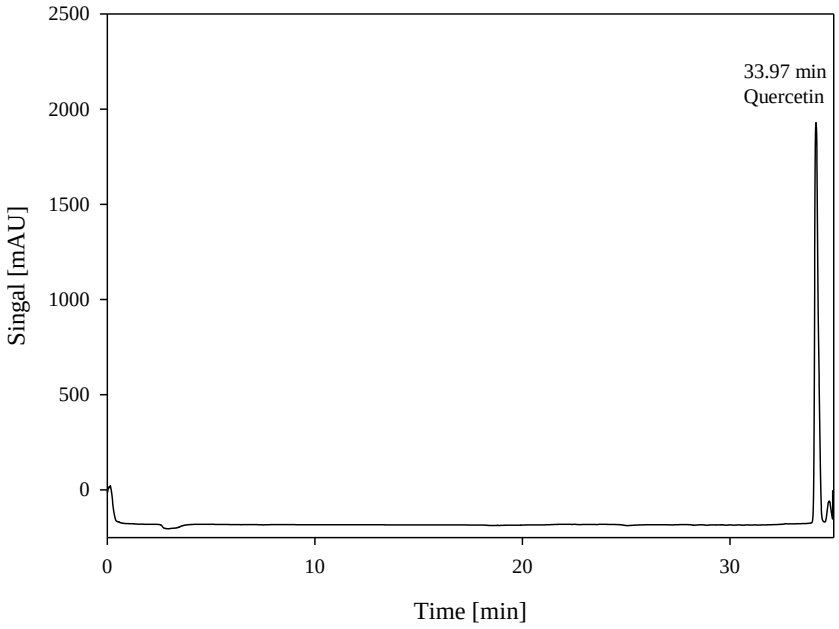


Figure 42: HPLC-DAD chromatogram of an organic extract from a *Pseudomonas sp.* CB3 culture treated with quercetin.
HPLC-DAD analysis at 380 nm of organic extract taken after 72 h of cultivation of *Pseudomonas sp*. CB3 and MPI9 in liquid media containing 500 µM quercetin. The peak for quercetin could be detected after 33.97 min at 380 nm.

The two *Arthrobacter sp.* GB1 and CB4 strains were both able to degrade 500 µM quercetin, with differences in the duration. While *Arthrobacter sp.* GB1 was able to metabolize quercetin completely within 48 h of growth, *Arthrobacter sp.* CB4 required 72 h for a complete degradation under the same conditions. Nevertheless, both bacterial strains revealed an accumulation of a compound in the aqueous phase of the culture extracts with a retention time of 23 min and UV absorption at 270 nm (Figure 43).

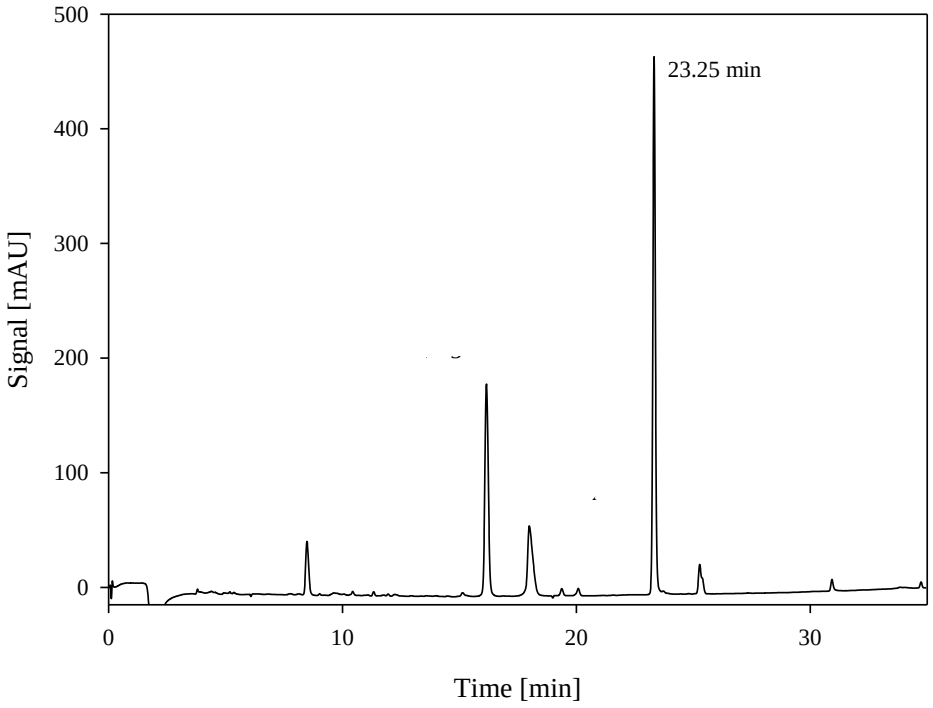


Figure 43: HPLC-DAD chromatogram of an aqueous extract from an *Arthrobacter sp*. GB1 culture treated with quercetin.
HPLC-DAD analysis at 270 nm of the aqueous extract taken after 6 h of cultivation of *Arthrobacter sp.* GB1 in liquid media containing 500 µM quercetin. A peak for a quercetin-derived degradation product could be detected at 23.25 min at 270 nm. *Artrhobacter sp*. CB4 extracts showed a similar chromatogram.

The accumulating substance and quercetin differ in their UV spectra (Figure 44). Several publications describe the degradation of quercetin by gut bacteria, via protocatechuic acid (Pillai and Swarup, 2002). Therefore, the degradation product found after incubation with the *Arthrobacter sp.* could be the protocatechuic acid. Indeed, the UV spectrum of this metabolite is identical to the one of the protocatechuic acid standard (Figure 44). Thus, the two *Arthrobacter sp.* Strains GB1 and CB4 degraded quercetin via protocatechuic acid as known from gut bacteria.

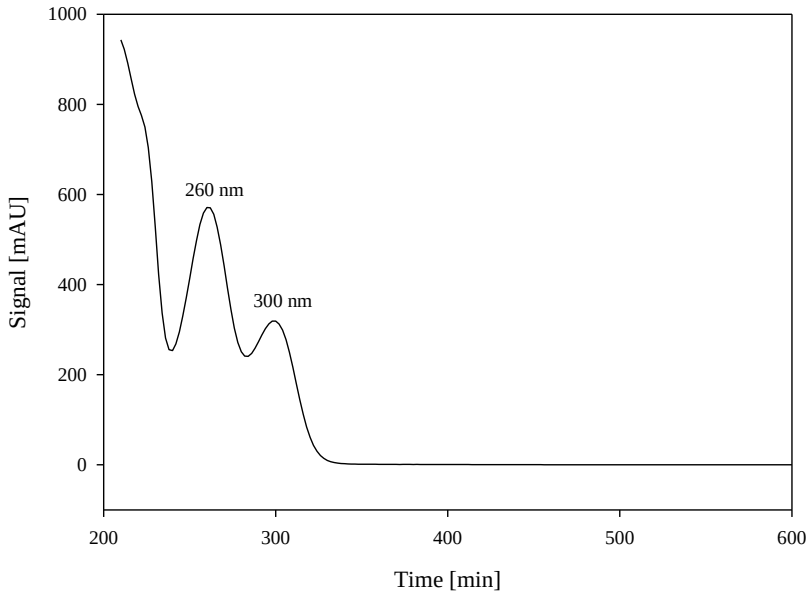


Figure 44: UV spectrum of protocatechuic acid.
HPLC-DAD UV spectrum of bacterial quercetin-derived protocatechuic acid with its characteristic absorption maxima at 260 nm and 300 nm.

4.8.4. Incubation of Soil Bacteria with BOA-derived Acetamidophenol

Since none of the tested bacterial strains were capable to degrade BOA, the group of tested soil organisms was enlarged and the strains *Arthrobacter sp.* GB1, CB4, MPI761, MPI762, MPI763, MPI764 and *Pseudomonas sp.* CB3 and MPI9 were tested for conversion of the known microbial BOA degradation product 2-acetamidophenol (AAP). This study highlights the importance of networking in microbiomes. Bacterial strains differ in their enzymatic capability to detoxify certain metabolites and may require therefore other members of the microbiome to perform all reaction steps necessary for degradation and detoxification. Therefore, it was possible that BOA is first converted into AAP (by unknown members of the microbiota) before AAP is further metabolized by one of the isolated soil bacteria. Incubation of *Arthrobacter sp.* MPI764 with AAP followed by HPLC analysis resulted in the detection of three new peaks, P1, P2, and P3, which might represent possible conversion products (Figure 45). The identification of the products was not possible with HPLC-DAD and required other methods for structure elucidation.

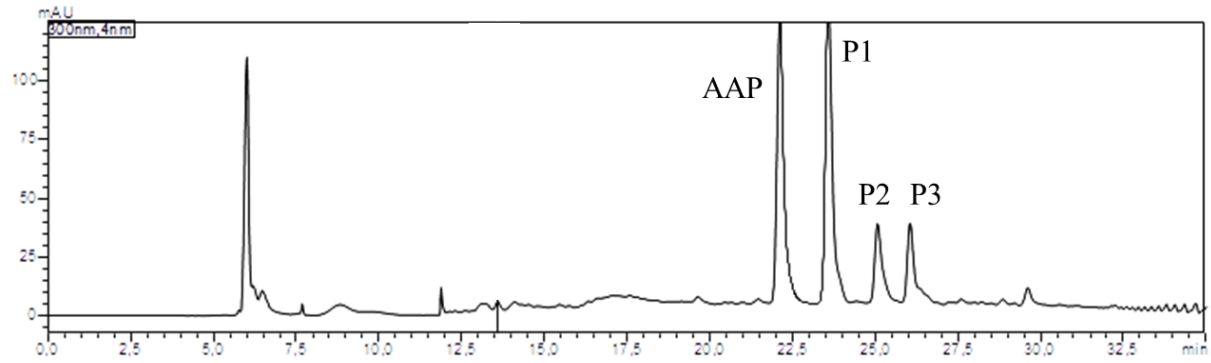


Figure 45: HPLC-DAD chromatogram of a sample from an *Arthrobacter sp.* MPI764 culture treated with AAP.
HPLC-DAD analysis at 300 nm *Arthrobacter sp.* MPI764 AAP culture samples taken after 24 h of cultivation of *Arthrobacter sp.* MPI764 in liquid media containing 500 µM AAP. Peaks for three AAP-derived metabolites, P1, P2, and P3, could be detected. n = 3

Structural analysis and identification of the three AAP-derived products was performed by PD Dr. Margot Schulz (University of Bonn, Germany) and Prof. Dr. Laurent Bigler (University of Zurich, Switzerland) using NMR and Q-TOF analysis. P1 was identified as the nitroso derivative N-(2-hydroxy-5-nitrosophenyl) acetamide (Figure 46). P2 and P3 are nitro derivatives of AAP with P2 being N-(2-hydroxy-5-nitrophenyl) acetamide (5-N-AAP), and P3 is N-(2-hydroxy-3-nitrophenyl) acetamide (3-N-AAP) (Figure 46). Thus, *Arthrobacter sp.* MPI764, which was not able to degrade BOA, may pave the way for subsequent reactions by the synthesis of molecules with a higher reactivity compared to AAP. This result highlights the importance of different bacterial species with different metabolic capacities in the microbiome.

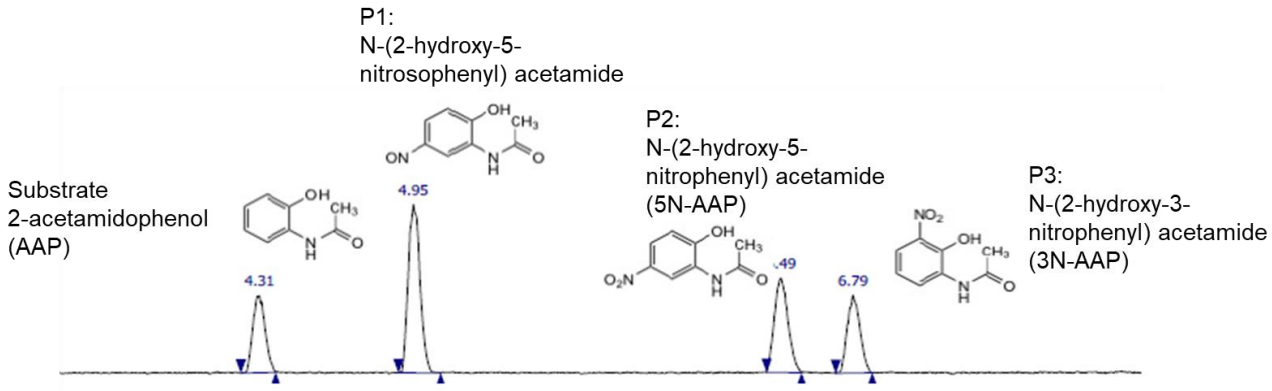


Figure 46: Identification of AAP-derived degradation products of *Arthrobacter sp.* MPI 764.
LC chromatogram of an aqueous extract from an *Arthrobacter sp.* MPI764 culture treated with AAP. Structural identification of the compounds was achieved via Q-TOF MS and NMR (data not shown) by PD Dr. Margot Schulz and Prof. Dr. Laurent Bigler. The AAP-derived degradation products of *Arthrobacter sp.* MPI764 are: P1: N-(2-hydroxy-5-nitrosophenyl) acetamide; P2: N-(2-hydroxy-5-nitrophenyl); P3: N-(2-hydroxy-3-nitrophenyl) acetamide

4.9. Extraction of Plant Secondary Metabolites from Soil

Next-generation sequencing, bacterial growth, and metabolite degradation experiments suggest interactions of the plant secondary metabolites with the soil microbiota. Since the plant secondary metabolites BOA, gramine, and quercetin were added by mixing the pure substances into the soil, their availability and metabolic fate in the soil had to be determined. Therefore, 10 µmol of the pure substances were added and extracted immediately at t0 (control) and after 2 days of incubation with the soil. The metabolites were extracted and measured by HPLC, and concentrations were calculated by use of external standard curves of each of the three metabolites. Within two days, most of the added BOA was either degraded or converted into a new substance (Figure 47). Only 1.50 ± 0.26 µmol BOA could be recovered after two days (Figure 47). A new substance occurring upon addition of BOA to soil, which was absent from the control or other treatments, was identified as APO exhibiting a characteristic UV spectrum. APO is a derivative of BOA, and it was previously synthesized by cyclic condensation *in vitro* (Voloshchuk et al., 2020) A standard curve of APO was used to determine the concentration which was 0.89 ± 0.17 µmol APO in the soil at 2 days after BOA application (Figure 47).

Only a minor portion of the added gramine could be recovered in the t0 samples (Figure 47). After two days, only 0.09 ± 0.03 µmol gramine were measured in the soil (Figure 47), but HPLC-DAD analysis indicated possible degradation products, which could not be identified due to their low concentrations. Therefore, most of the gramine was probably lost due to strong association with soil particles.

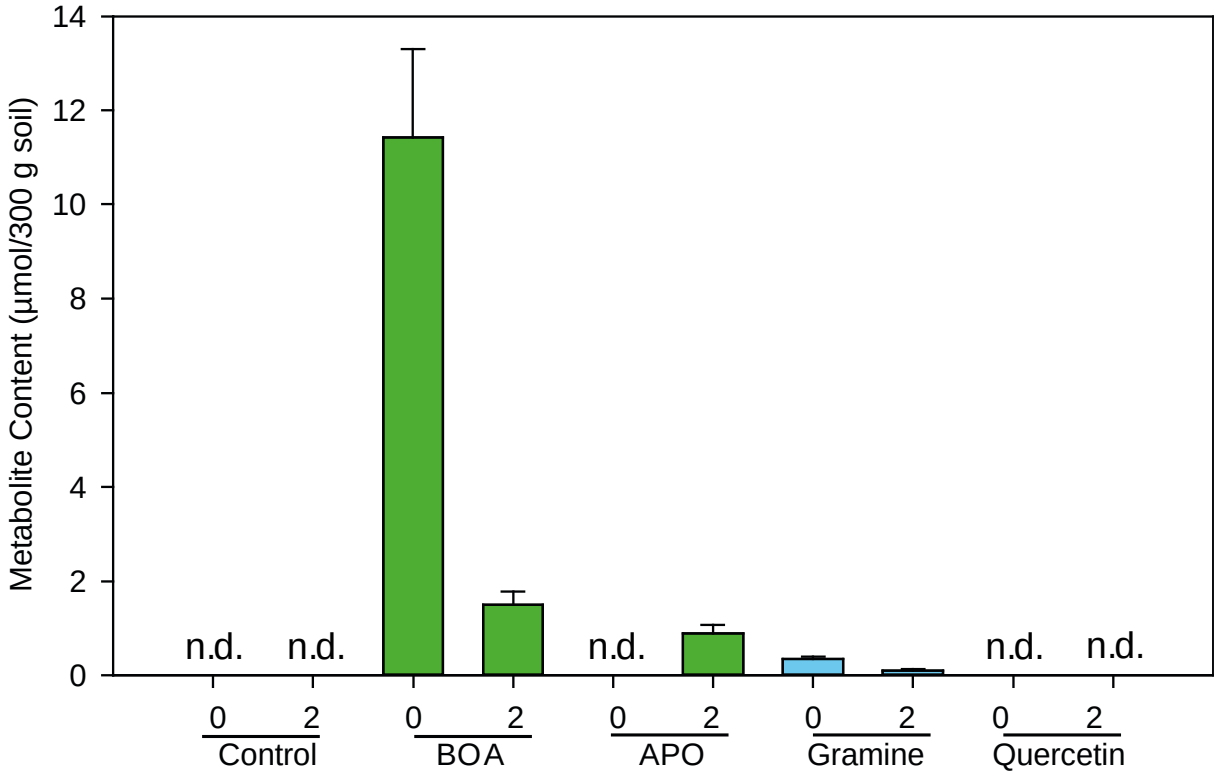


Figure 47: Extraction of BOA, gramine, and quercetin after two days of incubation of the soil with the metabolites.
10 µmol of the plant secondary metabolites BOA, gramine, and quercetin were mixed into 300 g soil and extracted right away (0) or after two days (2) and quantified by HPLC-DAD with standard curves of the substances. 2-aminophenoxazinone (APO) was found after two days in soil with BOA. n = 3, mean ± SD; n.d. = not detected; Figure from Schütz et al., 2021.

The extracts of soil after incubation with gramine were analyzed by UHPLC-MS/MS by Dr. Diana Hofmann and Dr. Björn Thiele at the Forschungszentrum Jülich (Germany). After the addition of gramine to the soil, residual gramine was detected after 9.97 min and the accumulating substance at 15.38 min was identified as I3A (Figure 48), the same substance which was previously detected after exposure of the soil bacterium *Arthrobacter sp. GB1* to gramine in liquid media (Figure 36). The fragmentation pattern of the presumed degradation product of gramine in soil (peak eluting at 15.38 min in Figure 48) contained the masses for I3A fragmentation as shown in Figure 36.

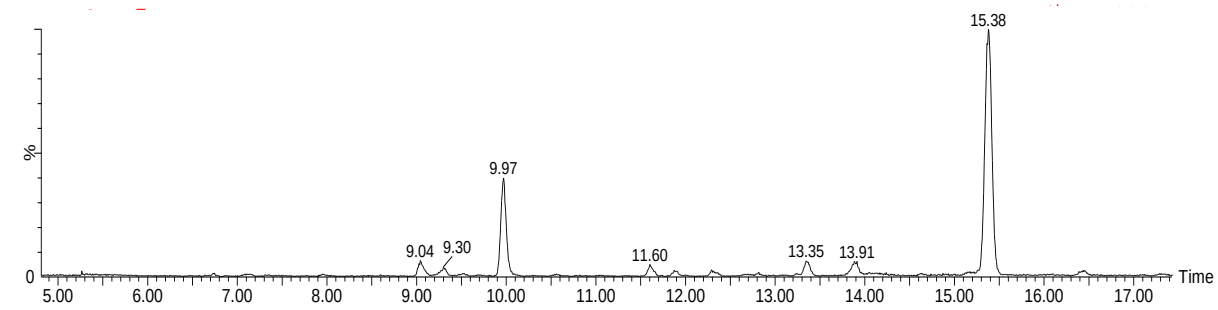


Figure 48: UHPLC chromatogram of an organic soil extract after gramine application.
Soil treated with 10 µmol gramine was extracted after two days with acidic methanol. Methanol extracts were analyzed via UHPLC MS/MS. LC chromatogram of an organic extract of a soil sample revealed two signals. At 9.97 min residual gramine was found and at 15.38 min a signal accumulated representing indole-3-carboxaldehyde (identified via MS/MS).

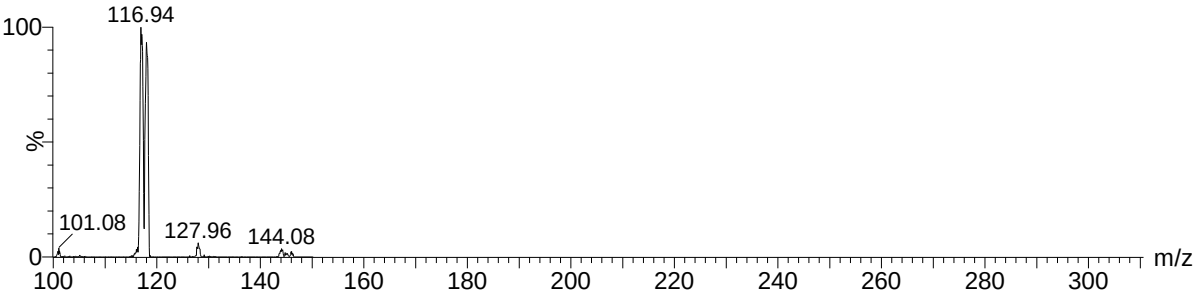


Figure 49: UHPLC MS/MS spectrum of the degradation product of gramine in soil.
UHPLC MS/MS spectrum of the substance eluting after 15.38 min (Figure 48). The fragmentation pattern (144.08 m/z, 127.96 m/z, 116.96 m/z and 101.08 m/z) revealed characteristic masses for indole-3-carboxaldehyde.

Quercetin could not be recovered after the addition to soil (Figure 47), a phenomenon often described in the literature (Terzano et al., 2015). This might also be due to binding to soil particles. However, several new compounds related to quercetin were present in the sample, which are currently under investigation. One compound was already identified as rutin, a glycosylated form of quercetin, indicating that soil microorganisms may convert the compound into a more hydrophilic one by glycosylation.

4.10. Phosphate Solubilization by Soil Bacteria

In soil, the macronutrient phosphorus is mostly present in forms that are not available to plants. Solubilization of phosphate is a key event for the survival and proliferation of plants. Therefore, bacterial strains capable to solubilize phosphate play an important role in plant-microbe interactions. *Arthrobacter sp.* GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9 were studied for their capability of phosphate solubilization using Pikovskayas agar containing 1 g apatite, an insoluble tricalcium phosphate ($Ca_3(PO_4)_2$). This medium allows the visualization of phosphate solubilization by the formation of a halo ring surrounding the bacteria.

All four organisms were tested in triplicates. Figure 50 shows the solubilization representatively for one of the replicates, while for the calculation of the average halo zone size several plates were used (Table 8). Incubation of the two *Arthrobacter sp*. GB1 and CB4 on Pikovskayas agar did not result in the formation of a halo zone and therefore, it can be assumed that these organisms are not able to solubilize phosphate from apatite (Figure 50). The *Pseudomonas sp*. showed a clear halo zone surrounding the bacteria, indicating the capability to solubilize phosphate (Figure 50). With an average halo zone size of 2.75 ± 0.37 mm (n=8), the phosphate solubilization of *Pseudomonas sp*. CB3 appeared to be distinctively weaker compared to *Pseudomonas sp*. MPI9 producing a halo zone with an average diameter of 7.29 ± 0.72 mm (Table 8). While quantitative phosphate solubilization is not possible using Pikovskayas agar, large differences in halo zone size might nevertheless be an indicator for more efficient solubilization of *Pseudomonas sp.* MPI9 than CB3, although the size of the colony is also important.

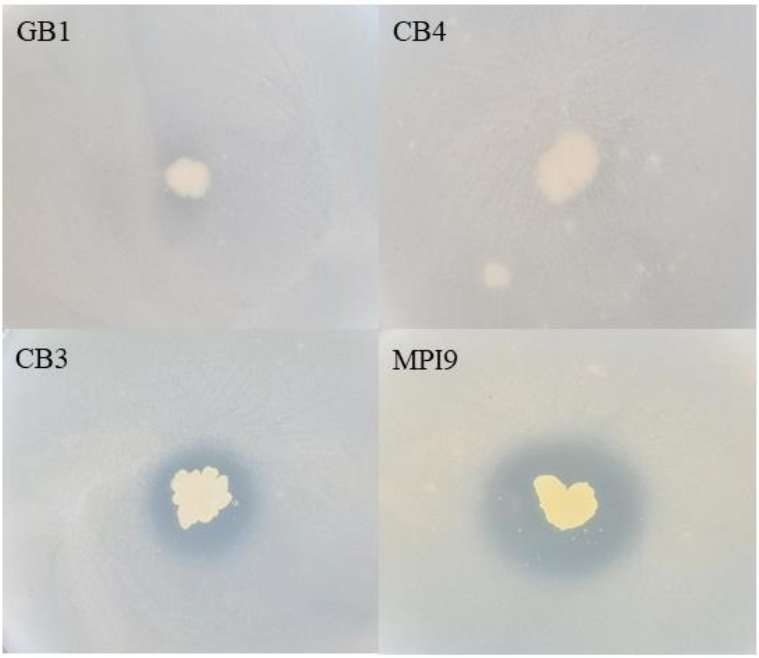


Figure 50: Phosphate solubilization of *Arthrobacter sp.* GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9.
Arthrobacter sp. GB1 and CB4 and *Pseudomonas sp*. CB3 and MPI9 were cultivated on agar containing tricalcium phosphate. The capability to solubilize phosphate was visualized by a halo surrounding the bacterial colony.

Table 8: Halo size of phosphate solubilizing soil bacteria.
Phosphate solubilization of *Arthrobacter sp.* GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9 was determined by the diameter [mm] of the colony surrounding halo. n = 3, mean ± SD.

Organism	Average halo ring size [mm]
Arthrobacter sp. GB1	-
Arthrobacter sp. CB4	-
Pseudomonas sp. CB3	2.75 ± 0.37
Pseudomonas sp. MPI9	7.29 ± 0.72

4.11. Impact of Soil Bacteria, Plant Secondary Metabolites, and Their Degradation Products on Plant Growth

4.11.1. Interactions of Plants with Soil Bacteria

While bacterial phosphate solubilization could improve the supply of plants with phosphate, additional properties of the bacteria may support or inhibit plant growth. To study these effects, *Arthrobacter sp.* GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9 were co-cultivated with *A. thaliana*, and the impact of the bacterial strains on the plants was determined. Evidently, both *Arthrobacter sp.* Strains caused the death of *A. thaliana* after germination as shown in Figure 51. Also, *Pseudomonas sp*. MPI9, the most successful phosphate-solubilizing bacterium tested in this study, caused the death of *A. thaliana* as soon as the seeds germinated (Figure 51). *Pseudomonas sp*. CB3 was the only organism with a growth-promoting effect on *A. thaliana* (Figure 51). Determination of shoot fresh weight without roots (140.78 ± 38.81 mg) revealed a slight increase when *A. thaliana* was cultivated with *Pseudomonas sp.* CB3, compared to the control (123.59 ± 30.5 mg) (Table 9). Despite an increase in shoot fresh weight, exposure of *A. thaliana* resulted in a decrease of root length to 6.23 ± 0.96 cm when inoculated with *Pseudomonas sp.* CB3, compared to the control (12.01 ± 2.63 cm) (Figure 51).

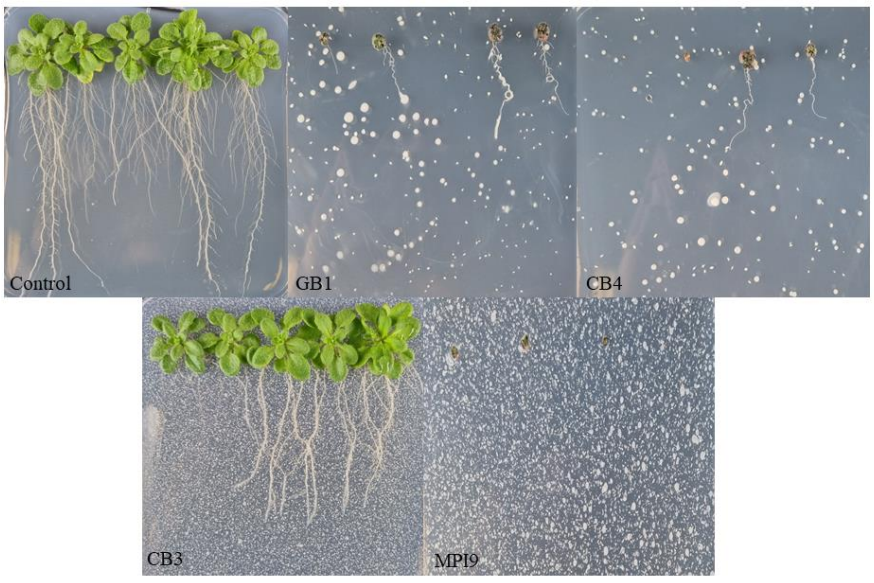


Figure 51: Co-cultivation of *A. thaliana* with soil bacteria *Arthrobacter sp. GB1 and CB4 and* Pseudomonas sp. *CB3 and MPI9*.
Co-cultivation of *A. thaliana* with the soil bacteria *Arthrobacter sp.* GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9 on MS media. Sterile seeds were grown for 21 days at a 16 h light/ 8 h dark ratio, 55% humidity, and 120 µmol m^{-2} s^{-1} light intensity.

Table 9: Shoot fresh weight and root length of *A. thaliana* co-cultivation with soil bacteria.
After 21 days of co-cultivation of *A. thaliana* with *Arthrobacter sp.* GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9 on MS media, roots were removed and the fresh weight [mg] of the shoots and root length [cm] was determined. Plants were grown at a 16 h light/ 8 h dark ratio, 55% humidity and 120 µmol m^{-2} s^{-1} light intensity, n = 20 – 30, mean ± SD.

Treatment	Shoot Fresh weight [mg]	Root length [cm]
Control	123.6 ± 30.5	12.0 ± 2.6
Arthrobacter sp. GB1	-	-
Arthrobacter sp. CB4	-	-
Pseudomonas sp. CB3	140.8 ± 38.8	6.2 ± 1.0
Pseudomonas sp. MPI9	-	-

4.11.2. Incubation of *A. thaliana* with BOA, Gramine, and Quercetin

When BOA, gramine, and quercetin are added to the soil, the compounds may influence not only microorganisms but also plants. Therefore, defined concentrations of BOA, gramine, or quercetin were applied to *A. thaliana*. Application of 500 µM of one of the compounds to seeds prevented germination or resulted in the death of the plants shortly after germination (data not shown). Lowering the concentration to 100 µM of any of the metabolites allowed the growth of the plants for 21 days. No visual differences could be observed in the plants treated with the different metabolites, compared to the control (Figure 52). The shoot fresh weight and root length for all treatments were about similar to the control, thus no significant changes could be found (Table 10).

Figure 52: Co-cultivation of *A. thaliana* with BOA, gramine, and quercetin.
Co-cultivation of *A. thaliana* on MS media with 100 µM BOA, gramine, quercetin, or DMSO as control. Plants were grown for 21 days at a 16 h light/ 8 h dark regime, 55% humidity and 120 µmol m^{-2} s^{-1} light intensity.

Table 10: Shoot fresh weight and root length of *A. thaliana* after co-cultivation with BOA, gramine, or quercetin.
After 21 days, co-cultivation of *A. thaliana* with 100 µM BOA, gramine, quercetin, or DMSO as control on MS media, roots were removed, and shoot fresh weight [mg] and root length of plants determined. Plants were grown at a 16 h light/ 8 h dark regime, 55% humidity and 120 µmol m^{-2} s^{-1} light intensity. n = 20 – 30, mean ± SD.

Treatment	Shoot fresh weight [mg]	Root length [cm]
Control	113.78 ± 33.55	10.88 ± 2.12
100 µM BOA	111.98 ± 27.95	11.83 ± 1.88
100 µM Gramine	107.26 ± 31.25	11.37 ± 1.60
100 µM Quercetin	110.08 ± 34.78	10.94 ± 2.75

Since the bacterial gramine degradation product I3A was not only found in liquid cultures but also after the addition of gramine to soil, further co-cultivation experiments of *A. thaliana* and I3A were performed. Concentrations of 100 µM and 500 µM of I3A resulted in the death of *A. thaliana* seedlings after germination. Therefore, co-cultivation required further adaptions. Since I3A is related to auxins, the concentrations were lowered to typical concentrations of auxin used in phytohormone experiments, i.e., 0.25 µM, 0.5 µM, 3.5 µM, and 7 µM (Figure 53). The shoot fresh weight of *A. thaliana* seedlings was increased with 0.25 µM I3A, and a strong increase

was observed after growth on 0.5 µM I3A. Shoot fresh weight was doubled to 105.48 ± 6.81 mg, compared to the control (51.59 ± 5.13 mg) when plants were grown on 0.5 µM I3A (Figure 53). With 7 µM I3A, growth was again similar to the control (Figure 53). This result strongly suggested that I3A might exert an auxin-like function.

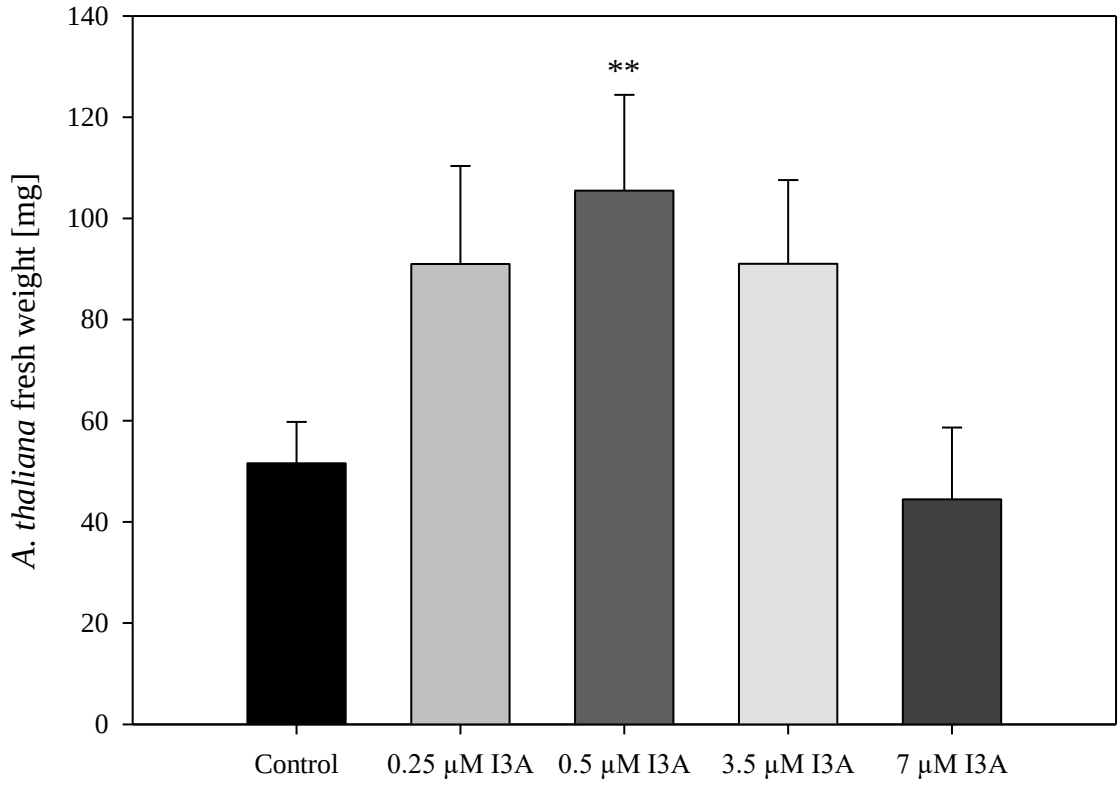


Figure 53: Co-cultivation of *A. thaliana* plants with different concentrations of indole-3-carboxaldehyde (I3A).
Co-cultivation of *A. thaliana* seedlings with 0.25 µM I3A, 0.5 µM I3A, 3.5 µM I3A, 7 µM I3A, and DMSO as control on MS media. Plants were grown for 21 days at a 16 h light/ 8 h dark ratio, 55% humidity and 120 µmol m-2 s-1 light intensity. Then, the shoot fresh weight was determined after the removal of the roots. n = 20 – 30, mean ± SD. Student's t-test ($P < 0.05$ = *, $P < 0.01$ = **). Only treatment with 0.5 µM I3A was significantly impacted compared with the control.

4.11.3. Changes in Gene Expression of *A. thaliana* after Exposure to 5-N-AAP

The conversion of AAP to 5-N-AAP by soil bacteria, as demonstrated in Figure 46, may result in the bioactivation of AAP, since nitroaromatic compounds are known to have high biological activity. Since the conversion of 5-NO-AAP to 5-N-AAP might be a source of nitrogen oxide, the expression of several selected genes responsive to NO was studied in *A. thaliana*. These genes include the terpene synthases *TPS02* and *TPS04* (Chen et al., 2003) and the tryptophan synthase *TRYPS02* (Liu et al., 2018), which are known to be upregulated by NO. Further genes included the tocopherol cyclase *VTE1* whose expression is up- or downregulated by NO, depending on exposure time, and the cytosolic nitrate reductases *NIA1* and *NIA2* which were included to determine the impact of 5-N-AAP on nitrate reduction (Costa-Broseta et al., 2021). It is possible that during the process of AAP catabolism, nitrated AAP is deaminated and ammonium is released. Therefore, the two cytosolic glutamine synthase genes *GLN1.1* and *GLN1.2* were also included in the expression study (Yao et al., 2019).

Three-week-old *A. thaliana* plants were incubated for 0.5 h, 1 h, 3 h, 5 h, or 24 h with 1 mM 5-N-AAP. After extraction of RNA and cDNA synthesis, the expression pattern of the genes described above was studied by qPCR. The strongest response to 5-N-AAP was observed for terpene synthase *TPS04*. After 30 min of incubation, *TPS04* was expressed higher than 4.5-fold compared to the control (Figure 54). Nevertheless, the TPS04 gene was only expressed in the early stages of the incubation since the expression dropped to 2.8-fold after 1 h and even resulted in a 3.3-fold downregulation after 24 h (Figure 54). Interestingly, this effect seemed to be specific for *TPS04* since *TPS02* showed no response throughout the incubation (data not shown). *VTE1* expression was 1.6-fold upregulated after 30 min. This effect was only observed within the first hours of exposure of *A. thaliana* plants to 5-N-AAP since *VTE1* was slightly downregulated after 24 h (Figure 54). *GLN1.1* and *GLN1.2* were slightly downregulated throughout the incubation, indicating a possible negative impact of 5-N-AAP on the expression of the genes (Figure 54). Nevertheless, the gene expression study of the selected genes did not show strong responses, except for *TPS04.* From the lack of *GLN1.1* and *GLN1.2* expression, it can be speculated that no ammonium was released during the incubation with 5-N-AAP.

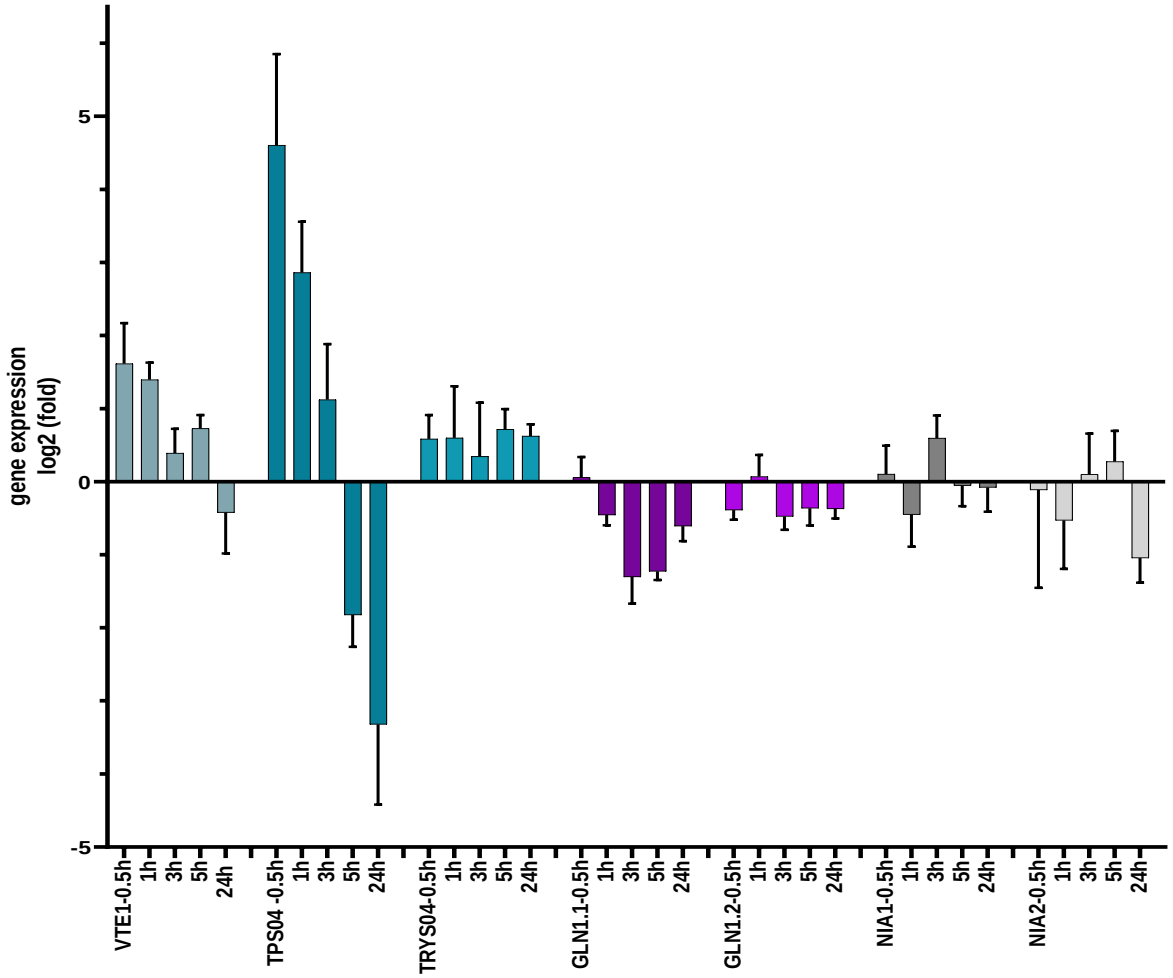


Figure 54: Gene expression studies of *A. thaliana* plants exposed to N-(2-hydroxy-5-nitrophenyl) (5-N-AAP).
21 day old *A. thaliana* plants were incubated for 0.5 h, 1 h, 3 h, 5 h, or 24 h with 1 mM 5-N-AAP, and afterwards harvested for RNA extraction and gene expression studies by qPCR (log2- ΔΔCt). Changes in expression of tocopherol cyclase *VTE1*, geranyl linalool synthase *TPS04*; cytosolic nitrate reductases *NIA1* and *NIA2*; tryptophan synthase *TRYPS02*; cytosolic glutamine synthases *GLN1.1* and *GLN 1.2*. Gene expression was calculated against control plants that were not exposed to 5-N-AAP. n = 3, mean ± SD.

4.11.4. Changes in Gene Expression of *Z. mays* Plants upon Exposure to Different BOA-OH Isomers

Plant secondary metabolites affect not only the soil microbiota but also different plants. Especially the impact of secondary metabolites on crop plants plays an important role in agriculture. For example, the cultivation of barley may lead to an accumulation of BOA in the soil. This substance cannot only be converted into APO, as shown before but can also be hydroxylated during detoxification processes at position 5 or 6 by enzymes present in a number of plants. The impact of BOA-derived detoxification products is well studied in the

benzoxazinone-containing model plant maize, including numerous enzymes for BOA-6-OH polymerization and degradation at the root surface (Schulz et al., 2012; Schulz et al., 2018). A high catalase activity during the early stages of exposure of maize to the metabolite suggests possible damage to the lipid membrane through peroxidation (Schulz et al., 2018). Therefore, genes involved in oxidative stress, lipid repair, and plant immunity response were chosen to investigate the impact of BOA isomers on maize. To cover ROS stress-related responses, superoxide dismutase 2 (*SOD2*), which plays an important role in superoxide radical transfer (Gond et al., 2015), was included in the study. The catalase *CAT1* was described to be involved in response to oxidative stress (Mylona et al., 2007), and *CAT3* in response to ROS, H_2O_2, and xenobiotics (Redinbaugh et al., 1990). To assess possible lipid restorations in the maize roots, oleoyl desaturases *FAD2.1* and *FAD2.2* were included (Dar et al., 2017). Pathogenic responses after exposure of maize to BOA-OH isomers were determined by including the well-studied pathogen response genes *PR1*, *PR4*, *NPR1*, and *POX12* (Backer et al., 2019; Hemetsberger et al., 2012; Sajad et al., 2018).

After incubation of *Z. mays* with BOA-4-OH, BOA-5-OH, BOA-6-OH, or BOA-7-OH, gene expression of the genes described above was determined in roots via qPCR. The expression of the ROS stress-related *SOD2* gene was increased with all isomers up to 8-fold within 30 min up to 6 h of incubation as shown in Figure 55. After 24 h incubation, the expression of *SOD2* was still increased with all isomers but then dropped to approximately 4-fold higher than the control (data not shown). While expression of *FAD2.1* seemed less pronounced throughout the treatments, the expression of *FAD2.2* was strongly increased in the maize roots. Upon incubation with BOA-6-OH, *FAD2.2* was increased from 30 min to 6 h up to 4-fold, and incubation with BOA-5-OH resulted in a 7-fold or even higher expression (Figure 55). While *CAT1* showed a low response pattern during the treatments, *CAT3* expression was slightly increased after incubation with BOA-4-OH or BOA-6-OH and up to 2- to 3-fold after 6 h incubation with BOA-5-OH or BOA-7-OH (Figure 55). The pathogen-related genes *PR1*, *PR4*; *NPR1*, and *POX12* showed an overall low response to the BOA-OH isomers. Incubation of maize for 6 h with BOA-5-OH and BOA-6-OH resulted in a 2-fold higher expression of *PR4*, indicating possible stimulations of pathogen-related defense mechanisms (Figure 55). *PR1* on the other hand showed a response to BOA-4-OH and BOA-7-OH after 24 h of incubation (data not shown).

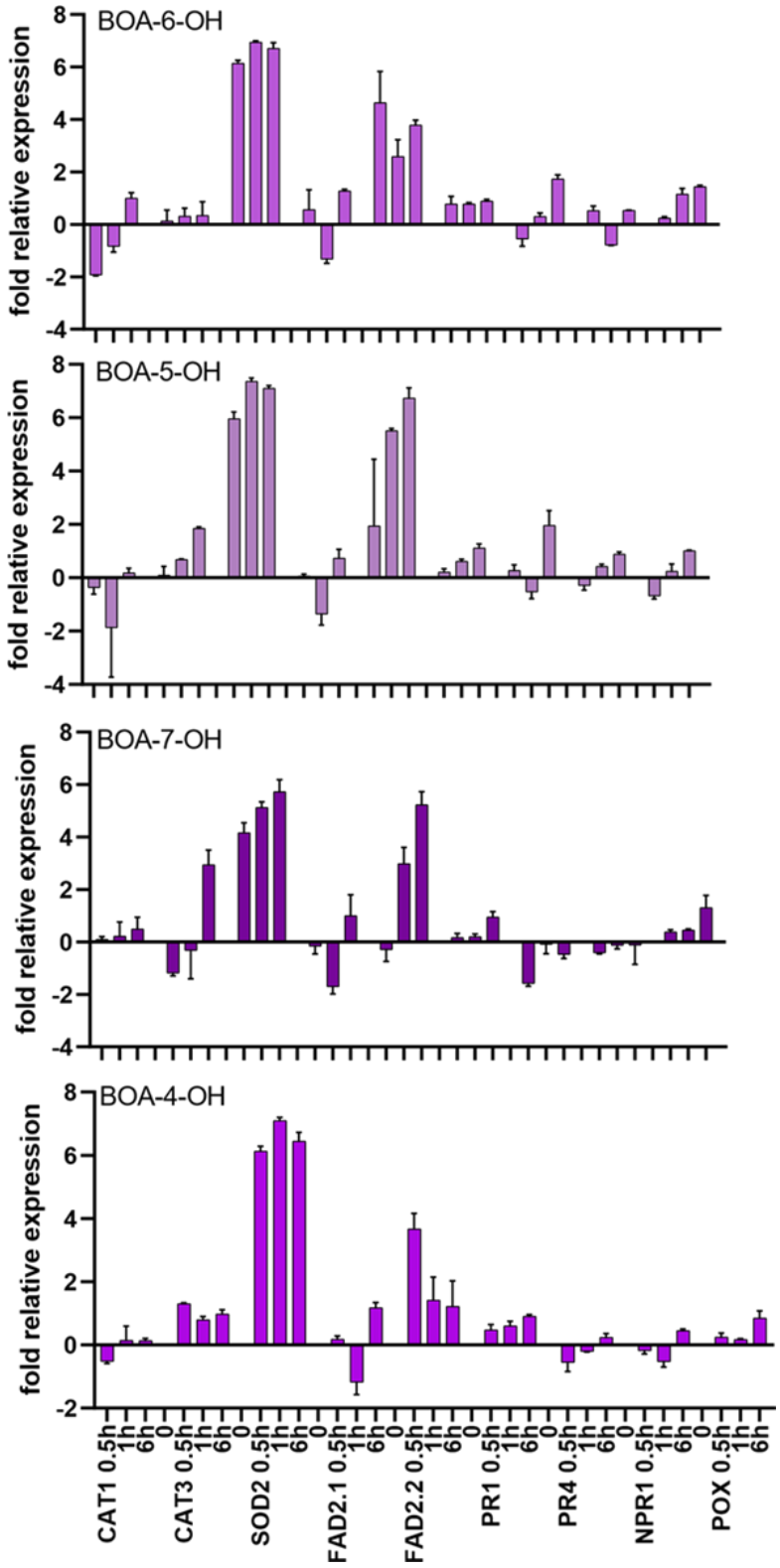


Figure 55: Gene expression of *Z. mays* roots upon exposure to BOA-OH isomers.
Gene expression of selected genes was analyzed in *Z. mays* roots after 0 h, 0.5 h, 1 h, and 6 h incubation with the BOA-OH isomers BOA-4-OH, BOA-5-OH, BOA-6-OH, and BOA-7-OH. Relative transcript abundance based on fold changes (log2-ΔΔCt) is shown for ROS-related genes *SOD2*, *CAT1*, and *CAT3*, lipid repair genes *FAD2.1* and *FAD2.2*, and pathogen response genes *PR1*, *PR4*, *NPR1*, and *POX12*. n = 3, mean ± SD. Figure published in Laschke et al., 2022.

4.12. Impact of Plant Secondary Metabolites on Bacterial Lipids

Bacteria lack a cell wall and therefore, their membrane represents the barrier that first gets in contact with environmental influences. Therefore, exposure of bacteria to BOA, gramine, or quercetin results in first contact of the lipid bilayer with the metabolites which might affect the membrane. While it is known that bacteria adapt to stress conditions like phosphate deprivation and heat with changes in their lipid composition, the impact of plant secondary metabolites on the lipids is not well studied. *Arthrobacter sp.* GB1 and CB4 and *Pseudomonas sp.* CB4 and MPI9 were incubated for 12 h with 500 µM of either BOA, gramine, or quercetin. Control conditions were selected by adding the same amount of DMSO. Total lipid extracts were analyzed via Q-TOF MS/MS, and lipids present in the bacterial strains were identified by their characteristic fragmentation patterns. The two *Arthrobacter sp.* GB1 and CB4 have the same lipid composition. Both strains contained monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), phosphatidylinositol (PI), and phosphatidylglycerol (PG) as their main lipids. The two *Pseudomonas sp.* CB3 and MPI9 contained only PG and phosphatidylethanolamine (PE) as their main lipids.

PG was identified using the fragmentation pattern of a reference standard (Figure 56, A). The parental ion was measured as an ammonium adduct with a total mass of 712.3958 m/z (Figure 56, A). The characteristic loss of the head group (neutral loss) could be measured with 189.0243 m/z resulting in a DAG with the size of 523.3715 m/z, allowing the identification of PG (Figure 56, A). According to the fragmentation pattern of the PG molecular species presented here, the PG molecule could be identified as 30:0 PG with a total mass of 694.4783 m/z (Figure 56, A).

The fragmentation pattern of PI as an ammonium adduct with a mass of 828.4335 m/z resulted in a 551.3981 m/z DAG (Figure 56, B). Calculation of the neural loss of 277.0354 m/z led to the identification of the lipid as a 32:0 PI species with a total mass of 810.5256 m/z (Figure 56, B).

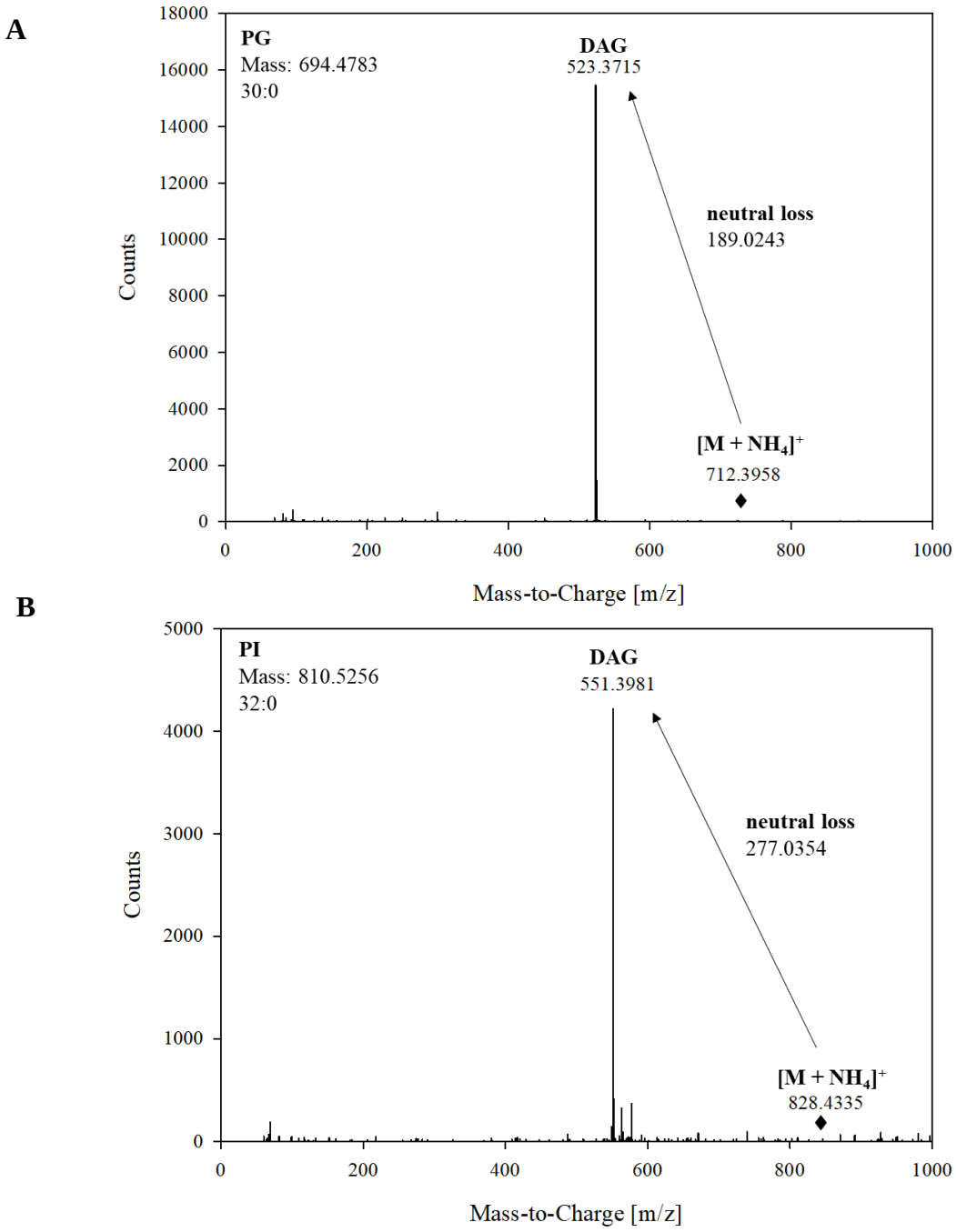


Figure 56: Q-TOF MS/MS fragmentation pattern of phospholipids isolated from *Arthrobacter sp.* GB1.

A: Fragmentation pattern of the ammonium adduct of the 30:0 PG species with a mass of 712.3958 m/z in a diacylglycerol (DAG) with 523.3715 m/z. The loss of the head group (neutral loss) of 189.0243 m/z is characteristic for PG.

B: Fragmentation pattern of the ammonium adduct of the 32:0 PI species with a mass of 828.4335 m/z in a diacylglycerol (DAG) with 551.3981 m/z. The loss of the head group (neutral loss) of 277.054 m/z is characteristic for PI. The lipids were isolated from *Arthrobacter sp.* GB1.

PE with a mass of 690.4566 m/z, was present in its protonated form and not as an ammonium adduct (Figure 57). Fragmentation of the parental ion resulted in a DAG with a mass of 549.4290 m/z, revealing a neutral loss of 141.0276 m/z (Figure 57). By the loss of a head group of 141.0276 m/z, the lipid was confirmed to be PE 32:1 with a total mass of 689.4994 m/z (Figure 57).

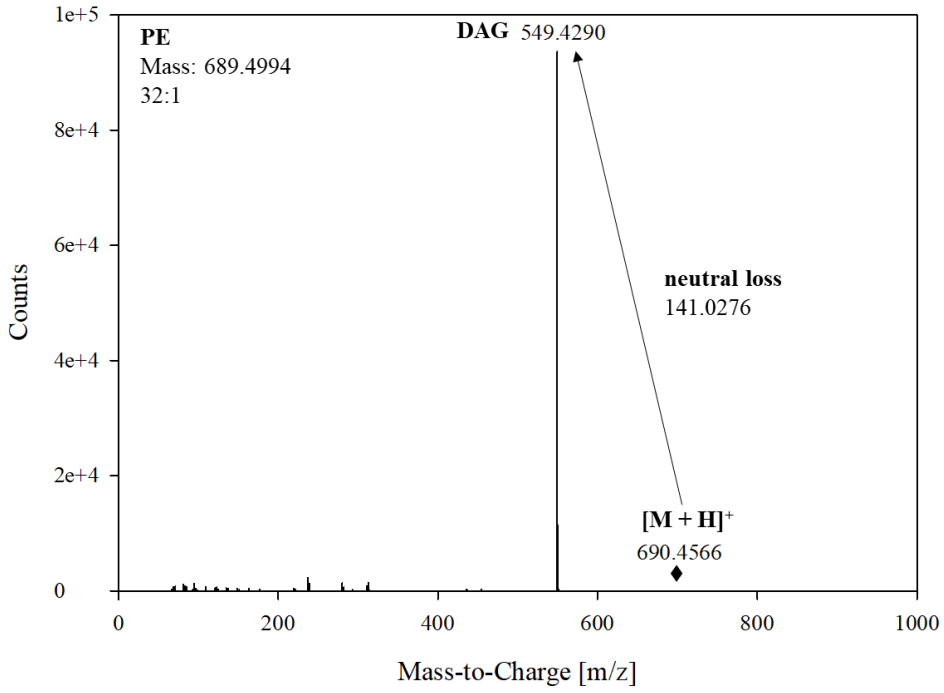


Figure 57: Q-TOF MS/MS fragmentation pattern of phosphatidylethanolamine (PE) isolated from *Pseudomonas sp.* CB3.
Fragmentation pattern of the protonated 32:1 PE species with a mass of 690.4566 m/z in a diacylglycerol (DAG) with 549.4290 m/z. The loss of the head group (neutral loss) of 141.0276 m/z is characteristic for PE. The lipid was isolated from *Pseudomonas sp.* CB3.

The parental ion of MGDG with a total mass of 732.4267 m/z was also fragmented as an ammonium adduct (Figure 58, A). The fragmentation pattern resulted in DAG with a mass of 535.4114 m/z indicating a neutral loss of 197.0153 m/z (Figure 58, A). This neutral loss is characteristic for the loss of the head group of MGDG, therefore this lipid could be identified as a 31:1 MGDG with a total mass of 714.5280 m/z (Figure 58, A).

The lipid with the highest mass of 882.4608 m/z could be identified as an ammonium adduct of DGDG (Figure 58, B). The loss of the head group with a size of 359.0906 m/z resulting in a DAG of 523.3702 m/z (Figure 58, B) is characteristic for DGDG. Nevertheless, the fragmentation pattern indicated a possible change in the sugar moiety of the DGDG. The difference to a reference DGDG fragmentation pattern (data not shown) suggested mannitol instead of glucose as the sugar. Since the identification of the sugar moiety required further analysis and characterization the here identified lipid will be further referred to as DGDG.

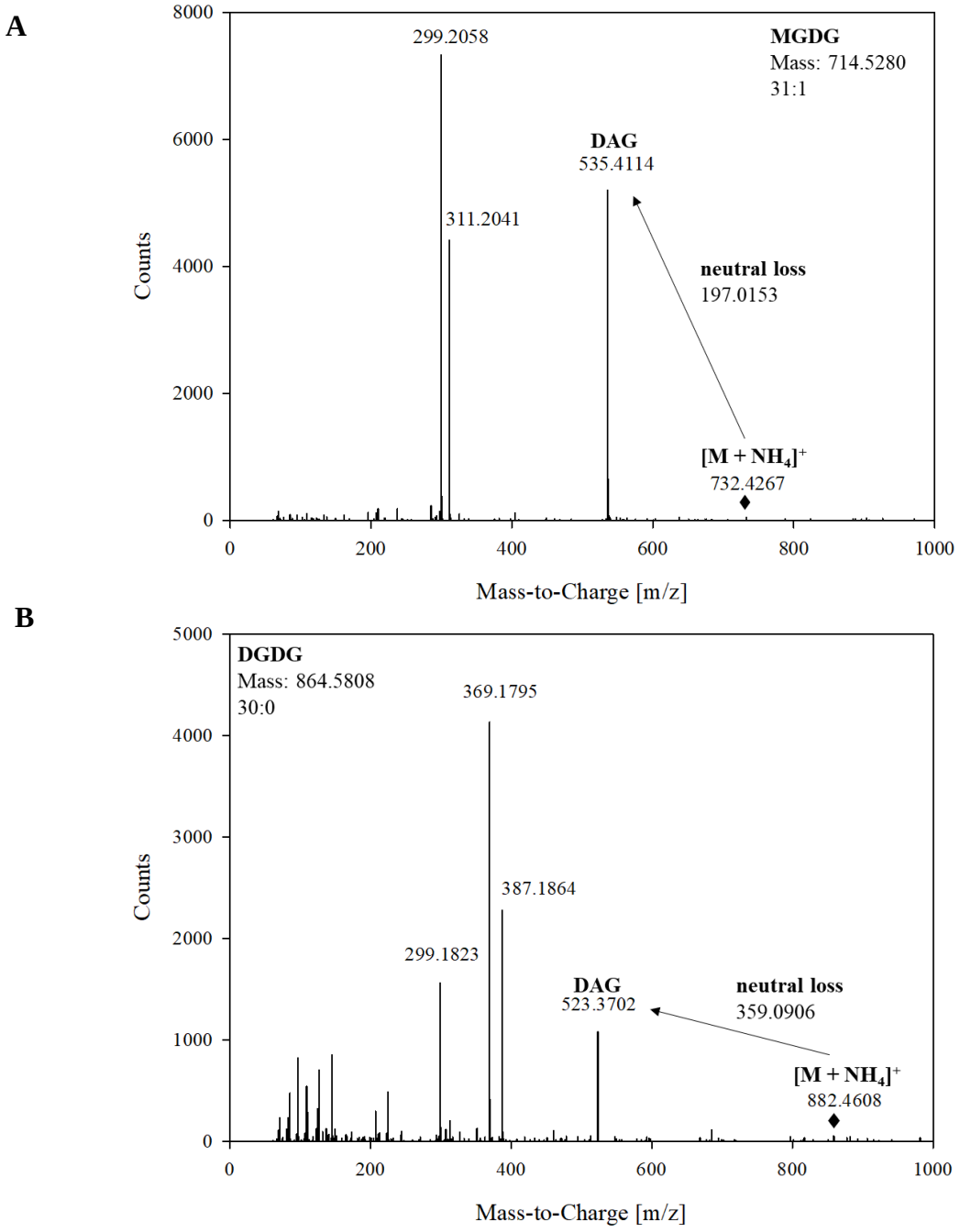


Figure 58: Q-TOF MS/MS fragmentation pattern of galactolipids isolated from *Arthrobacter sp.* GB1.

A: Fragmentation pattern of the ammonium adduct of the 31:1 MGDG species with a mass of 732.4267 m/z in a diacylglycerol (DAG) with 535.4114 m/z. The loss of the head group (neutral loss) of 197.0153 m/z is characteristic for MGDG.

B: Fragmentation pattern of the ammonium adduct of the 30:0 DGDG species with a mass of 882.4608 m/z in a diacylglycerol (DAG) with 523.3702 m/z. The loss of the head group (neutral loss) of 359.0906 m/z is characteristic for DGDG. The lipids were isolated from *Arthrobacter sp.* GB1.

Total lipid extracts were separated by TLC and subsequently visualized with iodine to compare the overall composition of the treatments. *Arthrobacter sp*. GB1 showed no changes in the lipid composition and no additional lipids to PI, PG, DGDG, and MGDG could be detected after exposure to BOA, gramine, or quercetin as shown in Figure 59 (A). The band 'b' in the red box in Figure 59 could be identified as residual quercetin. Similar to *Arthrobacter sp*. GB1, CB4 showed no changes or shifts in the overall lipid composition and the main lipids PI, PG, DGDG, and MGDG (Figure 59). The additional band accumulating below PG, highlighted with a red box and the letter 'a', in the gramine treated sample was identified as gramine (Figure 59, B). As already shown before, *Arthrobacter sp.* CB4 was not able to degrade gramine and therefore, it could be detected for CB4 but not for GB1. Similar to GB1, quercetin was accumulated in quercetin treated CB4 cells. The high concentration of quercetin in this lane disturbed the migration of the lipids (Figure 59 (B)). As previously shown, *Arthrobacter* sp. GB1 was able to degrade quercetin faster than CB4, and therefore, the amount of quercetin in GB1 was lower than in CB4, and lipid separation and visualization were not interfered with (Figure 59 (A)).

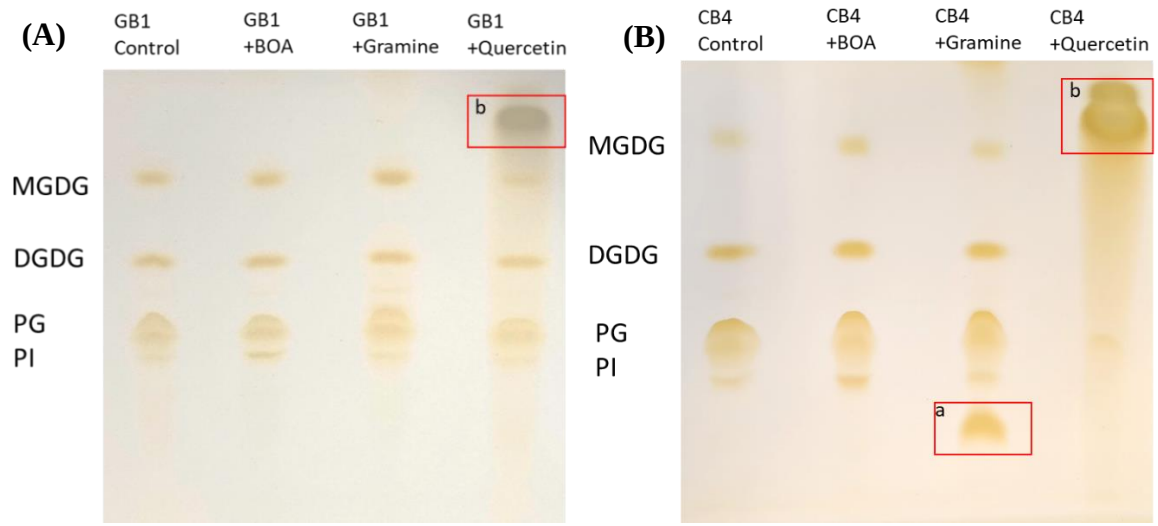


Figure 59: TLC separation of *Arthrobacter sp.* GB1 and CB4 lipid extracts after exposure to plant secondary metabolites.
Arthrobacter sp. GB1 **(A)** and CB4 **(B)** lipids were extracted after the cells were exposed to 500 µM BOA, gramine, quercetin, or DMSO as control for 12 h. After separation via TLC, the lipids were stained with iodine. The red box with the letter 'a' highlights gramine and 'b' highlights quercetin. MGDG: monogalactosyldiacylglycerol; DGDG: digalactosyldiacylglycerol; PG: phosphatidylglycerol; PI: phosphatidylinositol.

The *Pseudomonas sp.* CB3 and MPI9 lipid composition consist of the two phospholipids PG and PE (Figure 60). Q-TOF MS/MS analysis of the total lipid extract showed no accumulation of further lipids (data not shown). This result was also confirmed via TLC separation, where no additional bands accumulated. As shown in Figure 60, PG and PE could not be separated by the selected TLC method due to their high concentration and similar polarity. Nevertheless, gramine and quercetin were separated via TLC plates highlighting that the organisms were not capable to degrade the compounds within the given time.

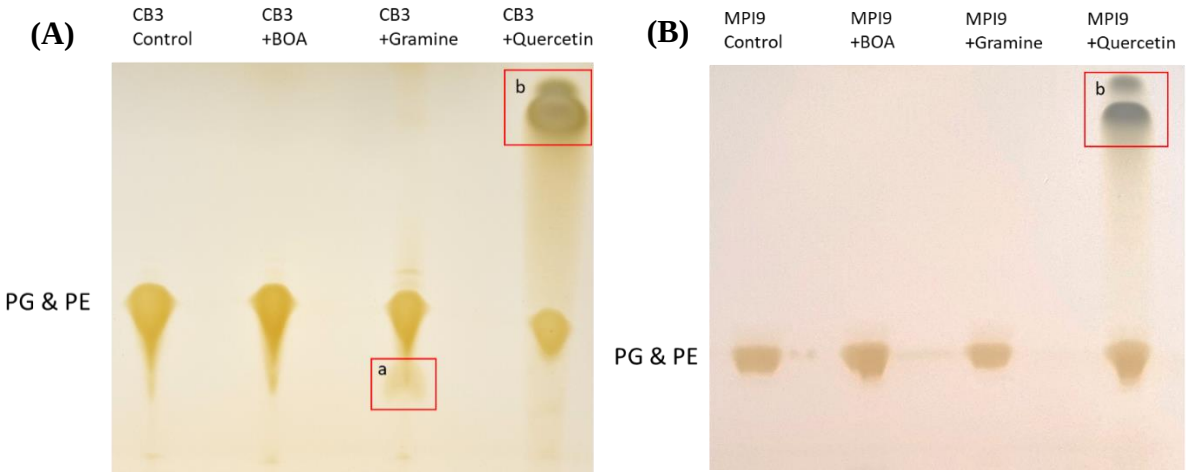


Figure 60: TLC of *Pseudomonas sp.* CB3 and MPI9 lipid extracts after exposure to plant secondary metabolites.
Pseudomonas sp. CB3 **(A)** and MPI9 **(B)** lipids were extracted after the cells were exposed to 500 µM BOA, gramine, quercetin or DMSO as control for 12 h. Upon separation via TLC the lipids were stained with iodine. Red box with the letter 'a' highlights gramine and the letter 'b' highlights quercetin. PG: phosphatidylglycerol; PE: phosphatidylethanolamine.

4.12.1. Impact of BOA, Gramine and Quercetin on Bacterial Fatty Acids

The phospholipid and glycolipid composition was not strongly changed after incubations with BOA, gramine, or quercetin (Figure 59 and Figure 60). Nevertheless, the fatty acid compositions might be affected. Stress-induced alterations in the fatty acid composition of bacterial membranes are known. Exposure of *Arthrobacter sp*. to phenolic compounds resulted in a shift in the anteiso/iso fatty acid ratio forming a more rigid membrane (Unell et al., 2007). Fatty acids of total phospholipids and glycolipids were converted into FAMEs and measured by GC-MS.

Indeed, the fatty acid composition of the two *Arthrobacter sp.* GB1 and CB4 was changed (Figure 61 and Figure 62) but only after exposure to 500 µM quercetin. An about 8% decrease

of the main fatty acid 15:0anteiso was found (Figure 61). Next, 16:0, 17:0anteiso and 18:0 increased by 2.16 ± 0.11 mol%, 2.87 ± 1.80 mol% and 0.23 ± 0.06 mol%, (Figure 61). Thus, a shift to longer chain fatty acids occurred. This effect was more pronounced with *Arthrobacter sp.* CB4 (Figure 62). The main fatty acid 15:0anteiso was reduced (63.97 ± 0.27 mol%) compared to the control (76.62 ± 0.57 mol%) (Figure 62). Reduction of about 13 mol% of the main fatty acid led to a strong increase of 16:0 by 7.48 ± 2.02 mol%, 17:0anteios by 5.68 ± 1.12 mol%, and 18:0 by 0.74 ± 0.20 mol% (Figure 62). The shift in the fatty acid composition indicated that the membrane adapts to the presence of quercetin.

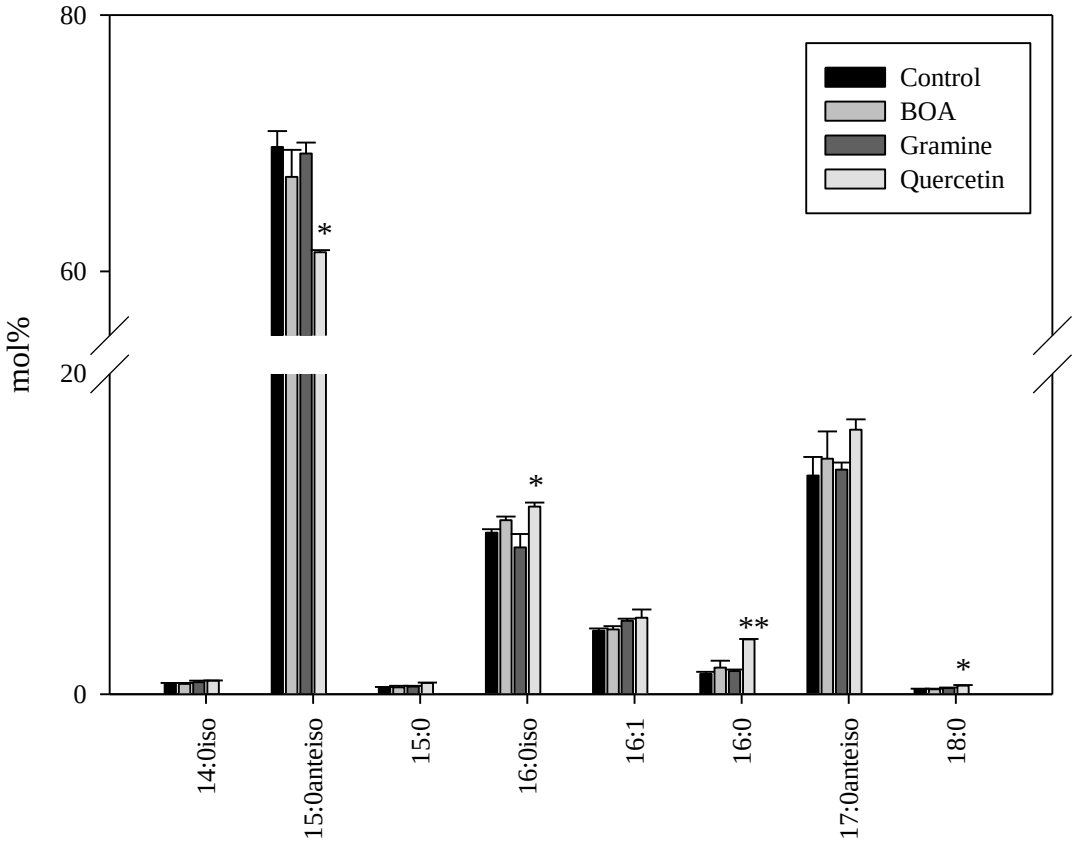


Figure 61: Fatty acid (FA) composition of *Arthrobacter sp.* GB1 after exposure to plant secondary metabolites.
Changes in FA composition of *Arthrobacter sp.* GB1 were determined in mol% via GC-MS of FAMEs. Lipids were extracted from cells which were cultivated with either 500 µM BOA, gramine, quercetin or DMSO as control for 12 h. n = 2 – 3, mean ± SD. Student's t-test (P < 0.05 = *, P < 0.01 = **).

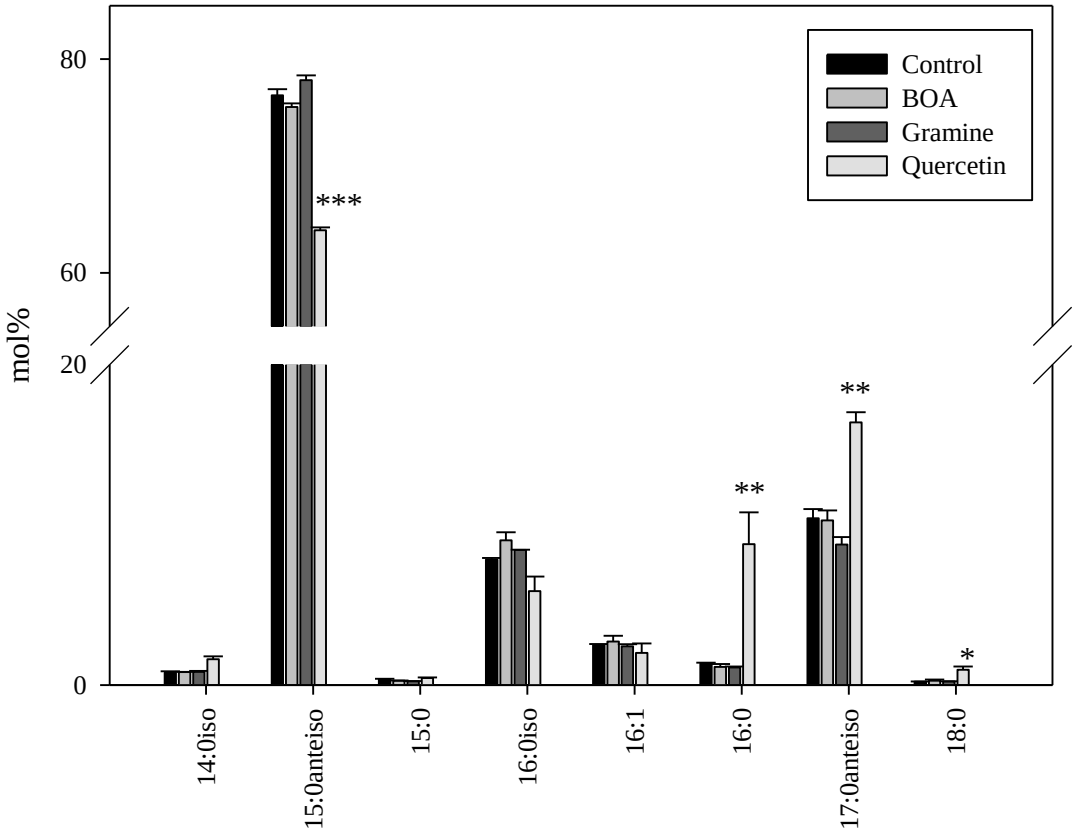


Figure 62: Fatty acid (FA) composition of *Arthrobacter sp.* CB4 after exposure to plant secondary metabolites.
Changes in FA composition of *Arthrobacter sp.* CB4 were determined in mol% via GC-MS after cells were cultivated with either 500 µM BOA, gramine, quercetin or DMSO as control for 12 h. n = 2 – 3, mean ± SD. Student's t-test (P < 0.05 = *, P < 0.01 = **, P < 0.001 = ***).

Both *Pseudomonas sp.*, CB3 as well as MPI9, did not show any response in their fatty acid composition when exposed to 500 µM BOA, gramine, or quercetin (Figure 63 and Figure 64). *Pseudomonas sp*. contains high amounts of 16:0, 17:0cyclo, and 18:1 fatty acids, which might be already sufficient protection against nonpolar phenolic compounds (Figure 63 and Figure 64).

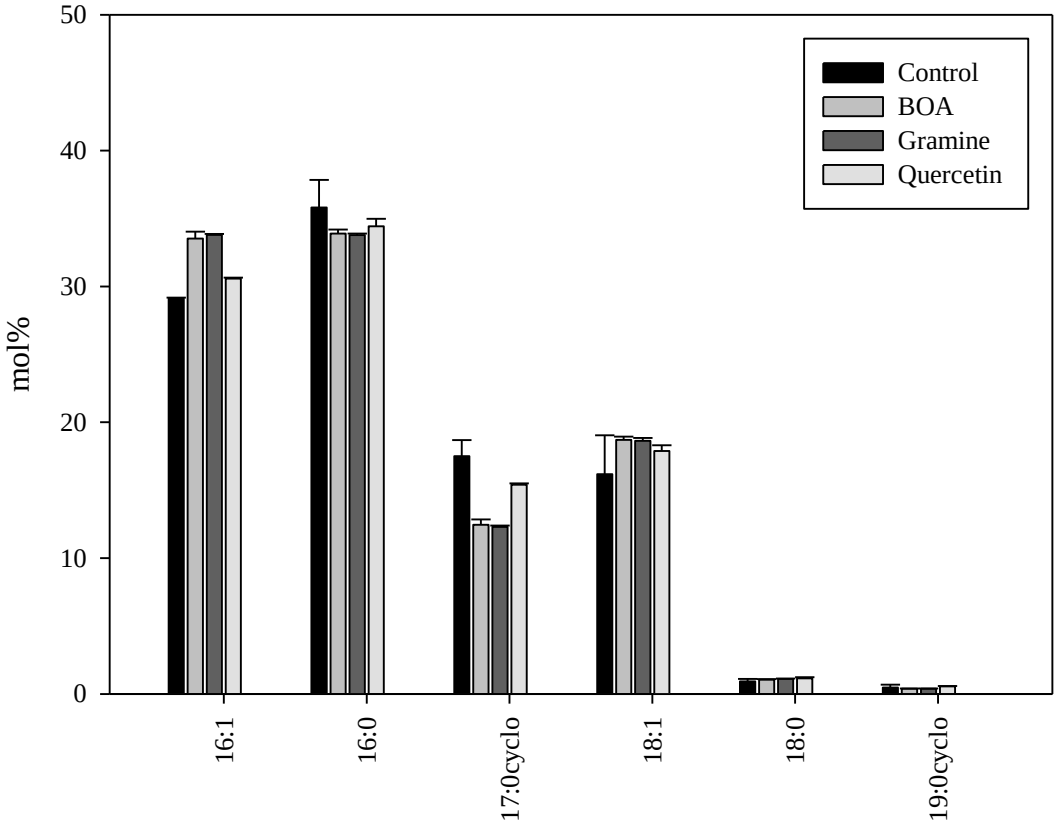


Figure 63: Fatty acid (FA) composition of *Pseudomonas sp.* CB3 after exposure to plant secondary metabolites.
Changes in FA composition of *Pseudomonas sp.* CB3 were determined in mol% via GC-MS after cells were cultivated with either 500 µM BOA, gramine, quercetin, or DMSO as control for 12 h. n = 2 – 3, mean ± SD.

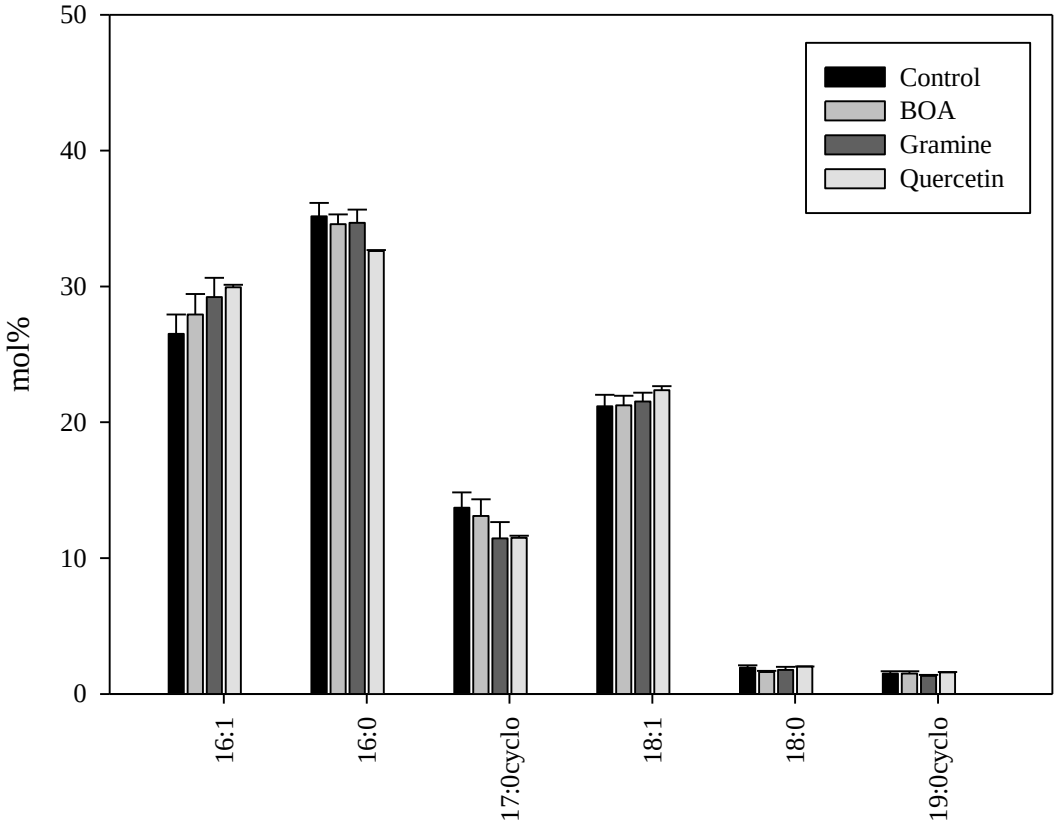


Figure 64: Fatty acid (FA) composition of *Pseudomonas sp*. MPI9 after exposure to plant secondary metabolites.
Changes in FA composition of *Pseudomonas sp.* MPI9 were determined in mol% via GC-MS after cells were cultivated with either 500 µM BOA, gramine, quercetin or DMSO as control for 12 h. n = 2 – 3, mean ± SD.

5. Discussion

The different approaches to study the role of plant secondary metabolites in plant-soil microbiota interactions shed light on their mode of action. BOA, gramine, and quercetin differed in their shaping of bulk soil microbiota with BOA mainly deterring organisms, gramine attracting potentially beneficial bacteria, and quercetin also mainly attracting bacterial strains but also deterring a number of soil organisms. This result highlights the importance of secondary metabolites in microbiota studies. Furthermore, degradation products of the initially utilized BOA, gramine, and quercetin indicate the use of the metabolites as an additional carbon source in the soil but also showed differences in their biological activity by either improving plant growth of certain plant species or harming others.

5.1. Relevance of the Amounts of Plant Secondary Metabolites Added to the Soil

In the early 1500s, the Swiss doctor and alchemist Theophrastus Bombast von Hohenheim, also known as Paracelsus, insisted on the importance of concentrations of substances. He then proposed the famous quote ‘Alle Dinge sind Gift, und nichts ist ohne Gift; alleine die Dosis machts, dass ein Ding kein Gift sei. (Poison is everything, and no thing is without poison. The dosage makes it either a poison or a remedy.)’. This quote is also important for today’s science since certain effects can be artificially achieved by increased concentrations of even daily consumed substances like sugars and salts.

To study the modulation of the soil microbiome by the plant secondary metabolites BOA, gramine and quercetin, concentrations were chosen according to values occurring in nature. Addition of secondary metabolites every other day for a period of 28 days to the soil resulted in an accumulating amount of approximately 0.4 µmol/g soil. This value is in agreement with other studies. The concentration of benzoxazinoids released into the soil by rye can vary between 0.5 – 5 kg/ha in field experiments (Barnes and Putnam, 1987; Reberg-Horton et al., 2005). Furthermore, measurements of root exudates showed that BOA can reach concentrations up to 30 µmol/g soil (Understrup et al., 2005). Concentrations between 2.032 – 5.290 µmol/g gramine were measured in young barley tissue (Maver et al., 2020). Data for quercetin concentrations in soil is less available. Studies showed that white clover released up to 0.5 µmol/g soil quercetin-glycoside (Carlsen et al., 2012). Wang et al. showed that up to 120 µmol/g soil of the quercetin-related flavonoid luteolin were released by peanut plants (Wang et al., 2018).

5.2. Absence of Fungi and Oomycetes in Experimental Soil

During the recent years, the methods for extraction of gDNA from soil were optimized and commercially available kits allowed an efficient and high yield of gDNA from soil. Nevertheless, challenges in the amplification of soil gDNA remained. DNA extracted from soil contains proteins as well as humic acid which inhibit PCRs by impacting DNA polymerase efficiencies (Wang et al., 2013). Therefore, digestion of soil gDNA with proteases and further purification is crucial for the amplification of microbial sequences. While these contaminations can be removed easily, more impactful contaminants are also present in gDNA extracted from soil. Despite optimization of extraction procedures, heavy metals, as well as organic substances, are bound in traces to the gDNA (Fortin et al., 2004). Negligible amounts of heavy metals and organic substances are sufficient to bind nucleic acids or even deactivate polymerases utilized in the PCR (Fortin et al., 2004). The gDNA extracted here was therefore purified several times with magnetic beads to reduce the contamination with substances inhibitory for PCR, and PCR products were digested with proteases. In addition to the purification, DMSO was added to the PCR assay to inhibit the formation of secondary structures of primers and gDNA. In combination with BSA, the PCR yield was increased successfully allowing the amplification of the bacterial 16S rRNA region. Nevertheless, fungal and oomycetal ITS1 regions could not be amplified in amounts that could be visualized on agarose gels despite several adaptations of the PCR conditions including further washing of gDNA, variations of DMSO and BSA concentrations, and utilization of different DNA polymerases. Since it was possible to amplify the ITS1 region from control fungal DNA under all tested conditions, it could be excluded that the reaction conditions were not adequate for the amplification of fungal DNA from soil samples. DNA concentrations of fungal and oomycetal PCR products were fluorometrically determined and were drastically lower, almost not detectable, compared to the bacterial products. Nevertheless, libraries of fungi and oomycetes were prepared and utilized for a first approach of next-generation sequencing which was not successful, and the test of the library revealed a too low amount of the reads with ~300 bp indicating the absence of fungi and oomycetes in the samples. Next generation sequencing was repeated with only the bacterial library and was, as expected, successful. According to the amplification of soil gDNA, fungi and oomycetes were absent at all timepoints (7 days, 14 days, 21 days, and 28 days) in the treated soil, but also in the control and therefore their absence could not be linked to the toxicity of the plant secondary metabolites BOA, gramine or quercetin.

5.3. PLFA Analysis Revealed Absence of Fungi and Oomycetes and Pointed to Stress Related Changes in Soil Bacterial Communities

Additionally to DNA-based methods, the analysis of PLFAs is a common and accepted method to determine the microbial biomass in soil, as well as changes in the composition. Already in the late 1990s, Bååth et al. were able to utilize the method of PLFA analysis to describe changes in the soil microbiome upon exposure to heavy metals (Bååth et al., 1998). Further studies with PLFA analysis allowed to show changes in the soil microbiota after changes in pH, water content, and also after growing genetically modified plants (Rousk et al., 2009; Williams and Rice, 2007; Blackwood and Buyer, 2004). With ~0.15 µg of total fatty acids per g soil, the overall microbial biomass was not impacted by the treatment with BOA, gramine, or quercetin compared to the control (Figure 26), highlighting that changes in the microbiome by the three secondary metabolites are exclusively due to shifts in the composition of the microorganisms. The absence of fungal marker fatty acids like 18:1Δ9 and 18:2Δ8,12 and cyanobacterial marker fatty acids 16:3Δ7,10,13 and 18:3Δ9,12,15 (Frostegård and Bååth, 1996; Kaiser et al., 2010; Ibekwe and Kennedy, 1998) supports the gDNA amplification results, indicating that amounts of fungi and oomycetes in soil are below the detection limit. Nevertheless, shifts in the overall fatty acid composition of the soil microbiota indicated treatment-dependent changes in the soil bacterial community. Since fatty acids measured here are common for the majority of bacterial species, no conclusions for changes of specific taxonomic groups can be made. The accumulation of 17:0cyclo fatty acids after treatment with the three secondary metabolites indicates stress responses of the bacterial species. Cyclopropane fatty acids are commonly synthesized by bacteria in the stationary phase because in liquid media the acidity increases during the stationary phase while the energy source is depleted (Wang and Cronan, 1994). Increasing acidity as well as other stresses presumably cause the stress-dependent synthesis of cyclopropane fatty acids to maintain bacterial survival (Arnold and Kasper, 1995; Brown et al., 1997; Shabala and Ross, 2008). Additionally, the accumulation of cyclopropane fatty acids results in changes in the membrane fluidity as well as a reduction in permeability (Poger and Mark, 2015). Based on this finding it became evident, that BOA, gramine, and quercetin impact the soil microbiota and cause stress in some bacterial species due to the allelopathic character of the compounds.

5.4. The Soil Carbon Content is Crucial for Fungal and Oomycetal Proliferation

The present approach to determine the impact of plant secondary metabolites on soil microbiomes differs from the majority of studies that are related to the field. While most studies used whole plants, of which some were genetically modified, to study the impact of chosen factors on the microbiome (Durán et al., 2018; Hu et al., 2018; Cotton et al., 2019), this project used the pure metabolites. The initial organic carbon content is crucial for the outcome since plants, which exude up to 20% of their photosynthesis products via roots into the soil (Haichar et al., 2008), are not grown in the soil throughout the treatment. Overall, the top part of most soils contains on average 0.5 – 3% organic carbon. A carbon content below 0.5% on the other hand represents desert-like soils. In this study, the utilized soil contained an initial carbon content of 0.279% similar to soil from desert areas and therefore causes harsh conditions for the microbiota. The low carbon content can be explained by the fact, that Cologne agriculture soil was not used for agricultural approaches for over 15 years. Therefore, it is very likely that fungi and oomycetes could not proliferate in the soil. Other studies highlight the importance of plants for fungal and oomycetal accumulation. Durán et al. used the same soil but introduced plants to it for their studies and were able to detect fungal species (Durán et al., 2018).

Summarizing the results of the gDNA amplification, PLFA analysis, and the initial carbon content measurements, it can be concluded, that the soil is deficient in fungi and oomycetes, which might be present in extremely low abundances or as spores for storage under the harsh conditions. The introduction of plants into Cologne agriculture soil allows the proliferation of fungi and oomycetes as shown by other studies.

5.5. Importance of Secondary Metabolites in the Shaping of the Soil Microbiota

The importance and impact of plant secondary metabolites as key substances for shaping the microbiome has been highlighted by many studies in the last years. It has been shown that interactions with probiotic rhizobacteria with plants stimulate the synthesis and exudation of scopoletin by the plant (Stringlis et al., 2018). Accumulation of scopoletin resulted in the beneficial shaping of the soil microbiota and increased plant growth and competitiveness by the attraction of favorable organisms (Stringlis et al., 2018). Furthermore, studies revealed the importance of coumarin synthesis during periods of iron deficiency. Coumarin accumulation resulted in the attraction of bacterial strains in the microbiota that are able to assist the plant by increasing iron availability and uptake (Harbort et al., 2020). Plants differ in their mechanisms

to deter or attract microorganisms and therefore in their soil microbiota. Monocultures or a loss in species diversity will therefore not only result in changes in the microbiomes but also will negatively impact yields in agriculture (Köberl et al., 2020; Korenblum et al., 2020; Li et al., 2019). This project focused on the role of the two indole metabolites BOA and gramine and the flavonoid quercetin in shaping a bulk soil microbiome.

5.5.1. Changes of the Soil Microbiome do not Depend on Exposure Time with BOA, Gramine, or Quercetin

Alpha diversity indices revealed significant changes in the soil microbiome if incubated with BOA or quercetin. Treatment with gramine on the other hand resembled the pattern of the control treatment for Shannon, Faith PD, and Evenness indices. This indicates that the species diversity after gramine treatment overlaps with the control and shifts in the overall diversity after BOA or quercetin treatment. These changes were not time-dependent as depicted in Figure 18. Nevertheless, slight changes over time suggest a clustering of the first two weeks and the last two weeks of soil incubation with secondary metabolites. The negligible difference in the time dependency of bacterial 16S rRNA gene community profiling could represent the actual mode of action in the field with living plants. Short reproduction cycles of bacterial strains result in the fast adaption of the communities to environmental changes or stresses. Furthermore, gramine, for example, is only synthesized in young plant tissues (Grün et al., 2005; Larsson et al., 2006) and therefore, its impact on the soil microbiome is limited to a short period of time. Generally, exudation of defense substances might require a fast response since plants might suffer from non-profitable interactions.

The PCA plot revealed further changes in the bacterial community structure (Figure 19). BOA treatment clustered separately from gramine and quercetin but overlapped with late timepoints of control treatment. Early timepoints of gramine treatment were slightly separated from late timepoints which overlapped with early timepoints of the control. Quercetin treatment, on the other hand, was separated from all treatments for all timepoints. The absence of plant material also causes the absence of additional carbon sources for bacterial growth. Over time, the initial carbon content of the control soil was further depleted, and the bacterial community faced harsher conditions, which explained the separation of early control timepoints (t7 and t14) from late timepoints (t21 and t28). Overlap of BOA with late control timepoints suggested its possible toxicity to soil bacteria. BOA is poorly metabolized by bacterial strains and so far, only few reports were published on BOA degrading bacteria (Friebe et al., 1998; Kettle et al.,

2015; Glenn et al., 2016). While BOA may not be utilized as an additional carbon source by soil bacteria, gramine and quercetin showed different clustering patterns (Figure 19). The overlap of early timepoints of the control with late timepoints of gramine treatment suggests a possible metabolization of gramine but also indicates high diversity of the bacterial community. After an initial change in the soil bacteria composition, the adapted community might be able to degrade gramine which serves as an additional carbon source, since the treatment overlapped with the control conditions where the initial carbon content was at its highest concentration. Since quercetin treatment did not overlap with late control timepoints nor any BOA timepoints, it can be assumed that the soil bacteria were also able to metabolize quercetin. Nevertheless, the metabolization of quercetin resulted in changes in bacterial community structure that were not related to any other treatment indicating a specific microbiome shaping of this flavonoid. This hypothesis is in accordance with other studies, that highlighted the importance of metabolic active bacterial strains that contribute to changes in soil parameters and subsequently in microorganisms' community structures by degradation or detoxification of diverse substances (Pileggi et al., 2020; Korenblum et al., 2020).

5.6. BOA, Gramine, and Quercetin Differ in Their Toxicity on Phylum Level

Changes in the soil bacteria community level agreed with the alpha indices and PCA level predictions. The bacterial community structure of control and gramine treatment was similar, while the patterns of the treatment with BOA and quercetin were changed (Figure 20). Only Patescibacteria were drastically decreased after gramine treatment, with a slight increase of Proteobacteria. Maver et al., on the other hand, were able to show that the cultivation of gramine-containing barley cultivars resulted in an inhibition of a broad spectrum of microorganisms in the rhizosphere but the impact of gramine on bulk soil remained to be clarified (Maver et al., 2021). Chloroflexi decreased drastically in relative abundance after BOA and quercetin (~2%) treatment compared to the control and gramine where the phylum remained at ~10%. These results revealed the strong toxicity of BOA and quercetin on bacterial species of Chloroflexi. Additionally, the relative abundance of Actinobacteriota was increased after exposure of soil to BOA compared to the other treatments, while upon treatment of soil with quercetin Actinobacteria were decreased to the lowest level of all treatments with a strong increase of Proteobacteria relative abundance. Changes on the phyla level already highlighted the differential impacts of the three secondary metabolites on the bacterial community structure. The data suggests that the flavonoid quercetin is mainly metabolized by members of

Proteobacteria, which dominate the bulk soil. While gramine is mainly toxic to Patescibacteria, analysis on the phylum level suggests, that a variety of bacterial strains from different phyla are able to cope with this metabolite. The strong accumulation of Actinobacteriota after BOA treatment suggests high tolerance of bacterial strains of this phylum. No studies have been conducted so far to assess the mode of action of BOA, gramine, or quercetin on the bulk soil microbiota. Nevertheless, several studies have been carried out with *Z. mays* plants (mutated in their benzoxazinoid synthesis), revealing the negative effects of benzoxazinoids on specific bacteria (Hu et al., 2018; Kudjordjie et al., 2019; Cotton et al., 2019).

5.7. BOA Mainly Functions by Deterring Microorganisms in the Soil

In-depth analysis revealed that BOA impacted overall the soil microbiome with negative effects. Only 10 ASVs were increased after the treatment and 11 ASVs were decreased. The majority of increased ASVs belonged to *Arthrobacter* species and the *Arthrobacter*-related strains *Pseudarthrobacter* and *Paenarthrobacter* (Figure 22). Changes in the relative abundance of selected ASVs showed the highest increase after BOA treatment for *Arthrobacter* ASV2, *Pseudarthrobacter* ASV1, and unclassified *Micrococcaceae*. Especially unclassified *Micrococcaceae* reached a relative abundance of ~30% after 28 days, dominating the bulk soil. *Arthrobacter* ASV5 and *Pseudomonas* ASV4 showed a high relative abundance for the early timepoints of soil treatment, and the content decreased during the course of incubation. This might indicate that these bacterial strains were not able to degrade BOA and suffered under starvation once the initial carbon was depleted. The genera of *Arthrobacter* and related organisms belong to, *Micrococcaceae*, a family widespread in bulk soil and known for its drought and starvation resistant members (Knief et al., 2020). Furthermore, Deutch et al. showed that especially *Paenarthrobacter* and related strains played an important role in xenobiotic degradation (Deutch et al., 2018). In agreement with those findings, it is very likely that the few ASVs accumulating after BOA treatment belonged to the group of bacterial strains that are able to metabolize BOA and use it as a carbon source. BOA's toxicity was especially visible for Chloroflexi (4 ASVs) and Proteobacteria (3 ASVs). The decrease of a *Mycobacterium* ASV agrees with several studies that were able to show that organisms from this genus are sensitive to BOA (Atwal et al., 1992; Schütz et al., 2019).

Bacterial interactions with BOA were further analyzed in liquid cultures. Growth of two *Arthrobacter sp.* (GB1 and CB4) and two *Pseudomonas sp.* (CB3 and MPI9) in minimal media, containing glucose as a carbon source, was not impaired or increased upon exposure to BOA

(Figure 27 – Figure 30). According to the generation time G, all tested strains were tolerant to BOA but a metabolization of the substance was unlikely since the OD_{600} did not increase compared to control cultures. This highlights the complex interactions in soil microbiota and shows that organisms that are not able to degrade the substances can still be important for plants since they are able to survive in the presence of BOA. Furthermore, this study highlights that member of the microbiota can be tolerant to allelochemicals despite not being able to degrade those.

5.7.1. BOA Detoxification – A Complex Network

None of the tested bacterial strains were able to cleave the heterocycle and further degrade BOA. Nevertheless, BOA conversion products were found after two days in soil. Since the whole amount of applied BOA was extracted right after the addition, it can be excluded that BOA binds to particles in the soil (Figure 47). After two days, only a minor proportion of the initially applied BOA was extracted, and utilizing HPLC-DAD, the BOA conversion product phenoxazinone (APO) could be detected. APO results from oxidative dimerization of AP and can be found in soil for up to several months (Zikmudova et al., 2002). In fact, studies showed that APO is more toxic than BOA, and its impact on the modification of the soil microbiome has to be accounted for (Fomsgaard et al., 2004; Macías et al., 2005). Due to the relatively low APO concentration in soil after two days, it can be assumed that a part of the initially applied BOA was degraded. Takenaka et al. (1988), identified a *Pseudomonas sp*. that degraded AP. Since it can be assumed that at a certain point AP was present in the soil, the degradation of AP by *Pseudomonas* sp. cannot be excluded. The presence of BOA-derived compounds in the soil highlights the highly complex interactions in microbiota. Conversion of the secondary metabolite into more toxic or easier metabolized compounds might be the key factor for these interactions. Therefore, the bacterial/fungal AP conversion product AAP was used to further understand the interplay of soil bacteria. In fact, *Arthrobacter sp.* MPI764, unable to degrade BOA, converted AAP into its nitrated forms 3-N-AAP and 5-N-AAP (Figure 45 and Figure 46). Nitrated metabolites are known to be more bioactive and therefore, it can be assumed that the presence of BOA in soil will impact microorganisms as well as other plants.

5.7.2. BOA-Derived Metabolites Impact Plants and Possibly Predators

While BOA did not affect *A. thaliana* at concentrations up to 100 µM, BOA-derived substances revealed interesting insights into complex interactions of the secondary metabolites with other plants on expression levels. Bacterial-derived nitrated AAP (5-N-AAP) resulted in a strong upregulation of the expression of geranyllinalool synthase *TPS04* (Figure 54). The induction of *TPS04* upregulation in Arabidopsis was reported after exposure to fungal pathogens, moths, or bacterial pathogens like *Pseudomonas syringae macilicola* (Herde et al., 2008). The upregulation of this enzyme results in the accumulation of geranyllinalool which, on the other hand, may function as a defense compound against herbivores and other pathogenic organisms since it is capable to interfere with the sphingolipid metabolism as shown recently (Li et al., 2021). By inhibiting the serine *C*-palmitoyltransferase, geranyllinalool might inhibit the ceramide synthesis in the herbivores. Since the exposure to 5-N-AAP resulted in such a strong defense response in *A. thaliana,* it can be assumed that it has negative effects on the growth of other plants and might be helpful for the 5-N-AAP producing bacteria to compete against other organisms.

Additionally to bacteria and fungi, plants can modify BOA. One of the products is the toxic hydroxylation product BOA-6-OH (Schulz et al., 2012; Schulz et al., 2018). To assess the full spectrum of the impact of BOA-6-OH, its isomers BOA-4-OH, BOA-5-OH and BOA-7-OH were included in gene expression studies with the benzoxazinoid producing crop plant *Z. mays*. All isomers resulted in a fast increase in the expression of *SOD2* (Figure 55). The increase of this gene in maize plants was already described after aphid-related stress (Sytykiewicz, 2014). Upregulation of *SOD2* highlights the necessity of superoxide radical detoxification and thus the stimulation of ROS in root tips by the allelochemicals. This result highlights the importance of detoxification of BOA-6-OH by glycosylation in certain plants (Hofman et al., 2006), and the relevance of harmful effects of BOA-related compounds not only on soil bacteria but also on other plants. Furthermore, enhanced expression of *FAD2-2,* a desaturase involved in linoleic acid synthesis, strengthened the importance of the detoxification of BOA-6-OH and its isomers. Increased linoleic acid synthesis in *Z. mays* root tips might be induced due to damage to the plant membrane by the BOA-OH isomers. BOA-6-OH therefore might result in membrane damage which requires fast repair to maintain plant survival, possibly by increasing the linoleic acid synthesis (Hernández et al., 2009). While this study is the first to describe increased *FAD2-2* induction by allelochemicals, Dar et al. (2017) showed that expression of *FAD2* played an important role in cold stress-related reactions of the plants.

The analysis of plant interaction with metabolites highlighted the importance of plant secondary metabolites and their degradation products not only in the shaping of the microbial community but also by pointing out their toxic effects on other plants.

5.8. Gramine Functions as an Additional Carbon Source for Soil Bacteria

Different than BOA, treatment of Cologne agriculture soil with the indole-derived gramine resulted in an increase of a total of 35 ASVs, indicating its function by attraction of potentially beneficial bacteria (Figure 22). Besides *Pseudarthrobacter* ASV2 and two Bdellovibrionota ASVs, gramine attracted exclusively members of Proteobacteria. Especially *Massilia* ASV3 showed a high relative abundance compared to the other organisms that were increased after gramine treatment. Within the course of the incubation, the relative abundance of *Massilia* ASV was decreased to control treatment level. This might result from the absence of a carbon source and indicated that the organism was not capable to degrade gramine. Accumulation of *Novosphingobium* and *Massilia* ASVs supported the hypothesis of attraction of beneficial bacteria. Bacteria from these genera are known for their auxin and siderophore production and therefore might contribute to plant growth and fitness (Ofek et al., 2012; Rangjaroen et al., 2017). The accumulation of *Bdellivibrio* and *Bacteriovorax* ASVs on the other hand highlighted a new mechanism of microbiome shaping. Davidov et al. (2006) were able to show that organisms from these genera can be predators that feed on other bacterial strains. While this requires further analysis, it may be assumed that these organisms are attracted by gramine to feed on bacteria that are potentially pathogenic for the plant, and therefore indirectly maintain plant survival. With only five Proteobacteria ASVs of which four were *Pseudomonas* ASVs, gramine treatment resulted in the decrease of only a few microorganisms, indicating its main role in bacterial attraction (Figure 23). Only few studies have been conducted to determine bacterial responses to gramine, but in 1990, Sepulveda and Corcuera showed, that gramine impaired the growth of the plant pathogen *P. syringae*. Therefore, it can be assumed that the decreased *Pseudomonas* ASVs may be pathogens for barley. By attraction of a number of beneficial bacteria of the Proteobacteria family, the increase of predator bacteria that might feed on plant pathogens, and the decrease of potential harmful *Pseudomonas* strains, the mode of action in microbiota shaping differed compared to BOA and its role to increase plant competitiveness by plant-bacteria interactions might be revealed.

Arthrobacter sp. (GB1 and CB4) and two *Pseudomonas sp.* (CB3 and MPI9) were utilized to investigate the mode of action of gramine further. Since gramine appeared to function by the attraction of bacteria, it was assumed that bacterial strains could metabolize gramine, different than BOA. The growth rate μ and the generation time G were not impacted by the presence of gramine in the liquid medium, and all tested organisms were able to tolerate gramine (Figure 27 – Figure 30). Surprisingly, the *Pseudomonas* strains tested here were also not negatively affected by gramine as suggested by the next generation sequencing data. Nevertheless, *Arthrobacter sp.* GB1 OD_{600} was increased after 24 h incubation and the death phase started later compared to the control, BOA, and quercetin treatment. This finding supports the hypothesis that bacterial strains are more likely attracted by gramine as an additional carbon source.

5.8.1. Gramine Detoxification via Degradation by Soil Bacteria

As suggested by an increased OD_{600}, degradation of gramine by *Arthrobacter sp.* GB1 could be monitored by HPLC-DAD. Interestingly, *Arthrobacter sp.* CB4 was not able to degrade gramine. This indicates that gramine metabolization is strain-specific, or possibly, that strain GB1 is adapted to gramine detoxification by plasmid uptake. *Arthrobacter sp.* CB4 was isolated from control soil that was not exposed to gramine, and GB1 was derived from soil, that was incubated for 28 days with gramine. While the secondary metabolite and its degradation products were fully metabolized within 24 h, I3A and I3C were detected as gramine-derived degradation products in the minimal medium during the growth of GB1. Ghini et al. (1991) suggested a possible degradation of gramine via its initial conversion to I3C as depicted in Figure 7. Nevertheless, bacterial degradation has not been shown so far, and enzymes involved in the first steps of degradation remain to be identified. Additionally to I3C, I3A was identified as a degradation product. Therefore, it can be concluded that the bacterial degradation of gramine results first in the carboxaldehyde I3A, followed by a subsequent conversion to the carboxylic acid I3C. Both compounds are related to the plant hormone auxins and might therefore play an important role during plant growth. Gramine is mainly synthesized in young barley tissue (Grün et al., 2005; Larsson et al., 2006; Hanson et al., 1983; Lovett et al., 1994). By attracting bacterial strains that can convert gramine to auxin-like compounds, the plants could support their growth by interactions with beneficial bacteria in the important and competitive stages of their life cycle. This hypothesis is supported by the findings depicted in Figure 48 and Figure 49. Two days after the addition of gramine, the bacterial degradation

product I3A was extracted from the soil. Therefore, the auxin-like compound could be available for further plant interactions.

5.8.2. The Bacterial Gramine-Product I3A Causes Increased Plant Growth

As described above, gramine mainly functions by the attraction of beneficial bacteria to improve plant growth. Additionally, to increased nutrient uptake resulting from interactions with beneficial bacterial strains, some bacterial strains support plants by the production of hormones (Ofek et al., 2012; Rangjaroen et al., 2017). Presence of gramine in the soil resulted in the accumulation of the auxin-like compound I3A by bacterial degradation of gramine. While gramine showed no improvement in plant growth at concentrations up to 100 µM and even resulted in plant death at 500 µM, I3A significantly increased the growth of *A. thaliana* at 0.5 µM (Figure 53). This supports the hypothesis of indirect auxin production by bacterial strains that are able to metabolize gramine. Currently, the possible mechanism of I3A function as an auxin remains to be unraveled. Expression studies utilizing known auxin-related genes in plants could help to further understand the role of I3A in beneficial bacterial-plant interactions. Recent studies emphasized the importance of I3A, produced by the gut microbiota of mammals, in interactions with other organisms. Zelante et al. (2021) were able to show that I3A derived from gut bacteria enhanced immunity reactions in the host and plays a role in the improvement of the mammal host´s physiology.

5.9. Quercetin Mainly Attracts Soil Bacteria but also Deters Specific Strains

Treatment of Cologne agriculture soil with the flavonoid quercetin resulted in the increase of 35 ASVs. The pattern was similar compared to gramine. Most of the increased bacteria belonged to the Proteobacteria, and only four ASVs to Actinobacteriota, including *Arthrobacter* species, and two Bdellovibrionota ASVs. *Predibacter* belongs to the group of bacterial strains that feed on other organisms (Davidov et al., 2006). Attraction of this predator genus might be necessary for the plant to avoid the overpopulation of bacteria in the rhizosphere. Quercetin attracted many organisms and therefore, a predator feeding on potentially pathogenic bacterial strains might be required to control the microbial population. Furthermore, many of the attracted bacterial strains were from the *Novosphingobium* and *Massilia* genera. Since these organisms are known to produce auxin and siderophores, it can be concluded that the flavonoid attracts mainly plant growth-promoting organisms (Ofek et al., 2012; Rangjaroen et al., 2017).

Besides the chemoattraction of several bacterial species, quercetin treatment also resulted in the decrease of 17 ASVs. Out of the three different treatments, the decrease in the number of ASVs was the strongest with quercetin (Figure 23). Similar to BOA, especially members of the Chloroflexi family were decreased upon treatment with quercetin. But also, three *Pseudomonas* ASVs, which were also decreased after gramine treatment, were significantly decreased after exposure of soil to quercetin. Especially, the *Sphingobium* ASV2 dominated the bulk soil microbiome after quercetin treatment with a relative abundance of ~27% after 28 days (Figure 24). This result indicates that this strain was able to use quercetin as an additional carbon source and propagate in soil. The high relative abundance of *Arthrobacter* ASV1 indicated, that some organisms from this genus are also able to metabolize quercetin. Metabolization of quercetin by soil bacteria might also be the reason for the decrease of many ASVs. Quercetin degradation occurs via protocatechuic acid (Ajiboye et al., 2017; Babich et al., 2003) which can cause oxidative stress in a number of strains and might therefore be the reason for its toxicity. The full mode of action and the role of quercetin in shaping the microbial community in soil might be explained by the chemoattraction of potentially plant growth promoting bacteria that are able to degrade quercetin via protocatechuic acid, which controls the population by causing oxidative stress in other organisms.

Exposure of two *Arthrobacter sp.* (GB1 and CB4) and two *Pseudomonas sp.* (CB3 and MPI9) strains revealed further interesting insights into the mode of action of quercetin (Figure 27 – Figure 30) supporting the hypothesis posed above. While the generation time G of *Arthrobacter sp.* GB1 was not changed in quercetin supplemented minimal media TSM, OD_{600} values in the first 6 h were strongly reduced compared to control, BOA, or gramine treatment. The growth rate was presumably not impacted due to an elongation of the lag phase of GB1 by 2 h. This highlights the need for the bacterial strain for adaption to the substance to be able to survive. Nevertheless, the OD_{600} after 24 h was higher compared to control and BOA cultures. This indicates a possible metabolization of the substance. *Arthrobacter sp.* CB4 on the other hand showed a different reaction to quercetin. Instead of an adaptation to the flavonoid by a longer lag phase like GB1, CB4 revealed a strongly increased generation time by 30 min. This also resulted in a constantly lower OD_{600} compared to other treatments within the first 6 h. Nevertheless, after 24 h, *Arthrobacter sp.* CB4 also showed higher OD_{600} values which can be explained by the recruitment of quercetin as an additional carbon source. While *Pseudomonas sp.* CB3 did not degrade quercetin, the generation time G was increased by 40 min indicating possible toxicity. But strains reached OD_{600} values after 24 h comparable to the control, and therefore, it can be concluded that CB4 and CB3 were not able to metabolize quercetin. The

strong response of bacterial strains in the first hours of cultivation with quercetin highlights the need for adaptation of the strains to quercetin.

5.9.1. Degradation of Quercetin by Soil Bacteria

As indicated by elevated OD_{600} values of the growth experiments, *Arthrobacter sp.* GB1 and CB4 were both able to degrade quercetin. HPLC-DAD monitoring of the culture extracts revealed a metabolization of quercetin via protocatechuic acid by GB1 and CB4. Pillai and Swarup (2002) demonstrated that quercetin is degraded by *P. putida*. As depicted in Figure 65, quercetin (I) is converted to naringenin (II) which is converted into an unstable product (III). This product is immediately converted to either phloroglucinol (IVa) or 3,4-dihydroxy cinnamic acid (IVb) (Figure 65). Phloroglucinol is not further metabolized by *P. putida* and accumulates in the media. Since phloroglucinol was not detected in *Arthrobacter sp.* GB1 and CB4 cultures, it can be assumed, that the two bacterial strains do not follow this pathway. Further metabolization of 3,4-dihydroxy cinnamic acid finally results in protocatechuic acid in *P. putida* (VII), and this part of the degradation might occur in *Arthrobacter sp.* GB1 and CB4 via the same pathway (Figure 65).

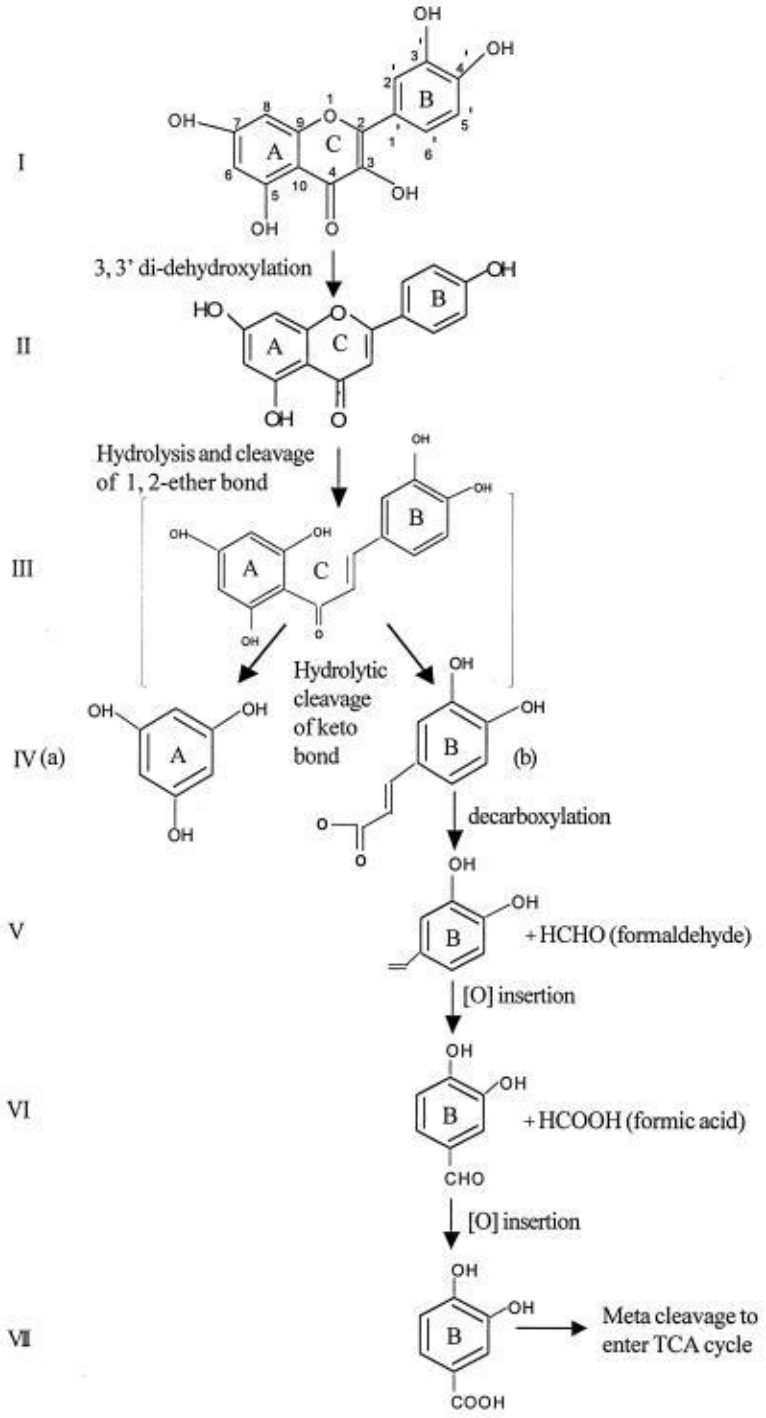


Figure 65: Degradation of quercetin by *P. putida* PML2.
P. putida degrades quercetin (I) via naringenin (II), an unstable intermediate (III), phloroglucinol (IVa), 3,4-dihydroxy cinnamic acid (IVb), 3,4-dihydroxy styrene (V), protocatechuic aldehyde (VI) and finally protocatechuic acid (VII) (Pillai and Swarup, 2002).

The degradation of quercetin via protocatechuic acid by *Arthrobacter sp.* GB1 and CB4 supports the hypothesis that the toxicity of quercetin might result from the oxidative stress caused by protocatechuic acid (Ajiboye et al., 2017; Babich et al., 2003). It also appeared, that even organisms that are able to metabolize protocatechuic acid need to adapt to the conditions

which resulted in lower cell density. *A. thaliana* plants exposed to 100 µM quercetin showed no reduction in their growth which supports the theory, that the toxicity of quercetin is initiated by the degradation of the compound.

5.10. The Ability to Solubilize Phosphate is not Exclusive to Plant Growth Promoting Bacteria

Phosphate solubilizing microbes (PSMs) are often described as plant beneficial organisms (Kalayu, 2019). While plants require interactions with other organisms for the uptake of phosphorous, this study highlighted the fact that not all PSMs are beneficial organisms. *Pseudomonas sp.* MPI9 solubilized phosphate with higher efficiency than *Pseudomonas sp.* CB3 but caused the death of *A. thaliana* upon germination in co-cultivation (Figure 50 and Figure 51). *Pseudomonas sp*. CB3 resulted in a slight increase of plant fresh weight indicating a possible plant growth promoting character. The specific interaction of *A. thaliana* highlights the importance of the modulation of the soil microbiota by plants. While the growth of *A. thaliana* required phosphate and therefore PSMs, plant survival can only be ensured if the plants are able to attract and deter suitable microorganisms. The study of the impact of BOA, gramine, and quercetin in this project showed, that these substances resulted in an increase in some but a decrease in other *Pseudomonas* ASVs, highlighting the very specific modes of action that require further studies.

5.11. Exposure of *Arthrobacter* Strains to Quercetin Resulted in Fatty Acid Adaptations to Ensure Bacterial Survival

While the overall lipid composition of all tested bacterial strains (*Arthrobacter sp.* GB1 and CB4 and *Pseudomonas sp.* CB3 and MPI9) was not changed upon exposure to BOA, gramine, or quercetin, *Arthrobacter* strains showed shifts in their fatty acid composition when cultivated with quercetin. In both strains, GB1 and CB4, the main fatty acid 15:0anteiso was reduced accompanied by an increase in the longer-chained fatty acids 16:0, 17:0anteiso, and 18:0 (Figure 61 and Figure 62). Since the change in the composition only occurred after the cells were exposed to quercetin, it became evident, that the *Arthrobacter sp.* adapted to the toxic compound by changing the composition and thus the fluidity of the cell membrane. In 1996, Escher et al. demonstrated that degradation of e.g., phenols by microorganisms requires mechanisms to cope with the toxicity of the substances. Adaptions in the membrane that

resulted in alterations in membrane fluidity were described upon changes in the temperature, and these changes were defined in 1974 as “homoeoviscous adaptations” by Sinensky. Furthermore, Unell et al. were able to show changes in the fatty acid composition of *Arthrobacter chlorophenolicus* when exposed to toxic concentrations of phenols (Unell et al., 2007). *Arthrobacter chlorophenolicus* changed the anteiso/iso ratio of fatty acids to maintain a rigid membrane and survive the fluidity increase caused by phenol. The increase of longer-chained fatty acids in *Arthrobacter sp.* GB1 and CB4 might result in a less rigid membrane to stop the permeation of quercetin into the cells. Interestingly, the reduction of the main fatty acid 15:0anteiso was much more pronounced in *Arthrobacter sp.* CB4 (Figure 62). HPLC-DAD monitoring of the quercetin degradation revealed that *Arthrobacter sp.* GB1 was able to degrade quercetin within 48 h, while CB4 needed 72 h to fully metabolize quercetin. Therefore, the CB4 cells were longer exposed to higher concentrations of quercetin. Despite the increase, the toxicity of quercetin remains questionable. It is yet unclear why the two *Pseudomonas* strains showed no response in their fatty acid composition when cultivated with quercetin. One reason could be that *Pseudomonas sp.* naturally contain more longer fatty acids than the two *Arthrobacter* strains and have therefore a less rigid membrane (Figure 63 and Figure 64). A more likely scenario is the presence of the quercetin degradation product protocatechuic acid. The two *Pseudomonas* strains were not able to degrade quercetin and therefore were never exposed to the compound. This hypothesis agrees with Wang et al. (2013) who were able to show that allelopathic effects of catechin were activated by further degradation of the substance to protocatechuic acid. Changes in the fatty acid composition of quercetin-degrading bacterial strains support the theory of quercetins allelochemical effect activation by degrading the compound into protocatechuic acid which causes oxidative stress.

6. Summary

This study revealed the impact of 2-benzoxazolinone (BOA), gramine, and quercetin on the microbiota in the bulk soil (Cologne agricultural soil) and investigated direct interactions of the plant secondary metabolites and their degradation products with soil bacteria and other plants. Only bacterial DNA from the soil could be analyzed because fungal and oomycetal DNA was below the detection limit. The absence of fungi and oomycetes from Cologne agricultural soil can be explained by the lack of initial carbon content as the soil had not been used for agriculture for over 15 years. The changes in the bacterial microbiota were followed by next-generation sequencing of soil DNA. In addition, surviving bacterial strains were isolated and characterized after treatment with BOA, gramine, or quercetin.

BOA mainly exhibited its function by deterring potentially unfavorable bacterial strains in the bulk soil microbiome resulting in the decrease in 11 amplicon sequence variants (ASVs) and an increase in 10 ASVs. While no bacterial strains were isolated that could degrade BOA, the known BOA-derived detoxification substances N-(2-hydroxy-5-nitrophenyl) acetamide (5-N-AAP), and the BOA-OH isomers BOA-4-OH, BOA-5-OH, BOA-6-OH, and BOA-7-OH revealed their allelopathic character in interactions with *A. thaliana* and *Z. mays*. The presence of 5-N-AAP caused an increased expression of geranyllinalool synthase, which is known to produce geranyllinalool, a potential defense compound interfering with sphingolipid metabolism in herbivores. Treatment of *Z. mays* roots with BOA-OH isomers on the other hand initiated the expression of ROS-related genes indicating the necessity of superoxide radical detoxification of the BOA-derived products.

Gramine exhibited its function in shaping the soil microbiota by mainly attracting potentially beneficial bacteria. While only five ASVs were decreased after gramine treatment, a total of 35 ASVs were increased in abundance. Therefore, gramine may serve as an additional carbon source for the bacteria in the soil. Indeed, one *Arthrobacter* strain was able to metabolize gramine via the degradation products indole-3-carboxaldehyde (I3A) and indole-3-carboxylic acid (I3C). The auxin-like compound I3A was also extracted two days after gramine application to bulk soil, and it is therefore available for interactions with other organisms including plants. Co-cultivation of *A. thaliana* with I3A resulted in an improvement in plant growth demonstrating its beneficial properties.

The treatment of bulk soil with the flavonoid quercetin resulted in an increase of the number of 35 ASVs but also a decrease of 17 ASVs. Therefore, quercetin might function by attracting beneficial bacteria, but it is also capable of shaping the soil microbiota by deterring potentially pathogenic strains. Two *Arthrobacter* strains were identified that were able to

degrade quercetin via protocatechuic acid and to use quercetin as a carbon source. Protocatechuic acid was thus identified as the toxic breakdown product of quercetin. Fatty acid analysis of bacterial strains that were able to degrade quercetin, revealed a decrease of the main fatty acid 15:0anteiso with a subsequent increase of longer-chained fatty acids. This alteration in the fatty acid composition is presumably associated with a decrease in membrane rigidity. A less rigid bacterial membrane might be necessary for the bacterial strains to cope with the quercetin degradation product protocatechuic acid, which is known to cause oxidative stress in other organisms.

This work thus demonstrates the complex roles of plant secondary metabolites during interactions with soil bacteria and plants. Further studies are required to decipher the functions of the metabolites on the different organisms.

7. References

Agarwal, N., Kolba, N., Khen, N., Even, C., Turjeman, S., Koren, O., et al. (2022). Quinoa soluble fiber and quercetin alter the composition of the gut microbiome and improve brush border membrane morphology in vivo (*Gallus gallus*). *Nutrients* 14. doi: 10.3390/nu14030448

Agler, M. T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.-T., Weigel, D., et al. (2016). Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol.* 14, e1002352. doi: 10.1371/journal.pbio.1002352

Ajiboye, T. O., Habibu, R. S., Saidu, K., Haliru, F. Z., Ajiboye, H. O., Aliyu, N. O., et al. (2017). Involvement of oxidative stress in protocatechuic acid-mediated bacterial lethality. *Microbiologyopen* 6. doi: 10.1002/mbo3.472

Albuquerque, M. B., Santos, R. C., Lima, L. M., Melo Filho, P. d. A., Nogueira, R. J. M. C., Câmara, C. A. G., et al. (2011). Allelopathy, an alternative tool to improve cropping systems. A review. *Agron. Sustain Dev.* 31, 379–395. doi: 10.1051/agro/2010031

Ali, S., Ganai, B. A., Kamili, A. N., Bhat, A. A., Mir, Z. A., Bhat, J. A., et al. (2018). Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiol. Res.* 212-213, 29–37. doi: 10.1016/j.micres.2018.04.008

Arnold, K. W., and Kaspar, C. W. (1995). Starvation- and stationary-phase-induced acid tolerance in *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 61, 2037–2039. doi: 10.1128/aem.61.5.2037-2039.1995

Atwal, A. S., Teather, R. M., Liss, S. N., and Collins, F. W. (1992). Antimicrobial activity of 2-aminophenoxazin-3-one under anaerobic conditions. *Can. J. Microbiol.* 38, 1084–1088. doi: 10.1139/m92-177

Bååth, E., Díaz-Raviña, M., Frostegård, S., and Campbell, C. D. (1998). Effect of metal-rich sludge amendments on the soil microbial community. *Appl. Environ. Microbiol.* 64, 238–245. doi: 10.1128/AEM.64.1.238-245.1998

Babich, H., Sedletcaia, A., and Kenigsberg, B. (2002). In vitro cytotoxicity of protocatechuic acid to cultured human cells from oral tissue: involvement in oxidative stress. *Pharmacol. Toxicol.* 91, 245–253. doi: 10.1034/j.1600-0773.2002.910505.x

Bailey, B. A., and Larson, R. L. (1989). Hydroxamic Acid glucosyltransferases from maize seedlings. *Plant Physiol.* 90, 1071–1076. doi: 10.1104/pp.90.3.1071

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57, 233–266. doi: 10.1146/annurev.arplant.57.032905.105159

Bais, H. P., Loyola-Vargas, V. M., Flores, H. E., and Vivanco, J. M. (2001). Root-specific metabolism: The biology and biochemistry of underground organs. *In Vitro Cell. Dev. Biol.-Plant* 37, 730–741. doi: 10.1007/s11627-001-0122-y

Bais, H. P., Park, S.-W., Weir, T. L., Callaway, R. M., and Vivanco, J. M. (2004). How plants communicate using the underground information superhighway. *Trends Plant Sci.* 9, 26–32. doi: 10.1016/j.tplants.2003.11.008

Barnes, J. P., and Putnam, A. R. (1987). Role of benzoxazinones in allelopathy by rye (*Secale cereale* L.). *J. Chem. Ecol.* 13, 889–906. doi: 10.1007/BF01020168

Bednarek, P., Pislewska-Bednarek, M., Svatos, A., Schneider, B., Doubsky, J., Mansurova, M., et al. (2009). A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science* 323, 101–106. doi: 10.1126/science.1163732

Berendsen, R. L., Pieterse, C. M. J., and Bakker, Peter A H M (2012). The rhizosphere microbiome and plant health. *Trends Plant Sci.* 17, 478–486. doi: 10.1016/j.tplants.2012.04.001

Bertin, C., Weston, L. A., and Kaur, H. (2007). Allelopathic Crop Development: Molecular and traditional plant breeding approaches. *Plant Breeding Rev*. J. Janick (Ed.). 231–258. doi: 10.1002/9780470380130.ch4

Blackwood, C. B., and Buyer, J. S. (2004). Soil microbial communities associated with Bt and non-Bt corn in three soils. *J. Environ. Qual.* 33, 832–836. doi: 10.2134/jeq2004.0832

Bligh, E. G., and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911–917. doi: 10.1139/o59-099

Bodenhausen, N., Horton, M. W., and Bergelson, J. (2013). Bacterial communities associated with the leaves and the roots of *Arabidopsis thaliana*. *PLoS One* 8, e56329. doi: 10.1371/journal.pone.0056329

Bogs, J., Ebadi, A., McDavid, D., and Robinson, S. P. (2006). Identification of the flavonoid hydroxylases from grapevine and their regulation during fruit development. *Plant Physiol.* 140, 279–291. doi: 10.1104/pp.105.073262

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37, 852–857. doi: 10.1038/s41587-019-0209-9

Boss, P. K., Davies, C., and Robinson, S. P. (1996). Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv Shiraz grape berries and the implications for pathway regulation. *Plant Physiol.* 111, 1059–1066. doi: 10.1104/pp.111.4.1059

Bravo, H. R., Iglesias, M. J., and Copaja, Sylvia V. and Argandona, Victor H (2010). Phytotoxicity of indole alkaloids from cereals. *Rev. Latinoam. Quím:*, pp.123-129.

Brown, J. L., Ross, T., McMeekin, T. A., and Nichols, P. D. (1997). Acid habituation of *Escherichia coli* and the potential role of cyclopropane fatty acids in low pH tolerance. *Intern: J: Food Microbiol:* 37, 163–173. doi: 10.1016/s0168-1605(97)00068-8

Browse, J., McCourt, P. J., and Somerville, C. R. (1986). Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. *Anal: Biochem:* 152, 141–145. doi: 10.1016/0003-2697(86)90132-6

Browse, J., McCourt, P. J., and Somerville, C. R. (1986). Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. *Anal: Biochem:* 152, 141–145. doi: 10.1016/0003-2697(86)90132-6

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren Themaat, E., and Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annu. Rev. Plant Biol.* 64, 807–838. doi: 10.1146/annurev-arplant-050312-120106

Cai, Q. N., Zhang, Q. W., and Cheo, M. (2004). Contribution of indole alkaloids to *Sitobion avenae* (F.) resistance in wheat. *J. Appl. Entomology* 128, 517–521. doi: 10.1111/j.1439-0418.2004.00770.x

Carlsen, S. C. K., Pedersen, H. A., Spliid, N. H., and Fomsgaard, I. S. (2012). Fate in soil of flavonoids released from white clover (*Trifolium repens* L.). *Appl: Environm: Soil Scie:* 2012, 1–10. doi: 10.1155/2012/743413

Castellarin, S. D., and Di Gaspero, G. (2007). Transcriptional control of anthocyanin biosynthetic genes in extreme phenotypes for berry pigmentation of naturally occurring grapevines. *BMC Plant Biol.* 7, 46. doi: 10.1186/1471-2229-7-46

Chang, Y.-Z., Lin, H.-C., Chan, S.-T., and Yeh, S.-L. (2012). Effects of quercetin metabolites on the enhancing effect of *β*-carotene on DNA damage and cytochrome P1A1/2 expression in benzoapyrene-exposed A549 cells. *Food Chem.* 133, 445–450. doi: 10.1016/j.foodchem.2012.01.060

Cheeke, Peter R. (1989). Toxicants of plant origin. Boca Rotan, Florida: *CRC Pr.*

Chen, F., Tholl, D., D'Auria, J. C., Farooq, A., Pichersky, E., and Gershenzon, J. (2003). Biosynthesis and emission of terpenoid volatiles from *Arabidopsis f*lowers. *Plant Cell* 15, 481–494. doi: 10.1105/tpc.007989

Corcuera, L. J. (1993). Biochemical basis for the resistance of barley to aphids. *Phytochemistry* 33, 741–747. doi: 10.1016/0031-9422(93)85267-U

Costa-Broseta, Á., Castillo, M., and León, J. (2021). Post-translational modifications of nitrate reductases autoregulates nitric oxide biosynthesis in Arabidopsis. *Int. J. Mol. Sci* 22. doi: 10.3390/ijms22020549

Cotton, T. E. A., Pétriacq, P., Cameron, D. D., Meselmani, M. A., Schwarzenbacher, R., Rolfe, S. A., et al. (2019). Metabolic regulation of the maize rhizobiome by benzoxazinoids. *ISME J.* 13, 1647–1658. doi: 10.1038/s41396-019-0375-2

Cseke, L. J., Kirakosyan, A., Kaufman, P. B., Warber, S., Duke, J. A., and Brielmann, H. L. (2016). Natural Products from Plants. *CRC Press.*

Dar, A. A., Choudhury, A. R., Kancharla, P. K., and Arumugam, N. (2017). The *FAD2* gene in plants: occurrence, regulation, and role. *Front. Plant Sci.* 8, 1789. doi: 10.3389/fpls.2017.01789

Davidov, Y., Friedjung, A., and Jurkevitch, E. (2006). Structure analysis of a soil community of predatory bacteria using culture-dependent and culture-independent methods reveals a hitherto undetected diversity of *Bdellovibrio*-and-like organisms. *Environ. Microbiol.* 8, 1667–1673. doi: 10.1111/j.1462-2920.2006.01052.x

Deutch, C. E., Bui, A. P., and Ho, T. (2018). Growth of *Paenarthrobacter aurescens* strain TC1 on atrazine and isopropylamine during osmotic stress. *Ann. Microbiol.* 68, 569–577. doi: 10.1007/s13213-018-1364-9

Dick, R., Rattei, T., Haslbeck, M., Schwab, W., Gierl, A., and Frey, M. (2012). Comparative analysis of benzoxazinoid biosynthesis in monocots and dicots: independent recruitment of stabilization and activation functions. *Plant Cell* 24, 915–928. doi: 10.1105/tpc.112.096461

Dominati, E., Mackay, A., Green, S., and Patterson, M. (2014). A soil change-based methodology for the quantification and valuation of ecosystem services from agro-ecosystems: a case study of pastoral agriculture in New Zealand. *Ecol. Econ.* 100, 119–129. doi: 10.1016/j.ecolecon.2014.02.008

Durán, P., Thiergart, T., Garrido-Oter, R., Agler, M., Kemen, E., Schulze-Lefert, P., et al. (2018). Microbial interkingdom interactions in roots promote Arabidopsis survival. *Cell* 175, 973-983.e14. doi: 10.1016/j.cell.2018.10.020

Einhellig, F. A. (1996). Interactions involving allelopathy in cropping systems. *Agron. J.* 88, 886–893. doi: 10.2134/agronj1996.00021962003600060007x

Escher, B. I., Snozzi, M., and Schwarzenbach, R. P. (1996). Uptake, speciation, and uncoupling activity of substituted phenols in energy transducing membranes. *Environ. Sci. Technol.* 30, 3071–3079. doi: 10.1021/es960153f

Fomsgaard, I. S., Mortensen, A. G., and Carlsen, S. C. K. (2004). Microbial transformation products of benzoxazolinone and benzoxazinone allelochemicals - A review. *Chemosphere* 54, 1025–1038. doi: 10.1016/j.chemosphere.2003.09.044

Fortin, N., Beaumier, D., Lee, K., and Greer, C. W. (2004). Soil washing improves the recovery of total community DNA from polluted and high organic content sediments. *J. Microbiol. Methods* 56, 181–191. doi: 10.1016/j.mimet.2003.10.006

Frey, M., Chomet, P., Glawischnig, E., Stettner, C., Grün, S., Winklmair, A., et al. (1997). Analysis of a chemical plant defense mechanism in grasses. *Science* 277, 696–699. doi: 10.1126/science.277.5326.696

Frey, M., Schullehner, K., Dick, R., Fiesselmann, A., and Gierl, A. (2009). Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants. *Phytochemistry* 70, 1645–1651. doi: 10.1016/j.phytochem.2009.05.012

Friebe, Vilich, Hennig, Kluge, and Sicker (1998). Detoxification of benzoxazolinone allelochemicals from wheat by G*aeumannomyces graminis* var. tritici, *G. graminis* var. graminis, *G. graminis* var. avenae, and *Fusarium culmorum*. *Appl. Environ. Microbiol.* 64, 2386–2391. doi: 10.1128/AEM.64.7.2386-2391.1998

Frostegrd, A., and Bth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils* 22, 59–65. doi: 10.1007/BF00384433

Gallagher, C. H., Koch, J. H., Moore, R. M., and Steel, J. D. (1964). Toxicity of *Phalaris tuberosa* for sheep. *Nature* 204, 542–545. doi: 10.1038/204542a0

Gans, J., Wolinsky, M., and Dunbar, J. (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science* 309, 1387–1390. doi: 10.1126/science.1112665

Ghini, A. A., Burton, G., and Gros, E. G. (1991). Biodegradation of the indolic system of gramine in *Hordeum vulgare*. *Phytochemistry* 30, 779–784. doi: 10.1016/0031-9422(91)85251-T

Gierl, A., and Frey, M. (2001). Evolution of benzoxazinone biosynthesis and indole production in maize. *Planta* 213, 493–498. doi: 10.1007/s004250100594

Glenn, A. E., Davis, C. B., Gao, M., Gold, S. E., Mitchell, T. R., Proctor, R. H., et al. (2016). Two horizontally transferred xenobiotic resistance gene clusters associated with detoxification of benzoxazolinones by *Fusarium* Species. *PLoS One* 11, e0147486. doi: 10.1371/journal.pone.0147486

Goelz, M. F., Rothenbacher, H., Wiggins, J. P., Kendall, W. A., and Hershberger, T. V. (1980). Some hematological and histopathological effects of the alkaloids gramine and hordenine on meadow voles (*Microtus pennsylvanicus)*. *Toxicology* 18, 125–131. doi: 10.1016/0300-483X(80)90075-X

Gond, S. K., Bergen, M. S., Torres, M. S., and White, J. F. (2015). Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defense gene expression in maize. *Microbiol Res* 172, 79–87. doi: 10.1016/j.micres.2014.11.004

Gross, D.H., Lehmann, H.-R., Shutte, P.M. (1974). Zur Biosynthese des Gramins. *Biochem. Physiol. Pflanzen*, Pages 281-287.

Grün, S., Frey, M., and Gierl, A. (2005). Evolution of the indole alkaloid biosynthesis in the genus *Hordeum*: distribution of gramine and DIBOA and isolation of the benzoxazinoid biosynthesis genes from *Hordeum lechleri*. *Phytochemistry* 66, 1264–1272. doi: 10.1016/j.phytochem.2005.01.024

Haichar, F. e. Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., Balesdent, J., et al. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *ISME J.* 2, 1221–1230. doi: 10.1038/ismej.2008.80

Hanson, A. D., Ditz, K. M., Singletary, G. W., and Leland, T. J. (1983). Gramine accumulation in leaves of barley grown under high-temperature stress. *Plant Physiol.* 71, 896–904. doi: 10.1104/pp.71.4.896

Harbort, C. J., Hashimoto, M., Inoue, H., Niu, Y., Guan, R., Rombolà, A. D., et al. (2020). Root-secreted coumarins and the microbiota interact to improve iron nutrition in Arabidopsis. *Cell Host Microbe* 28, 825-837.e6. doi: 10.1016/j.chom.2020.09.006

Havsteen, B. (1983). Flavonoids, a class of natural products of high pharmacological potency. *Biochem: Pharmacol:* 32, 1141–1148. doi: 10.1016/0006-2952(83)90262-9

Hawes, M. C., Gunawardena, U., Miyasaka, S., and Zhao, X. (2000). The role of root border cells in plant defense. *Trends Plant Sci.* 5, 128–133. doi: 10.1016/S1360-1385(00)01556-9

Heipieper, H. J., Weber, F. J., Sikkema, J., Keweloh, H., and Bont, J. A. de (1994). Mechanisms of resistance of whole cells to toxic organic solvents. *Trends Biotechnol:* 12, 409–415. doi: 10.1016/0167-7799(94)90029-9

Hemetsberger, C., Herrberger, C., Zechmann, B., Hillmer, M., and Doehlemann, G. (2012). The *Ustilago maydis* effector Pep1 suppresses plant immunity by inhibition of host peroxidase activity. *PLoS Pathol.* 8, e1002684. doi: 10.1371/journal.ppat.1002684

Herde, M., Gärtner, K., Köllner, T. G., Fode, B., Boland, W., Gershenzon, J., et al. (2008). Identification and regulation of TPS04/GES, an Arabidopsis geranyllinalool synthase catalyzing the first step in the formation of the insect-induced volatile C16-homoterpene TMTT. *Plant Cell* 20, 1152–1168. doi: 10.1105/tpc.106.049478

Hernández, M. L., Padilla, M. N., Mancha, M., and Martínez-Rivas, J. M. (2009). Expression analysis identifies *FAD2-2* as the olive oleate desaturase gene mainly responsible for the linoleic acid content in virgin olive oil. *J. Agric. Food Chem.* 57, 6199–6206. doi: 10.1021/jf900678z

Hiltner L. (1904). Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. *Arb. DLG*, 59–78.

Hiruma, K., Gerlach, N., Sacristán, S., Nakano, R. T., Hacquard, S., Kracher, B., et al. (2016). Root endophyte *Colletotrichum tofieldiae* confers plant fitness benefits that are phosphate status dependent. *Cell* 165, 464–474. doi: 10.1016/j.cell.2016.02.028

Hodnick, W. F., Kung, F. S., Roettger, W. J., Bohmont, C. W., and Pardini, R. S. (1986). Inhibition of mitochondrial respiration and production of toxic oxygen radicals by flavonoids. *Biochem. Pharmacol.* 35, 2345–2357. doi: 10.1016/0006-2952(86)90461-2

Hofmann, D., Knop, M., Hao, H., Hennig, L., Sicker, D., and Schulz, M. (2006). Glucosides from MBOA and BOA detoxification by *Zea mays* and *Portulaca oleracea*. *J. Nat. Prod.* 69, 34–37. doi: 10.1021/np0580762

Hong, Y., Hu, H.-Y., Xie, X., Sakoda, A., Sagehashi, M., and Li, F.-M. (2009). Gramine-induced growth inhibition, oxidative damage, and antioxidant responses in freshwater cyanobacterium *Microcystis aeruginosa*. *Aquat. Toxicol.* 91, 262–269. doi: 10.1016/j.aquatox.2008.11.014

Hu, L., Robert, C. A. M., Cadot, S., Zhang, X., Ye, M., Li, B., et al. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nat. Commun.* 9, 2738. doi: 10.1038/s41467-018-05122-7

Ibekwe, A. M., and Kennedy, A. (1998). Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. *FEMS Microbiol. Ecol.* 26, 151–163. doi: 10.1111/j.1574-6941.1998.tb00501.x

Ishizawa, K., Yoshizumi, M., Kawai, Y., Terao, J., Kihira, Y., Ikeda, Y., et al. (2011). Pharmacology in health food: metabolism of quercetin in vivo and its protective effect against arteriosclerosis. *J. Pharmacol. Sci.* 115, 466–470. doi: 10.1254/jphs.10R38FM

Jacyn Baker, C., Roberts, D. P., Mock, N. M., Whitaker, B. D., Deahl, K. L., and Aver'yanov, A. A. (2005). Apoplastic redox metabolism: synergistic phenolic oxidation and a novel oxidative burst. *Physiol. Mol. Plant Pathol.* 67, 296–303. doi: 10.1016/j.pmpp.2006.04.005

Kaiser, C., Frank, A., Wild, B., Koranda, M., and Richter, A. (2010). Negligible contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18:2ω6,9 and 18:1ω9. *Soil Biol. Biochem.* 42, 1650–1652. doi: 10.1016/j.soilbio.2010.05.019

Kalayu, G. (2019). Phosphate Solubilizing Microorganisms: Promising approach as biofertilizers. *Intern. J. Agrony.* 1–7. doi: 10.1155/2019/4917256

Kanehisa, K., Tsumuki, H., Kawada, K., and Rustamani, M. A. (1990). Relations of gramine contents and aphid populations on barley lines. *Appl. Entomol. Zool* 25, 251–259. doi: 10.1303/aez.25.251

Kelly SR, Garo, E., Jensen, P. R., Fenical, W., and Pawlik JR (2005). Effects of Caribbean sponge secondary metabolites on bacterial surface colonization. *Aquat. Microb. Ecol.* 40, 191–203. doi: 10.3354/ame040191

Kettle, A. J., Batley, J., Benfield, A. H., Manners, J. M., Kazan, K., and Gardiner, D. M. (2015). Degradation of the benzoxazolinone class of phytoalexins is important for virulence of *Fusarium pseudograminearum* towards wheat. *Mol. Plant Pathol.* 16, 946–962. doi: 10.1111/mpp.12250

Knief, C., Bol, R., Amelung, W., Kusch, S., Frindte, K., Eckmeier, E., et al. (2020). Tracing elevational changes in microbial life and organic carbon sources in soils of the Atacama Desert. *Global Planet. Change* 184, 103078. doi: 10.1016/j.gloplacha.2019.103078

Köberl, M., Wagner, P., Müller, H., Matzer, R., Unterfrauner, H., Cernava, T., et al. (2020). Unraveling the complexity of soil microbiomes in a large-scale study subjected to different agricultural management in Styria. *Front. Microbiol.* 11, 1052. doi: 10.3389/fmicb.2020.01052

Kolde, R. (2019). pheatmap: Pretty heatmaps. R Package Version 1.0.12. Available online at: https://CRAN.R-project.org/package=pheatmap. (Accessed May 14, 2021).

Koo, B.-., Adriano, D. C., Bolan, N. S., and Barton, C. D. (2005). Root exudates and microorganisms. Hillel, D., Ed., *Ency. Soils Environ*, 421–428. doi: 10.1016/B0-12-348530-4/00461-6

Kopittke, P. M., Menzies, N. W., Wang, P., McKenna, B. A., and Lombi, E. (2019). Soil and the intensification of agriculture for global food security. *Environ. Int.* 132, 105078. doi: 10.1016/j.envint.2019.105078

Korenblum, E., Dong, Y., Szymanski, J., Panda, S., Jozwiak, A., Massalha, H., et al. (2020). Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. *Proc. Natl. Acad. Sci. USA* 117, 3874–3883. doi: 10.1073/pnas.1912130117

Kruse, M., Strandberg, M., and Strandberg, B. (2000). Ecological effects of allelopathic plants: a review. *National Environmental Research Institute, Silkeborg, Denmark*. 66 pp. – *NERI Technical Report No*. 315.

Kudjordjie, E. N., Sapkota, R., Steffensen, S. K., Fomsgaard, I. S., and Nicolaisen, M. (2019). Maize synthesized benzoxazinoids affect the host associated microbiome. *Microbiome* 7, 59. doi: 10.1186/s40168-019-0677-7

Larsson, K. A. E., Zetterlund, I., Delp, G., and Jonsson, L. M. V. (2006). N-Methyltransferase involved in gramine biosynthesis in barley: cloning and characterization. *Phytochemistry* 67, 2002–2008. doi: 10.1016/j.phytochem.2006.06.036

Laschke, L., Schütz, V., Schackow, O., Sicker, D., Hennig, L., Hofmann, D., et al. (2022). Survival of plants during short-term BOA-OH exposure: ROS-related gene expression and detoxification reactions are accompanied with fast membrane lipid repair in root tips. *J. Chem. Ecol.* 48, 219–239. doi: 10.1007/s10886-021-01337-z

Le Zhao, Zhang, Q., Ma, W., Tian, F., Shen, H., and Zhou, M. (2017). A combination of quercetin and resveratrol reduces obesity in high-fat diet-fed rats by modulation of gut microbiota. *Food Funct.* 8, 4644–4656. doi: 10.1039/C7FO01383C

Leighton, V., Niemeyer, H. M., and Jonsson, L. M. (1994). Substrate specificity of a glucosyltransferase and an N-hydroxylase involved in the biosynthesis of cyclic hydroxamic acids in Gramineae. *Phytochemistry* 36, 887–892. doi: 10.1016/S0031-9422(00)90457-6

Leland, T. J., and Hanson, A. D. (1985). Induction of a specific N-methyltransferase enzyme by long-term heat stress during barley leaf growth. *Plant Physiol.* 79, 451–457. doi: 10.1104/pp.79.2.451

Li, J., Halitschke, R., Li, D., Paetz, C., Su, H., Heiling, S., et al. (2021). Controlled hydroxylations of diterpenoids allow for plant chemical defense without autotoxicity. *Science* 371, 255–260. doi: 10.1126/science.abe4713

Li, X., Jousset, A., Boer, W. de, Carrión, V. J., Zhang, T., Wang, X., et al. (2019). Legacy of land use history determines reprogramming of plant physiology by soil microbiome. *ISME J.* 13, 738–751. doi: 10.1038/s41396-018-0300-0

Liu, C., Sun, Q., Zhao, L., Li, Z., Peng, Z., and Zhang, J. (2018). Heterologous expression of the transcription factor EsNAC1 in Arabidopsis enhances abiotic stress resistance and retards growth by regulating the expression of different target genes. *Front. Plant Sci.* 9, 1495. doi: 10.3389/fpls.2018.01495

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Lotito, S. B., Zhang, W.-J., Yang, C. S., Crozier, A., and Frei, B. (2011). Metabolic conversion of dietary flavonoids alters their anti-inflammatory and antioxidant properties. *Free Radic. Biol. Med.* 51, 454–463. doi: 10.1016/j.freeradbiomed.2011.04.032

Lovett, J. V., Hoult, A. H., and Christen, O. (1994). Biologically active secondary metabolites of barley. IV. Hordenine production by different barley lines. *J. Chem. Ecol.* 20, 1945–1954. doi: 10.1007/BF02066235

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, et al. (2004). ARB: a software environment for sequence data. *Nucleic Acids Res.* 32, 1363–1371. doi: 10.1093/nar/gkh293

Maag, D., Erb, M., Köllner, T. G., and Gershenzon, J. (2015). Defensive weapons and defense signals in plants: some metabolites serve both roles. *Bioessays* 37, 167–174. doi: 10.1002/bies.201400124

Macías, F. A., Oliveros-Bastidas, A., Marín, D., Castellano, D., Simonet, A. M., and Molinillo, J. M. G. (2005). Degradation studies on benzoxazinoids. Soil degradation dynamics of (2R)-2-O-beta-D-glucopyranosyl-4-hydroxy-(2H)- 1,4-benzoxazin-3(4H)-one (DIBOA-Glc) and its degradation products, phytotoxic allelochemicals from Gramineae. *J. Agric. Food Chem.* 53, 554–561. doi: 10.1021/jf048702l

Magoč, T., and Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27, 2957–2963. doi: 10.1093/bioinformatics/btr507

Mandal, S., van Treuren, W., White, R. A., Eggesbø, M., Knight, R., and Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microb. Ecol. Health Dis.* 26, 27663. doi: 10.3402/mehd.v26.27663

Martino, C., Morton, J. T., Marotz, C. A., Thompson, L. R., Tripathi, A., Knight, R., et al. (2019). A novel sparse compositional technique reveals microbial perturbations. *mSystems* 4. doi: 10.1128/mSystems.00016-19

Mathesius, U. (2018). Flavonoid functions in plants and their interactions with other organisms. *Plants (Basel)* 7. doi: 10.3390/plants7020030

Matsuo, H., Taniguchi, K., Hiramoto, T., Yamada, T., Ichinose, Y., Toyoda, K., et al. (2001). Gramine increase associated with rapid and transient systemic resistance in barley seedlings induced by mechanical and biological stresses. *Plant Cell Physiol.* 42, 1103–1111. doi: 10.1093/pcp/pce139

Maver, M., Escudero-Martinez, C., Abbott, J., Morris, J., Hedley, P. E., Mimmo, T., et al. (2021). Applications of the indole-alkaloid gramine modulate the assembly of individual members of the barley rhizosphere microbiota. *PeerJ* 9, e12498. doi: 10.7717/peerj.12498

Maver, M., Miras-Moreno, B., Lucini, L., Trevisan, M., Pii, Y., Cesco, S., et al. (2020). New insights in the allelopathic traits of different barley genotypes: middle Eastern and Tibetan wild-relative accessions vs. cultivated modern barley. *PLoS One* 15, e0231976. doi: 10.1371/journal.pone.0231976

McNear Jr., D. H. (2013). The rhizosphere - roots, soil, and everything in between. *Nature Educat. Knowl*. 4(3):1

Mudd, S. H. (1961). 3-Aminomethylindole and 3-methylaminomethylindole: new constituents of barley. *Nature* 189, 489. doi: 10.1038/189489a0

Musajo, L., and Rodighiero, G. (1962). The skin-photosensitizing furocoumarins. *Experientia* 18, 153–161. doi: 10.1007/BF02151700

Mylona, P. V., Polidoros, A. N., and Scandalios, J. G. (2007). Antioxidant gene responses to ROS-generating xenobiotics in developing and germinated scutella of maize. *J. Exp. Bot.* 58, 1301–1312. doi: 10.1093/jxb/erl292

Nabavi, S. M., Šamec, D., Tomczyk, M., Milella, L., Russo, D., Habtemariam, S., et al. (2020). Flavonoid biosynthetic pathways in plants: Versatile targets for metabolic engineering. *Biotechnol. Adv.* 38, 107316. doi: 10.1016/j.biotechadv.2018.11.005

Niculaes, C., Abramov, A., Hannemann, L., and Frey, M. (2018). Plant Protection by Benzoxazinoids-recent insights into biosynthesis and function. *Agronomy* 8, 143. doi: 10.3390/agronomy8080143

Niemeyer, H. M. (1988). Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defense chemicals in the Gramineae. *Phytochemistry* 27, 3349–3358. doi: 10.1016/0031-9422(88)80731-3

Niemeyer, H. M. (2009). Hydroxamic acids derived from 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one: key defense chemicals of cereals. *J. Agric. Food. Chem.* 57, 1677–1696. doi: 10.1021/jf8034034

Ofek, M., Hadar, Y., and Minz, D. (2012). Ecology of root colonizing *Massilia* (Oxalobacteraceae). *PLoS One* 7, e40117. doi: 10.1371/journal.pone.0040117

Pachter, I. J., Zacharias, D. E., and Ribeiro, O. (1959). Indole alkaloids of *Acer saccharinum* (the Silver Maple), *Dictyoloma incanescens, Piptadenia colubrina*, and *Mimosa hostilis*. *J. Org. Chem.* 24, 1285–1287. doi: 10.1021/jo01091a032

Parks, D. H., Tyson, G. W., Hugenholtz, P., and Beiko, R. G. (2014). STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics* 30, 3123–3124. doi: 10.1093/bioinformatics/btu494

Petrussa, E., Braidot, E., Zancani, M., Peresson, C., Bertolini, A., Patui, S., et al. (2013). Plant flavonoids-biosynthesis, transport, and involvement in stress responses. *Int. J. Mol. Sci.* 14, 14950–14973. doi: 10.3390/ijms140714950

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiology*, 362–370.

Pileggi, M., Pileggi, S. A. V., and Sadowsky, M. J. (2020). Herbicide bioremediation: from strains to bacterial communities. *Heliyon* 6, e05767. doi: 10.1016/j.heliyon.2020.e05767

Pillai, B. V. S., and Swarup, S. (2002). Elucidation of the flavonoid catabolism pathway in *Pseudomonas putida* PML2 by comparative metabolic profiling. *Appl. Environ. Microbiol.* 68, 143–151. doi: 10.1128/AEM.68.1.143-151.2002

Poger, D., and Mark, A. E. (2015). A ring to rule them all: the effect of cyclopropane fatty acids on the fluidity of lipid bilayers. *J. Phys. Chem. B.* 119, 5487–5495. doi: 10.1021/acs.jpcb.5b00958

Pritsos, C. A., Ahmad, S., Bowen, S. M., Elliott, A. J., Blomquist, G. J., and Pardini, R. S. (1988). Antioxidant enzymes of the black swallowtail butterfly, *Papilio polyxenes*, and their response to the prooxidant allelochemical, quercetin. *Arch. Insect Biochem. Physiol.* 8, 101–112. doi: 10.1002/arch.940080204

Pruesse, E., Peplies, J., and Glöckner, F. O. (2012). SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28, 1823–1829. doi: 10.1093/bioinformatics/bts252

Raetz, C. R., and Dowhan, W. (1990). Biosynthesis and function of phospholipids in *Escherichia coli*. *J. Biol. Chem.* 265, 1235–1238. doi: 10.1016/S0021-9258(19)40001-X

Rajniak, J., Giehl, R. F. H., Chang, E., Murgia, I., Wirén, N. von, and Sattely, E. S. (2018). Biosynthesis of redox-active metabolites in response to iron deficiency in plants. *Nat. Chem. Biol.* 14, 442–450. doi: 10.1038/s41589-018-0019-2

Ramos, J. L., Duque, E., Gallegos, M.-T., Godoy, P., Ramos-Gonzalez, M. I., Rojas, A., et al. (2002). Mechanisms of solvent tolerance in gram-negative bacteria. *Annu. Rev. Microbiol.* 56, 743–768. doi: 10.1146/annurev.micro.56.012302.161038

Rangjaroen, C., Sungthong, R., Rerkasem, B., Teaumroong, N., Noisangiam, R., and Lumyong, S. (2017). Untapped endophytic colonization and plant growth-promoting potential of the genus *Novosphingobium* to optimize rice cultivation. *Microbes Environ.* 32, 84–87. doi: 10.1264/jsme2.ME16112

Rao, J. R., Sharma, N. D., Hamilton, J. T., Boyd, D. R., and Cooper, J. E. (1991). Biotransformation of the pentahydroxy flavone quercetin by *Rhizobium loti* and *Bradyrhizobium* Strains (Lotus). *Appl. Environ. Microbiol.* 57, 1563–1565. doi: 10.1128/aem.57.5.1563-1565.1991

Reberg-Horton, S. C., Burton, J. D., Danehower, D. A., Ma, G., Monks, D. W., Murphy, J. P., et al. (2005). Changes over time in the allelochemical content of ten cultivars of rye (*Secale cereale* L.). *J. Chem. Ecol.* 31, 179–193. doi: 10.1007/s10886-005-0983-3

Redinbaugh, M. G., Sabre, M., and Scandalios, J. G. (1990). The distribution of catalase activity, isozyme protein, and transcript in the tissues of the developing maize seedling. *Plant Physiol.* 92, 375–380. doi: 10.1104/pp.92.2.375

Reimann, J. E., and Byerrum, R. U. (1964). Studies on the biosynthesis of 2,4-dihydroxy-7-methoxy-2H-1,4benzoxazin-3-one. *Biochemistry* 3, 847–851. doi: 10.1021/bi00894a021

Rice, C. P., Park, Y. B., Adam, F., Abdul-Baki, A. A., and Teasdale, J. R. (2005). Hydroxamic acid content and toxicity of rye at selected growth stages. *J. Chem. Ecol.* 31, 1887–1905. doi: 10.1007/s10886-005-5933-6

Rice, E. L. (1974). Allelopathy. *New York: Academic Press.*

Rice, E. L. (1984). Allelopathy (2). *New York: Academic Press.*

Rousk, J., Brookes, P. C., and Bååth, E. (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl. Environ. Microbiol.* 75, 1589–1596. doi: 10.1128/AEM.02775-08

Santangelo, R., Silvestrini, A., and Mancuso, C. (2019). Ginsenosides, catechins, quercetin and gut microbiota: current evidence of challenging interactions. *Food Chem. Toxicol.* 123, 42–49. doi: 10.1016/j.fct.2018.10.042

Schmid, N. B., Giehl, R. F. H., Döll, S., Mock, H.-P., Strehmel, N., Scheel, D., et al. (2014). Feruloyl-CoA 6'-hydroxylase1-dependent coumarins mediate iron acquisition from alkaline substrates in Arabidopsis. *Plant Physiol.* 164, 160–172. doi: 10.1104/pp.113.228544

Schneider, H., Schwiertz, A., Collins, M. D., and Blaut, M. (1999). Anaerobic transformation of quercetin-3-glucoside by bacteria from the human intestinal tract. *Arch. Microbiol.* 171, 81–91. doi: 10.1007/s002030050682

Schullehner, K., Dick, R., Vitzthum, F., Schwab, W., Brandt, W., Frey, M., et al. (2008). Benzoxazinoid biosynthesis in dicot plants. *Phytochemistry* 69, 2668–2677. doi: 10.1016/j.phytochem.2008.08.023

Schulz, M., Hofmann, D., Sicker, D., Hennig, L., Schütz, V., Preusche, M., et al. (2018). *Pantoea ananatis* converts MBOA to 6-methoxy-4-nitro-benzoxazolin-2(3H)-one (NMBOA) for cooperative degradation with its native root colonizing microbial consortium. *Natural Prod. Commun.* 13, 1934578. doi: 10.1177/1934578X1801301010

Schulz, M., Marocco, A., and Tabaglio, V. (2012). BOA detoxification of four summer weeds during germination and seedling growth. *J. Chem. Ecol.* 38, 933–946. doi: 10.1007/s10886-012-0136-4

Schulz, M., Marocco, A., Tabaglio, V., Macias, F. A., and Molinillo, J. M. G. (2013). Benzoxazinoids in rye allelopathy - from discovery to application in sustainable weed control and organic farming. *J. Chem. Ecol.* 39, 154–174. doi: 10.1007/s10886-013-0235-x

Schütz, V., Bigler, L., Girel, S., Laschke, L., Sicker, D., and Schulz, M. (2019). Conversions of benzoxazinoids and downstream metabolites by soil microorganisms. *Front. Ecol. Evol.* 7. doi: 10.3389/fevo.2019.00238

Schütz, V., Frindte, K., Cui, J., Zhang, P., Hacquard, S., Schulze-Lefert, P., et al. (2021). Differential impact of plant secondary metabolites on the soil microbiota. *Front. Microbiol.* 12, 666010. doi: 10.3389/fmicb.2021.666010

Sepulveda, B. A., and Corcuera, L. J. (1990). Effect of gramine on the susceptibility of barley leaves to *Pseudomonas syringae*. *Phytochemistry* 29, 465–467. doi: 10.1016/0031-9422(90)85098-Z

Shabala, L., and Ross, T. (2008). Cyclopropane fatty acids improve *Escherichia coli* survival in acidified minimal media by reducing membrane permeability to H^+ and enhanced ability to extrude H^+. *Res. Microbiol.* 159, 458–461. doi: 10.1016/j.resmic.2008.04.011

Sicker, D., Frey, M., Schulz, M., and Gierl. A. (2000). Role of natural benzoxazinones in the survival strategy of plants. *Int. Rev. Cytol.* 198, 319–346. doi: 10.1016/s0074-7696(00)98008-2

Sicker, D., and Schulz, M. (2002). Benzoxazinones in plants: occurrence, synthetic access, and biological activity. *Studies Nat. Prod. Chem.*, 185–232. doi: 10.1016/S1572-5995(02)80037-0

Siebers, M., Brands, M., Wewer, V., Duan, Y., Hölzl, G., and Dörmann, P. (2016). Lipids in plant-microbe interactions. *Biochim. Biophys. Acta.* 1861, 1379–1395. doi: 10.1016/j.bbalip.2016.02.021

Siebers, M., Rohr, T., Ventura, M., Schütz, V., Thies, S., Kovacic, F., et al. (2018). Disruption of microbial community composition and identification of plant growth promoting microorganisms after exposure of soil to rapeseed-derived glucosinolates. *PLoS One* 13, e0200160. doi: 10.1371/journal.pone.0200160

Sikkema, J., Bont, J. A. de, and Poolman, B. (1995). Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev.* 59, 201–222. doi: 10.1128/mr.59.2.201-222.1995

Sinensky, M. (1974). Homeoviscous adaptation-a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 71, 522–525. doi: 10.1073/pnas.71.2.522

Singh, P., Arif, Y., Bajguz, A., and Hayat, S. (2021). The role of quercetin in plants. *Plant Physiol. Biochem.* 166, 10–19. doi: 10.1016/j.plaphy.2021.05.023

Sohlenkamp, C., and Geiger, O. (2016). Bacterial membrane lipids: diversity in structures and pathways. *FEMS Microb. Rev.* 40, 133–159. doi: 10.1093/femsre/fuv008

Stringlis, I. A., Yu, K., Feussner, K., Jonge, R. de, van Bentum, S., van Verk, M. C., et al. (2018). MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proc. Natl. Acad. Sci. USA* 115, E5213-E5222. doi: 10.1073/pnas.1722335115

Suzuki, H., Sasaki, R., Ogata, Y., Nakamura, Y., Sakurai, N., Kitajima, M., et al. (2008). Metabolic profiling of flavonoids in *Lotus japonicus* using liquid chromatography Fourier transform ion cyclotron resonance mass spectrometry. *Phytochemistry* 69, 99–111. doi: 10.1016/j.phytochem.2007.06.017

Sytykiewicz, H. (2014). Differential expression of superoxide dismutase genes in aphid-stressed maize (*Zea mays* L.) seedlings. *PLoS One* 9, e94847. doi: 10.1371/journal.pone.0094847

Szwed, M., Wiczkowski, W., Szawara-Nowak, D., Obendorf, R. L., and Horbowicz, M. (2019). Allelopathic influence of common buckwheat root residues on selected weed species. *Acta. Physiol. Plant.* 41. doi: 10.1007/s11738-019-2885-y

Tabaglio, V., Gavazzi, C., Schulz, M., and Marocco, A. (2008). Alternative weed control using the allelopathic effect of natural benzoxazinoids from rye mulch. *Agron. Sustain. Dev.* 28, 397–401. doi: 10.1051/agro:2008004

Takenaka, S., Murakami, S., Shinke, R., and Aoki, K. (1998). Metabolism of 2-aminophenol by *Pseudomonas sp*. AP-3: modified meta-cleavage pathway. *Arch. Microbiol.* 170, 132–137. doi: 10.1007/s002030050624

Terzano, R., Cuccovillo, G., Gattullo, C. E., Medici, L., Tomasi, N., Pinton, R., et al. (2015). Combined effect of organic acids and flavonoids on the mobilization of major and trace elements from soil. *Biol. Fertil. Soils* 51, 685–695. doi: 10.1007/s00374-015-1009-0

Thornton, H. G. (1922). On the development of a standardised agar medium for counting soil bacteria, with especial regard to the repression of spreading colonies. A*nn. Appl.Biol.* 9, 241–274. doi: 10.1111/j.1744-7348.1922.tb05958.x

Understrup, A. G., Ravnskov, S., Hansen, H. C. B., and Fomsgaard, I. S. (2005). Biotransformation of 2-benzoxazolinone to 2-amino-(3H)-phenoxazin-3-one and 2-acetylamino-(3H)-phenoxazin-3-one in soil. *J. Chem. Ecol.* 31, 1205–1222. doi: 10.1007/s10886-005-4257-x

Unell, M., Kabelitz, N., Jansson, J. K., and Heipieper, H. J. (2007). Adaptation of the psychrotroph *Arthrobacter chlorophenolicus* A6 to growth temperature and the presence of phenols by changes in the anteiso/iso ratio of branched fatty acids. *FEMS Microbiol. Lett.* 266, 138–143. doi: 10.1111/j.1574-6968.2006.00502.x

Venturelli, S., Petersen, S., Langenecker, T., Weigel, D., Lauer, U. M., and Becker, C. (2016). Allelochemicals of the phenoxazinone class act at physiologically relevant concentrations. *Plant Signal Behav.* 11, e1176818. doi: 10.1080/15592324.2016.1176818

Voges, M. J. E. E. E., Bai, Y., Schulze-Lefert, P., and Sattely, E. S. (2019). Plant-derived coumarins shape the composition of an Arabidopsis synthetic root microbiome. *Proc. Natl. Acad. Sci. USA* 116, 12558–12565. doi: 10.1073/pnas.1820691116

Voloshchuk, N., Schütz, V., Laschke, L., Gryganskyi, A. P., and Schulz, M. (2020). The *Trichoderma viride* F-00612 consortium tolerates 2-amino-3H-phenoxazin-3-one and degrades nitrated benzo[d]oxazol-2(3H)-one. *Chemoecology* 30, 79–88. doi: 10.1007/s00049-020-00300-w

Wang, A. Y., and Cronan, J. E. (1994). The growth phase-dependent synthesis of cyclopropane fatty acids in *Escherichia coli* is the result of an RpoS(KatF)-dependent promoter plus enzyme instability. *Mol. Microbiol.* 11, 1009–1017. doi: 10.1111/j.1365-2958.1994.tb00379.x

Wang, C.-M., Li, T.-C., Jhan, Y.-L., Weng, J.-H., and Chou, C.-H. (2013). The impact of microbial biotransformation of catechin in enhancing the allelopathic effects of *Rhododendron formosanum*. *PLoS One* 8, e85162. doi: 10.1371/journal.pone.0085162

Wang, H.-W., Tang, M.-J., Su, C.-L., Zhang, W., Xu, R.-S., Guan, Y.-X., et al. (2018). The Alleopathic compound luteolin from peanut litter affects peanut nodule formation and the rhizosphere microbial community. *Agron. J.* 110, 2587–2595. doi: 10.2134/agronj2018.03.0149

Wang, Y., Feng, J., Wang, G. P., Lv, X. G., and Wang, X. Y. (2013). A modified protocol for microbial DNA extraction and purification in crude oil-spilled marsh soil. *AMM* 340, 353–357. doi: 10.4028/www.scientific.net/AMM.340.353

Weber, F. J., and Bont, J. A. de (1996). Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. *Biochim. Biophys. Acta* 1286, 225–245. doi: 10.1016/s0304-4157(96)00010-x

Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H.-E., et al. (2002). Profiling membrane lipids in plant stress responses. Role of phospholipase D alpha in freezing-induced lipid changes in Arabidopsis. *J. Biol. Chem.* 277, 31994–32002. doi: 10.1074/jbc.M205375200

Welti, R., Shah, J., Li, W., Li, M., Chen, J., Burke, J. J., et al. (2007). Plant lipidomics: discerning biological function by profiling plant complex lipids using mass spectrometry. *Front. Biosci.* 12, 2494–2506. doi: 10.2741/2250

Wickham, H., François, R., Henry, L., and Müller, K. (2020). dplyr: a grammar of data manipulation. R Package Version 1.0.2.

Williams, M. A., and Rice, C. W. (2007). Seven years of enhanced water availability influences the physiological, structural, and functional attributes of a soil microbial community. *Appl. Soil Ecol.* 35, 535–545. doi: 10.1016/j.apsoil.2006.09.014

Willis, R. J. (1985). The historical bases of the concept of allelopathy. *J. Hist. Biol.* 18, 71–102. doi: 10.1007/BF00127958

Winkel-Shirley, B. (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol.* 126, 485–493. doi: 10.1104/pp.126.2.485

Wippich, C., and Wink, M. (1985). Biological properties of alkaloids. Influence of quinolizidine alkaloids and gramine on the germination and development of powdery mildew, *Erysiphe graminis* f. sp. *hordei*. *Experientia* 41, 1477–1479. doi: 10.1007/BF01950046

Woodward, M. D., Corcuera, L. J., Schnoes, H. K., Helgeson, J. P., and Upper, C. D. (1979). Identification of 1,4-benzoxazin-3-ones in maize extracts by gas-liquid chromatography and mass spectrometry. *Plant Physiol.* 63, 9–13. doi: 10.1104/pp.63.1.9

Wouters, F. C., Blanchette, B., Gershenzon, J., and Vassão, D. G. (2016). Plant defense and herbivore counter-defense: benzoxazinoids and insect herbivores. *Phytochem. Rev.* 15, 1127–1151. doi: 10.1007/s11101-016-9481-1

Yao, J., Luo, J.-S., Xiao, Y., and Zhang, Z. (2019). The plant defensin gene *AtPDF2.1* mediates ammonium metabolism by regulating glutamine synthetase activity in *Arabidopsis thaliana*. *BMC Plant Biol.* 19, 557. doi: 10.1186/s12870-019-2183-2

Yoshino, S., Hara, A., Sakakibara, H., Kawabata, K., Tokumura, A., Ishisaka, A., et al. (2011). Effect of quercetin and glucuronide metabolites on the monoamine oxidase-A reaction in mouse brain mitochondria. *Nutrition* 27, 847–852. doi: 10.1016/j.nut.2010.09.002

Zelante, T., Puccetti, M., Giovagnoli, S., and Romani, L. (2021). Regulation of host physiology and immunity by microbial indole-3-aldehyde. *Curr. Opin. Immunol.* 70, 27–32. doi: 10.1016/j.coi.2020.12.004

8. Supplementary Information

8.1. Barcode/Illumina Adapter Primer

Oligonucleotides for the addition of barcodes and Illumina Adapters (Durán et al., 2018) used in this study are provided in Supplementary Table 1 - 3. The primers were synthesized by Integrated DNA Technologies (IDT) (Belgium).

Supplementary Table 1: Bacterial 16S rRNA oligonucleotides for the addition of barcodes and Illumina Adapters (Durán et al., 2018).

Name	P7	Index/ Barcode	Temp/Null	1192R
B5-1	CAAGCAGAAGACGGCATACGAGAT	TCCCTTGTCTCC	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-2	CAAGCAGAAGACGGCATACGAGAT	ACGAGACTGATT	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-3	CAAGCAGAAGACGGCATACGAGAT	ACCGGTATGTAC	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-4	CAAGCAGAAGACGGCATACGAGAT	TGCATACACTGG	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-5	CAAGCAGAAGACGGCATACGAGAT	TGGTCAACGATA	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-6	CAAGCAGAAGACGGCATACGAGAT	ATCGCACAGTAA	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-7	CAAGCAGAAGACGGCATACGAGAT	GTCGTGTAGCCT	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-8	CAAGCAGAAGACGGCATACGAGAT	TACAGCGCATAC	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-9	CAAGCAGAAGACGGCATACGAGAT	ATCCTTTGGTTC	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-10	CAAGCAGAAGACGGCATACGAGAT	AGTCGAACGAGG	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-11	CAAGCAGAAGACGGCATACGAGAT	ACCAGTGACTCA	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-12	CAAGCAGAAGACGGCATACGAGAT	CCAATACGCCTG	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-13	CAAGCAGAAGACGGCATACGAGAT	GCAACACCATCC	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-14	CAAGCAGAAGACGGCATACGAGAT	AGTCGTGCACAT	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-15	CAAGCAGAAGACGGCATACGAGAT	AGTTACGAGCTA	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-16	CAAGCAGAAGACGGCATACGAGAT	TTGCGTTAGCAG	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-17	CAAGCAGAAGACGGCATACGAGAT	TACGAGCCCTAA	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-18	CAAGCAGAAGACGGCATACGAGAT	TGTCGCAAATAG	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-19	CAAGCAGAAGACGGCATACGAGAT	ACAATAGACACC	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC
B5-20	CAAGCAGAAGACGGCATACGAGAT	TCTCTACCACTC	CAGCCATTTAGTGTC	ACGTCATCCCCACCTTCC

B5-21	CAAGCAGAAGACGGCAT ACGAGAT	CGATCGAA CACT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-22	CAAGCAGAAGACGGCAT ACGAGAT	ATTGCAAG CAAC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-23	CAAGCAGAAGACGGCAT ACGAGAT	AGCGCTCA CATC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-24	CAAGCAGAAGACGGCAT ACGAGAT	TCGACCAA ACAC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-25	CAAGCAGAAGACGGCAT ACGAGAT	TGTGTTACT CCT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-26	CAAGCAGAAGACGGCAT ACGAGAT	TGCACAGT CGCT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-27	CAAGCAGAAGACGGCAT ACGAGAT	TTCTAGAGT GCG	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-28	CAAGCAGAAGACGGCAT ACGAGAT	ACACCTGC GATC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-29	CAAGCAGAAGACGGCAT ACGAGAT	ATTCCTCTC CAC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-30	CAAGCAGAAGACGGCAT ACGAGAT	CATCGACG AGTT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-31	CAAGCAGAAGACGGCAT ACGAGAT	CACCACAG AATC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-32	CAAGCAGAAGACGGCAT ACGAGAT	GGTCTTAGC ACC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-33	CAAGCAGAAGACGGCAT ACGAGAT	TATCGCGC GATA	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-34	CAAGCAGAAGACGGCAT ACGAGAT	CTCTACGA ACAG	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-35	CAAGCAGAAGACGGCAT ACGAGAT	CTCCTCCCT TAC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-36	CAAGCAGAAGACGGCAT ACGAGAT	CGTGTTATG TGG	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-37	CAAGCAGAAGACGGCAT ACGAGAT	ATTAGCAG CGTA	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-38	CAAGCAGAAGACGGCAT ACGAGAT	CAAGTTTCC GCG	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-39	CAAGCAGAAGACGGCAT ACGAGAT	CCTTGTTCA CCT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-40	CAAGCAGAAGACGGCAT ACGAGAT	AACCAGCA GATT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-41	CAAGCAGAAGACGGCAT ACGAGAT	CTAGAGCT CCCA	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-42	CAAGCAGAAGACGGCAT ACGAGAT	CACGCAGT CTAC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-43	CAAGCAGAAGACGGCAT ACGAGAT	ACAAACAT GGTC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-44	CAAGCAGAAGACGGCAT ACGAGAT	TCGAAACA TGCA	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-45	CAAGCAGAAGACGGCAT ACGAGAT	TTCCCACCC ATT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-46	CAAGCAGAAGACGGCAT ACGAGAT	AGCAGAAC ATCT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-47	CAAGCAGAAGACGGCAT ACGAGAT	GAAACATC CCAC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC

B5-48	CAAGCAGAAGACGGCAT ACGAGAT	CTGTCAGTG ACC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-49	CAAGCAGAAGACGGCAT ACGAGAT	CGGATCTA GTGT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-50	CAAGCAGAAGACGGCAT ACGAGAT	TTCTCCATC ACA	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-51	CAAGCAGAAGACGGCAT ACGAGAT	ATTTAGGA CGAC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-52	CAAGCAGAAGACGGCAT ACGAGAT	GGTTTAAC ACGC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-53	CAAGCAGAAGACGGCAT ACGAGAT	AGACAGTA GGAG	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-54	CAAGCAGAAGACGGCAT ACGAGAT	GCAGATTTC CAG	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-55	CAAGCAGAAGACGGCAT ACGAGAT	AGATGATC AGTC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-56	CAAGCAGAAGACGGCAT ACGAGAT	TATCACCG GCAC	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-57	CAAGCAGAAGACGGCAT ACGAGAT	CCAGATAT AGCA	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-58	CAAGCAGAAGACGGCAT ACGAGAT	GGTCTCCTA CAG	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-59	CAAGCAGAAGACGGCAT ACGAGAT	ACAGCTCA AACA	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-60	CAAGCAGAAGACGGCAT ACGAGAT	ATAGCGAA CTCA	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-61	CAAGCAGAAGACGGCAT ACGAGAT	AACCGCAT AAGT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-62	CAAGCAGAAGACGGCAT ACGAGAT	CTTGAGAA ATCG	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-63	CAAGCAGAAGACGGCAT ACGAGAT	CAGTCGTTA AGA	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC
B5-64	CAAGCAGAAGACGGCAT ACGAGAT	CTTCCAACT CAT	CAGCCATTTA GTGTC	ACGTCATCCCCA CCTTCC

Supplementary Table 2: Fungal ITS1 oligonucleotides for the addition of barcodes and Illumina Adapters (Durán et al., 2018).

Name	**P7**	**Index/ Barcode**	**Temp/Null**	**ITS2**
Ft-1	CAAGCAGAAGACGGCA TACGAGAT	GCGTTCTA GCTG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-2	CAAGCAGAAGACGGCA TACGAGAT	ATGTCACC GCTG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-3	CAAGCAGAAGACGGCA TACGAGAT	ACTCCTTGT GTT	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-4	CAAGCAGAAGACGGCA TACGAGAT	GATTCCGG CTCA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-5	CAAGCAGAAGACGGCA TACGAGAT	GTCACGGA CATT	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-6	CAAGCAGAAGACGGCA TACGAGAT	GGTGACTA GTTC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC

Ft-7	CAAGCAGAAGACGGCA TACGAGAT	GCGAGCGA AGTA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-8	CAAGCAGAAGACGGCA TACGAGAT	ACTTGGTG TAAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-9	CAAGCAGAAGACGGCA TACGAGAT	AACTAGTT CAGG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-10	CAAGCAGAAGACGGCA TACGAGAT	GCACACCT GATA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-11	CAAGCAGAAGACGGCA TACGAGAT	GCGACAAT TACA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-12	CAAGCAGAAGACGGCA TACGAGAT	GTATTTCG GACG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-13	CAAGCAGAAGACGGCA TACGAGAT	AGTAGCGG AAGA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-14	CAAGCAGAAGACGGCA TACGAGAT	GTTAAGCT GACC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-15	CAAGCAGAAGACGGCA TACGAGAT	ACCCAAGC GTTA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-16	CAAGCAGAAGACGGCA TACGAGAT	AGCAACAT TGCA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-17	CAAGCAGAAGACGGCA TACGAGAT	GATGTGGT GTTA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-18	CAAGCAGAAGACGGCA TACGAGAT	GTAGAGGT AGAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-19	CAAGCAGAAGACGGCA TACGAGAT	GGTTATTTG GCG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-20	CAAGCAGAAGACGGCA TACGAGAT	GTGTTAGA TGTG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-21	CAAGCAGAAGACGGCA TACGAGAT	AACTCGCG CTAC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-22	CAAGCAGAAGACGGCA TACGAGAT	CTTAAATG GGCA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-23	CAAGCAGAAGACGGCA TACGAGAT	TAGTGATG ACCA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-24	CAAGCAGAAGACGGCA TACGAGAT	GTCTCAAA GCAC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-25	CAAGCAGAAGACGGCA TACGAGAT	GGTATCAC CCTG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-26	CAAGCAGAAGACGGCA TACGAGAT	CTATGAGT CCAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-27	CAAGCAGAAGACGGCA TACGAGAT	AAGCCTCT ACGA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-28	CAAGCAGAAGACGGCA TACGAGAT	GTTACGTG GTTG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-29	CAAGCAGAAGACGGCA TACGAGAT	TAGGACGG GAGT	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-30	CAAGCAGAAGACGGCA TACGAGAT	AGACTTCT CAGG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-31	CAAGCAGAAGACGGCA TACGAGAT	AGTGACTG TCAA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-32	CAAGCAGAAGACGGCA TACGAGAT	GGTGAGCA AGCA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-33	CAAGCAGAAGACGGCA TACGAGAT	ATACAGCA TACG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC

Ft-34	CAAGCAGAAGACGGCA TACGAGAT	GGACAAGT GCGA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-35	CAAGCAGAAGACGGCA TACGAGAT	TGCAGATC CAAC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-36	CAAGCAGAAGACGGCA TACGAGAT	AGAACACG GAAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-37	CAAGCAGAAGACGGCA TACGAGAT	CCTTTATAG TCC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-38	CAAGCAGAAGACGGCA TACGAGAT	TCCCACGA AACA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-39	CAAGCAGAAGACGGCA TACGAGAT	TAGGCCAT GTAA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-40	CAAGCAGAAGACGGCA TACGAGAT	AGTCTAGA GTAC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-41	CAAGCAGAAGACGGCA TACGAGAT	TGGGTTAA CACA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-42	CAAGCAGAAGACGGCA TACGAGAT	GGAACTTA CTCG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-43	CAAGCAGAAGACGGCA TACGAGAT	CAGTTACC CAAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-44	CAAGCAGAAGACGGCA TACGAGAT	AACGGCTG GAAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-45	CAAGCAGAAGACGGCA TACGAGAT	TAGAGAAT GCTC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-46	CAAGCAGAAGACGGCA TACGAGAT	CTGGATTA CGGT	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-47	CAAGCAGAAGACGGCA TACGAGAT	CTAGTGAC CTAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-48	CAAGCAGAAGACGGCA TACGAGAT	GTGAGATA CCTA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-49	CAAGCAGAAGACGGCA TACGAGAT	GGTAACCT CTGA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-50	CAAGCAGAAGACGGCA TACGAGAT	AGCTTGAA TCAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-51	CAAGCAGAAGACGGCA TACGAGAT	CCAGTTCC AAAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-52	CAAGCAGAAGACGGCA TACGAGAT	CTAAAGTA GCAC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-53	CAAGCAGAAGACGGCA TACGAGAT	AGTGCTAG GTTA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-54	CAAGCAGAAGACGGCA TACGAGAT	TGTTCTGA GACG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-55	CAAGCAGAAGACGGCA TACGAGAT	AGAGCGGA ACAA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-56	CAAGCAGAAGACGGCA TACGAGAT	CTGGAACA TTAG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-57	CAAGCAGAAGACGGCA TACGAGAT	AATGGTTC AGCA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-58	CAAGCAGAAGACGGCA TACGAGAT	GTGACCCT GTCA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-59	CAAGCAGAAGACGGCA TACGAGAT	TTCACTGTG CGG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-60	CAAGCAGAAGACGGCA TACGAGAT	AACGAATA CCAC	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC

Ft-61	CAAGCAGAAGACGGCA TACGAGAT	ATGGTTCA CCCG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-62	CAAGCAGAAGACGGCA TACGAGAT	TAGCGGAA GACG	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-63	CAAGCAGAAGACGGCA TACGAGAT	CCGCTGAT GTCA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC
Ft-64	CAAGCAGAAGACGGCA TACGAGAT	GGAGATTG GAGA	CGTACTGTG GAGA	GCTGCGTTCTTCAT CGATGC

Supplementary Table 3: Oomycetal ITS1 oligonucleotides for the addition of barcodes and Illumina Adapters (Durán et al., 2018).

Name	**P7**	**Index/ Barcode**	**Temp/Null**	**5.8s-O**
Ot-1	CAAGCAGAAGACGGCA TACGAGAT	TCGGAATT AGAC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-2	CAAGCAGAAGACGGCA TACGAGAT	TGTGAATT CGGA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-3	CAAGCAGAAGACGGCA TACGAGAT	GGCCAGTT CCTA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-4	CAAGCAGAAGACGGCA TACGAGAT	AGTTGAGG CATT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-5	CAAGCAGAAGACGGCA TACGAGAT	CGGTCAAT TGAC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-6	CAAGCAGAAGACGGCA TACGAGAT	GCTCGAAG ATTC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-7	CAAGCAGAAGACGGCA TACGAGAT	CTCACCTA GGAA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-8	CAAGCAGAAGACGGCA TACGAGAT	ATGATGAG CCTC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-9	CAAGCAGAAGACGGCA TACGAGAT	TATACCGC TGCG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-10	CAAGCAGAAGACGGCA TACGAGAT	GTACGATA TGAC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-11	CAAGCAGAAGACGGCA TACGAGAT	GTGGTGGT TTCC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-12	CAAGCAGAAGACGGCA TACGAGAT	AGCTGTCA AGCT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-13	CAAGCAGAAGACGGCA TACGAGAT	TACTCGGG AACT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-14	CAAGCAGAAGACGGCA TACGAGAT	CACTCATC ATTC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-15	CAAGCAGAAGACGGCA TACGAGAT	TATCTATC CTGC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-16	CAAGCAGAAGACGGCA TACGAGAT	ATGTGTGT AGAC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-17	CAAGCAGAAGACGGCA TACGAGAT	GACATTGT CACG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-18	CAAGCAGAAGACGGCA TACGAGAT	ATCAGTAC TAGG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-19	CAAGCAGAAGACGGCA TACGAGAT	CAGAAATG TGTC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG

Ot-20	CAAGCAGAAGACGGCA TACGAGAT	ACGACTGC ATAA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-21	CAAGCAGAAGACGGCA TACGAGAT	TAGTTGAG CTGA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-22	CAAGCAGAAGACGGCA TACGAGAT	CCTATGCA CGGT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-23	CAAGCAGAAGACGGCA TACGAGAT	TGCAATGG TACC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-24	CAAGCAGAAGACGGCA TACGAGAT	ACAGACGA CGGA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-25	CAAGCAGAAGACGGCA TACGAGAT	GAACCAGT ACTC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-26	CAAGCAGAAGACGGCA TACGAGAT	TGGTAGTC TGAA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-27	CAAGCAGAAGACGGCA TACGAGAT	AGTCCTTT ATCC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-28	CAAGCAGAAGACGGCA TACGAGAT	TCAACGTG CTGC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-29	CAAGCAGAAGACGGCA TACGAGAT	TGATAATG CACG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-30	CAAGCAGAAGACGGCA TACGAGAT	CAACAGGT AACT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-31	CAAGCAGAAGACGGCA TACGAGAT	TAGCCTGT CGTG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-32	CAAGCAGAAGACGGCA TACGAGAT	AACAGGTC TCTG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-33	CAAGCAGAAGACGGCA TACGAGAT	CGCTAGGA TGTT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-34	CAAGCAGAAGACGGCA TACGAGAT	ACGGTGAA AGCG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-35	CAAGCAGAAGACGGCA TACGAGAT	AGGATCAG GGAA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-36	CAAGCAGAAGACGGCA TACGAGAT	GACAATTC CGAA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-37	CAAGCAGAAGACGGCA TACGAGAT	GAAGGTGA AGGT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-38	CAAGCAGAAGACGGCA TACGAGAT	TGACGGTT TAGC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-39	CAAGCAGAAGACGGCA TACGAGAT	AAGTGTGG TTGT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-40	CAAGCAGAAGACGGCA TACGAGAT	CTGTCGTG TCAG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-41	CAAGCAGAAGACGGCA TACGAGAT	CACATGGG TTTG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-42	CAAGCAGAAGACGGCA TACGAGAT	GGTCGAAT TGCT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-43	CAAGCAGAAGACGGCA TACGAGAT	TGTAGGTG TGCT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-44	CAAGCAGAAGACGGCA TACGAGAT	AGTTCATA CGGC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-45	CAAGCAGAAGACGGCA TACGAGAT	ATTGAAGT CTGG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-46	CAAGCAGAAGACGGCA TACGAGAT	GCTACAAG CCCT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG

Ot-47	CAAGCAGAAGACGGCA TACGAGAT	AACTCAAT AGCG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-48	CAAGCAGAAGACGGCA TACGAGAT	CCATATCC CGGA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-49	CAAGCAGAAGACGGCA TACGAGAT	AACTAAGG ACTC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-50	CAAGCAGAAGACGGCA TACGAGAT	GCGTGGTC ATTA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-51	CAAGCAGAAGACGGCA TACGAGAT	AGCTACTG CGTC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-52	CAAGCAGAAGACGGCA TACGAGAT	AATCCATG ACAG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-53	CAAGCAGAAGACGGCA TACGAGAT	AGATAGCT CGCT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-54	CAAGCAGAAGACGGCA TACGAGAT	ACCAGAAA TGTC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-55	CAAGCAGAAGACGGCA TACGAGAT	ACAGTTGT ACGC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-56	CAAGCAGAAGACGGCA TACGAGAT	ACGTCCAC TGTG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-57	CAAGCAGAAGACGGCA TACGAGAT	CTGGTTGG CATC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-58	CAAGCAGAAGACGGCA TACGAGAT	AAGTTAGT CCGC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-59	CAAGCAGAAGACGGCA TACGAGAT	CTGAATCT GGTG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-60	CAAGCAGAAGACGGCA TACGAGAT	AGAGGGTG ATCG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-61	CAAGCAGAAGACGGCA TACGAGAT	TCCCTCTG AGAG	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-62	CAAGCAGAAGACGGCA TACGAGAT	AGCGTTGT CCAA	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-63	CAAGCAGAAGACGGCA TACGAGAT	ATCTCGCT GGGT	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG
Ot-64	CAAGCAGAAGACGGCA TACGAGAT	GATTCGAG TGTC	GCCTGGAGT CATAG	AGCCTAGACATCC ACTGCTG

8.2. Impacts of BOA, Gramine and Quercetin on ASV Level

Relative abundance changes after treatment with BOA, gramine, or quercetin of bacterial genera were calculated and plotted by Dr. Katharina Frindte (University of Bonn, Germany).

Supplementary Table 4: Relative abundance changes after soil treatment with BOA, gramine, or quercetin of bacteria on ASV level
Mean relative frequencies and standard deviations (SD) are presented.

Genus	**Control: mean rel. freq. (%)**	**BOA: mean rel. freq. (%)**	**Gramine: mean rel. freq. (%)**	**Quercetin: mean rel. freq. (%)**	**Comment**
Pseudomonas	15.85 ± 17.64	26.84 ± 18.46	0.08 ± 0.08	9.68 ± 5.73	Inhibition by gramine
Nitrospira	1.54 ± 0,86	0.47 ± 0.22	0.76 ± 0.25	0.46 ± 0.28	Inhibition by all 3 compounds
Novosphingobium	0 ± 0	0 ± 0.02	0.63 ± 0.33	0.05 ± 0.07	Increase by gramine
Polaromonas	0.8 ± 0.31	0.61 ± 0.32	1.99 ± 0.4	0.41 ± 0.22	Increase by gramine
Ellin6067	0.56 ± 0.28	0.12 ± 0.11	0.44 ± 0.16	0.02 ± 0.04	Inhibition by BOA, quercetin
Arthrobacter	8.05 ± 2.46	14.8 ± 3.81	5.01 ± 1.76	6.33 ±2.17	Increase by BOA
Massilia	4.14 ± 2.5	1.78 ± 1.05	8.03 ± 1.8	3.64 ± 1.89	Increase by gramine
Asticcacaulis	0 ± 0.01	0 ± 0	1.49 ± 0.83	0 ± 0	Increase by gramine
Noviherbaspirillum	0.14 ± 0.01	0.38 ± 0.33	0.09 ± 0.12	1.94 ± 0.41	Increase by quercetin
Phenylobacterium	0.35 ± 0.14	0.13 ± 0.07	0.62 ± 0.11	0.95 ± 0.23	Increase by quercetin
Caenimonas	0.37 ± 0.19	0.18 ± 0.2	1.15 ± 0.75	0.21 ± 0.25	Increase by gramine
MND1	0.54 ± 0.33	0.08 ± 0.09	0.32 ± 0.26	0.08 ± 0.1	Inhibition by all 3 compounds
Bradyrhizobium	0.62 ± 0.24	0.31 ± 0.11	0.68 ± 0.18	1.47 ± 0.39	Increase by quercetin

mle1-7	0.44 ± 0.25	0.13 ± 0.11	0.32 ± 0.16	0.14 ± 0.17	Inhibition by all 3 compounds
P3OB-42	0.02 ± 0.03	0.05 ± 0.05	0.4 ± 0.29	0.02 ± 0.03	Increase by gramine
Gaiella	1.14 ± 0.66	0.46 ± 0.33	1.09 ± 0.41	0.51 ± 0.26	Inhibition by BOA, quercetin
Arenimonas	0.4 ± 0.25	0.11 ± 0.06	0.17 ± 0.08	0.01 ± 0.02	Inhibition by BOA, quercetin
Phyllobacterium	0.07 ± 0.07	0.01 ± 0.02	0.05 ± 0.05	0.43 ± 0.12	Increase by quercetin
Pseudarthrobacter	0.31 ± 0.37	1.41 ± 0.93	1.8 ± 1.18	0 ± 0	Increase by BOA, gramine
Lysobacter	1.11 ± 0.47	0.47 ± 0.21	0.75 ± 0.1	0.34 ± 0.21	Inhibition by all 3 compounds
Bacillus	0.82 ± 0.61	0.38 ± 0.23	0.85 ± 0.39	1.01 ± 0.45	Inhibition by BOA
Ramlibacter	0.16 ± 0.14	0.14 ± 0.13	0.71 ± 0.26	0.01 ± 0.03	Increase by gramine
Sphingomonas	5.81 ± 2.77	3.17 ± 0.67	5.4 ± 1.84	6.44 ± 1.55	
Sphingobium	0 ± 0.01	0 ± 0.01	0 ± 0.01	28.36 ± 12.19	Increase by quercetin
Streptomyces	1.49 ± 0.85	0.54 ± 0.19	1.18 ± 0.37	1.28 ± 0.48	Inhibition by BOA
Rhizobacter	0.53 ± 0.18	0.26 ± 0.09	2.27 ± 1.84	0.33 ± 0.22	Increase by gramine
Peredibacter	0.21 ± 0.17	0.05 ± 0.05	0.3 ± 0.19	1.0 ± 0.7	Increase by quercetin
Paenibacillus	0.39 ± 0.4	0.1 ±0.09	0.42 ± 0.24	0.55 ± 0.32	Inhibition by BOA
Flavobacterium	0.3 ± 0.21	0.06 ± 0.05	0.16 ± 0.11	0.1 ± 0.13	Inhibition by all 3 compounds
Paenarthrobacter	0.17 ± 0.17	0.43 ± 0.26	0 ± 0	0.45 ± 0.28	Increase by BOA, quercetin
Nocardioides					
Bdellovibrio	0.18 ± 0.19	0.18 ± 0.14	0.45 ± 0.26	0.11 ± 0.15	Increase by gramine

Mycobacterium	0.36 ± 0.21	0.12 ± 0.09	0.3 ± 0.12	0.48 ± 0.18	Inhibition by BOA
Cupriavidus	0.29 ± 0.34	0.97 ± 0.64	0.09 ± 0.08	0.7 ± 0.18	Increase by BOA, quercetin
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0 ± 0.02	0 ± 0	0.34 ± 0.41	0 ± 0	Increase by gramine
CL500-29_marine_group	0.08 ± 0.04	0.01 ± 0.01	0.05 ± 0.03	0.01 ± 0.02	Inhibition by BOA, quercetin
Janibacter	0 ± 0	0 ± 0	0 ± 0.01	0.37 ± 0.17	Increase by quercetin
Bosea	0 ± 0	0 ± 0	0 ± 0	0.11 ± 0.08	Increase by quercetin
Dongia	0.33 ± 0.14	0.08 ± 0.08	0.2 ± 0.12	0.37 ± 0.13	Inhibition by BOA, gramine
Altererythrobacter	0.01 ± 0.03	0 ± 0	0.03 ± 0.04	0.12 ± 0.08	Increase by quercetin
Brevundimonas	0.2 ± 0.06	0.08 ± 0.07	0.17 ± 0.08	0.25 ± 0.08	Inhibition by BOA
Pseudolabrys	0.16 ± 0.08	0.08 ± 0.08	0.27 ± 0.11	0.13 ± 0.06	Increase by gramine
Tardiphaga	0 ± 0	0 ± 0	0 ± 0	0.04 ± 0.05	Increase by quercetin
Sphingoaurantiacus	0 ± 0	0 ± 0	0 ± 0	0.02 ± 0.03	Increase by quercetin
Starkeya	0 ± 0	0 ± 0	0.01 ± 0.02	0 ± 0	Increase by gramine
Nordella	0.12 ± 0.1	0.03 ± 0.04	0.11 ± 0.07	0.13 ± 0.09	Inhibition by BOA
Geobacillus	0.12 ± 0.08	0.02 ± 0.03	0.1 ± 0.08	0.17 ± 0.09	Inhibition by BOA
Aquabacterium	0.08 ± 0.05	0.02 ± 0.03	0.12 ± 0.05	0.01 ±0.02	Inhibtion by BOA, quercetin
Piscinibacter	0.11 ± 0.07	0.05 ± 0.06	0.11 ± 0.06	0.02 ± 0.04	Inhibtion by BOA, quercetin

Terrimonas	0.1 ± 0.08	0.02 ± 0.03	0.02 ± 0.03	0.03 ± 0.04	Inhibition by all 3 compounds
Bacteriovorax	0.01 ± 0.02	0.03 ± 0.05	0.24 ± 0.16	0 ± 0	Increase by gramine
IS-44	0.16 ± 0.11	0.02 ± 0.03	0.11 ± 0.08	0.02 ± 0.05	Inhibtion by BOA, quercetin
Ga0074140	0.02 ± 0.02	0 ± 0	0 ± 0	0 ± 0	Inhibition by all 3 compounds

8.3. 16S rRNA Amplicon Sequences of Soil Bacteria

Sanger sequencing (GATC) was performed by Eurofins Genomics (Ebersberg, Germany), and the 16S rRNA sequences were blasted for bacterial identification using the databank of the National Center for Biotechnology Information (NCBI).

Bacteria isolated from control soil (CB)

CB1 *Pseudomonas sp.*

TTAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGA
GCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCG
CAAGGTTAAAACTCAAATGAATTGACGCACCTGTCTCAATGTCGGCGAGGCACCAATTAA
TCTCAAGAAAGTTCAAGAACCTTAAGGCCTGTTAACATTCATTGAACTTTCCAGAGATGG
ATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCAGTTGTCAGCTACTGTGGTG
AGATGTTGGGTTAAGTCCTGTAACGAGCGCAACCCTGTCCTTAGTTACCAGCACGTAATG
GTACCACTCTATGGCATTTTAAAGCTA

CB2 *Pseudomonas sp.*

AAACAGGATTTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGA
GCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCG
CAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT
AATTTGAAGCAAGTTCAAGAACCTTACCAGGCCGTGACATTCATTGAACTTTCCAGAGAT
GGATCGGTGCCTTCGGGAACATTGAGACAGGTGCGGCAAGGCAGTCACAGTTACTGTGC
GGCGATGTTGGGTCAAGTCCTCAAACGAGCGCAACCCTGGTCCATTAAGCCAGCACGCTA
TCAACCACTAGTGACATCGTCAAGCTA

CB3 *Pseudomonas sp.*

AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAG
CCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGACGGGAGCTAGGGGAGTACGGCCG
CAAGGTTAAAACTCAAATGAATTGACCGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTTGAAGCAAGGCGAAGAACCTTACCAGGCCGTAACATTCATTGAACTTTCCAGAGATG
GACCGGTGCCTCCGGGAACAGGAGACCCCCCGGCAATTCATTTGTCAGTAACCGTGCGGC
GATATTCCCCAAGTCCTCAAACTAGTGCAACCCTGTCCTTTAAGACAGCACGCTCCCAAC
CACTAGTGACATCGTCCG

CB4 ***Arthrobacter sp.***

AGGCGTAGTTGCTGCCAGTGTGTGATCGAATGCTGGTTTTCCGAACCAGGGTTGCGCTCG
TTGTGCGACTTAACGCAGCATCTCGCCACACGGCTTGACACAACCGAGTTGAACCGGGAA
ACCGACCACAAGTGAGGGACCTGATTCCAGGTCTTACCGGTTCGAACCAAGCCTTGGCAA
GGTTCTTCCCGTTGCAACCAATTAACCCGCACGCTCCGCCGCTTGTGCGGGCCCCCGTCAA
TTCCTTTGAGTTTTATCCTTGCGGCCGAACTCCCCAGGCGTCGCACTTAATGCGTTAGCTA
CGGCGCGGAAAACGTGGAATGTCCCCCACACCCACTGCCCAAAGTTTACGGCATGGACTA
CAAGGATATCAAAAACCAGTT

CB5 ***Pseudomonas sp.***

ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGCC
TTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAA
GGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATT
TGAAGCAAGTTCAAGAACCTTACCAGGCCTTAACATTCATTGAACTTTCCAGAGATGGAT
CGGTGCCTTCGGGAACAGTGCACAGGTGCGGCAAGGCAGTTGTCAGCAACTGTGCGGCG
ATATTGGGTCAGGTGCTCAAACTAGTGCAACCCTGTCCTTTAAGACAGCACGTTCCCAGG
GACTAGTGACATCGTCAAGGCC

CB6 ***Pseudomonas sp.***

ATACAGGATTAGATATCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAG
CCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGC
AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTTCAAGCAAGTTCAAGAACCTTAACAGGCCTTAACATTCAATGAACTTTCCAGAGATG
GATTGGTGCCTTCGGGAACATTGAGACAGGTGCGGCATGGCAGTTGTCAGCTACTGTGGT
GAGATGTTGGGTCAAGTCCTGAAACGAGTGCAACCCTTGTCCTTATAAGACAGCACGTAA
TGGTACCACTATAAGACGTCGTCCAGCCCG

CB7 ***Pseudomonas sp.***

TAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAG
CCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGC
AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTTGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATTCAATGAACTTTCCAGAGATG
GATTGGTGCCTTCGGGAACATTGAGACAGGTGCGGCAAGGCAGTTGTCAGCTCCTGTCCT
GAGATGTTGGGTCAAGTCCTGAAACGAGCGCAACCCTTCGCCTTAGTAGCCAGCACGTAC
CGGTACCACTAGTGACATCGTCCATGCCCC

CB8 ***Pseudomonas sp.***

ACAGGATTTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAGC
CTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCA
AGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA
TTTGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATTCATTGAACTTTCCAGAGATGG
ATTGGTGCCTTCGGGAACAGGAGACAGGTGCGGCAAGGCATTTGTGTTTTAACTGTGCTG
AGATATTGGGTCAGGTCCTCAAACGAGCGCAACCCTGTCCTTAGTAGCCAGCACGTTCTC
AACCACTAGTGACATTGTCCAGCTC

CB9 ***Pseudomonas sp.***

AAACAGGATATAGATATCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGA
GCCTTGAGCTATTAGTGGCGCAGCTAACGCATTAAGACGGGACCTAGGGGAGTATGGCC
GCAAGGGTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGCGAGCATGTGGTTT
AATTTGAAGCAAGTTCAAGAACCTTACCAGGCCTTAACATTCATTGAACTTTCCAGAGAT
GGATCGGTGCCTTCGGGAACAGGAGACAGGTGCGGCAATTCATTTGTGTTTTAACTGTGC
TGCGATATTGGGTCAGGTCCTCAAACTAGTGCAACCCTGTCCTTTAAGACAGCACGCTCC
CAACCACTAGTGACGTCGTCCAGCCGCG

CB10 ***Arthrobacter sp.***

ACAGGATTAGATACCCTGGTGGTCCATGCCGTAAAGTTTGGGCAGTGGGTGTGGGGGACA
TTCCACGTTTTCCGCGCCGTAGGTAACGCTTTAAGTGCGACGCCTGGGGAGTTCGGCCGC
AAGGATAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTA
TTTGGATGCAACGGGAAGAACCTTACCAAGGCTTGACATGGGCCGGACCGCCGCAGAAA
TGTGGTTTCTCCTTTGGGGCCGGTTCACAGGTGGTGCATGGTTTCGTCAGCCCGTGTCGTG
AGATTTTGGGTTAAGTCCCACAACGAGCGCAACCCTCGTTCCAAAAGCCAGAATTTAACC
ACACACTGCATGCAACTACCTGGCCT

CB11 ***Pseudomonas sp.***

TAACAGGATTATATATCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAG
CCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGC
AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTTGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCCAATGAACTTTCCAGAGATG
GATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGT
GAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGCTA
TGGTGGGCACTCTATGACATCGTCAGGCTC

CB12 ***Pseudomonas sp.***

AACAGGATTTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAG
CCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGC
AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTTGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATTCAATGAACTTTCCAGAGATG
GATTGGTGCCTTCGGGAACAGGAGACAGGTGCGGCAAGGCAGTTGTCAGCTACTGTGCTG
CGATATTGGGTCAAGTCCTCAAACTAGCGCAACCCTTTCCTTTAAGCCAGCACGCTCTCA
ACCACTAGTGACATCGTCAGGTCT

CB13 ***Pseudomonas sp.***

AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAG
CCTTGAGCTCTTAGTGGCGAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCA
AGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA
TTTGAAGCAACGCGAAGAACCTTACCAGGCCTTAACATTCATTGAACTTTCCAGAGATGG
ATTGGTGCCTTCGGGAACAGGAGACAGGTGCGGCAAGGCATTTGTGTTTTAACTGTGCTG
CGATATTGGGTCAGGTCCTCAAACTAGTGCAACCCTGTCCTTTAAGACAGCACGTTCCGG
TACCACTAGTGACATCGTCCAGTCC

CB14 ***Pseudomonas sp.***

TTAACTGGATTTGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGA
GCCTTGAGCTCTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCG
CAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT
AATTTGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATTCAATGAACTTTCCAGAGAT
GGATTGGTGCCTTCGGGAACATTGAGACAGGTGCTGCATGGCAGTCGTCAGCTCCTGTCG
TGAGATGTTGGGTTAAGTCCTGAAACGAGCGCAACCCTGTCCTTAGTAGCCAGCACGTAA
CGGTACCACTAGTGACATCGTCCCCGCCC

CB15 ***Arthrobacter sp.***

AAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTTCCAGCAGCCGCGGTTAT
ACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTC
GCGTCTGCCGTGAAAGTCCGGGGCTCAAATCCGGATCTGCGGTGGGTACGGGCAGACTA
GAGTGATGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGA
GGAACACCGATGGCGAAGGCAGGTCTCTGGGCATTAACTGACGCTGAGGAGCGAAAGCA
TGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGT
GTGGGGGACATTCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAG
TACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCA
TGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGGGCCGGACCG
GGCTGGAAACAGTCCTTCCCCTTTGGGGCCGGTTCACAGGTGGTGCATGGTTGTCGTCAG
CTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCCATGTTGC
CAGCGCGTAATGGCGGGACTTCATGAACTCGGCC

CB16 ***Arthrobacter sp.***

AGGTAAATTATCAACAAGGCGTCGACGGTAGCAGACATGAGAGGGTAACGGTCCATACT
GGGACTGAGACACAGCCCAGACTCCTACGGGAGGCAGCAGTGGGGATATTGCTCAATGG
GCGCAAGCCTGATGCAGCGACACCGCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCT
TTCAGTAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGT
GCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAG
AGCTCGTAGGCGGTTTGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCG
GTGGGTACGGGCAGACTAGAGTGATGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGA
AATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCATTAACTGA
CGCTGAGGAGCGAAAGCATGGGGGCGAACAGGATTAGATACCCTGGTAGTCCATGCCGT
AAACGTTGGGCACTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGCATT
AAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGG
CTTGACATGGACCGGACCGCCGCAGAAATGTGGTTTCCCCTTTGGGGCCGGTTCACAGGT
GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTCGTTCCATGTTGCCAGCGCGTAATGGCGGGACTCATGGAGATCCGCCC

CB17 ***Pseudomonas sp.***

TATTTTATTCAGGTGGTGAATGGGTGCGTATACCTAGGAATTTCCCTGTACTGGGGGACA
ACTTTTGAAAGGAACGCTAATACCGCATACTTCCTTCGGGAGAAAGCAGGGGACCTTCGG
GCCTTGCGCTATCAGATGAGCTTAGGTCGGATTAGTAGTTGGTGAGGTAATGGCTCACCA
AGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGG
TCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATC
CAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGA
AGGGCATTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAAC
TCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAAC
TGCATTCAAAACTGACAAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGG
TGAAATGCGTAGATATAGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACT
GACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
CGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCAT
TAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGG
CCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACAGG
TGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCG
CAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGCACTCTAAGGAGACGCAGCCC

CB18 ***Pseudomonas sp.***

GGCCCTACAAGTTCAACTAAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGC
ATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACA
GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAG
CGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGT
GACAAACCGGAGGAAGGTGGGGATGACGTAA

CB19 ***Arthrobacter sp.***

GGGGCGAAGTTGGGCCTAAGGTGTGGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAAC
GCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTAC
CAAGGCTTGACATGGGCCGGACCGGGCTGGAAACAGTCCTTCCCCTTTGGGGCCGGTTCA
CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTCGTTCCATGTTGCCAGCGCGTAATGGCGGGGACTCATGGGAGACTGCCG
GGGTCAACTCGGAGGAAGGTGGGGATGACGTAA

CB20 ***Paenibacillus sp.***

GGCGGGTTAGAGGAATGGCTAAGGTGTTAGGGGTTTCAATACCCTTGGTGCCGAAGTTAA
CACATTAAGCATTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGAC
GGGGACCCGCACAAGCAGTGCAGTATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTA
CCAGGTCTTGACATCCCTCTGACCGTCCTAGAGATAGGGCTTTCCTTCGGGACAGAGGAG
ACAGGTGGCGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTGATCTTAGTTGCCAGCACTTTGGGACGGCACTCTAGGACGACTGCCG
GTGACAAAACGGAGGAAGGTGGGGAGGAGTTTTCGCATTCAGGTCCGAAGTATAGGCGA
GGCTATGCCCCGCTCTGCTCCATCTAAGCGATGTCGACTGATGGCTACGCTTGATATCTCA
TGCTAGGCAGGTCTTGATCGATTTGTAGTGCGACAGCGATGGTGAGGTCGCAGACGCACG
ACATCCCGGTAGATTTCCTGCGCTACCTCAAGGACATGACCGCGAGCCGTTACCACTGTT
CTTCTAGCTCTGCAATTTCAAGGTGCGCTGTAGACGTTGTCGCACGTCTCTAGTGTATTAC
TGTTAGTCGGCACTACGACGGGGTTACAGTCACTCCGTACGCGCGGTCTCTGCCGTGCCG
TTCTGTCTTCTTCTTCCTTTGGGTTTTAAAAGCAGGTTGTATTTTTTGTATGTCGGCGGGAA
CGGTTGCCTCTTAGGTTCCCGGCTTCTCTGTCCGCTCCCCGATCATTTGTATAAGTTTTTCT
TTTTTTTAATTTTTTGTTTTTTTCCTTATCTCTTCGTTTCGCGTGGGAAGGCCTCCTCAGGCC
CCCCCCTCCTT

CB21 ***Streptomyces sp.***

TTCAAAAAATTAAGGTGGGACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCT
AACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCTAAGGATTTGA
AGGGGGACCGCACAAGCCTCGAAGAATGTGTCTTAATTCTACGCAACGGGAACAAACTT
ACCAAGGATTGACCTATTGCGGAAACCATCGCACACGGTGACCCCCTTGTGGTCGGTGAA
CGGGTGGGGCATGGCTGTCGTCAGCTCGTGCTGTGAGATGTTGGGTTAATCCCCGCGGGA
TGCAACACTTGTCCTGTGCTGCCCCCCCGACCTCCGCGGGGAGGCGGAGATCTGGGACAT
GGTCGGGACCTCTTTGGCCGACTTTGAATATGATCTATGACCTTCTCCACTCCTTCTCAAT
GCCGCGCTTGAGACCCAGCGATTGGCGCCGGCGTGACGGTACGCAGGCCCTCACTGACCA
ACGCCTGAGCCAGCACCGTGGCCCTCGTCGTGCCGACACCGCCAACGTCGTCGGTCTTCT
TGGCCACTTCCTTGACCAGGTAGACGCCATCTTCTCCTAGGCGTCCTCCAGTCATTACTGG
TAGCGAGAAAAAGGGAAAATATC

CB22 ***Arthrobacter sp.***

CCCCATCCCAACAGTTGGGAATAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCT
AACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCT
TACCAAGGCTTGACATGGACCGGACCGCCGCAGAAATGTGGTTTCCCCTTTGGGGCCGGT
TCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTCGTTCCATGTTGCCAGCGCGTAATGGCGGGGACTCATGGGAGACTG
CCGGGGTCAACTCAGAGGAAGGTTCCTATGACGATCCCCATCTTTTCACTTTATCTATGTT
TCACTCTCTATTTATATCTACAGAACCGTGCCCTGGAACTCGTTCTCATCATGCTGGGGTG
CTTTGTAAGTGAATCCAACATTTGCCGCCCCGAAAGTAGATACTGTCATGCAAACTACAA
CCCCTCTAACAGTTCCCTGCTACGAATAATAGGTTTAAAATCTTGTGACCCTGATCGCCCG
ATACGTGAATCATATTAAAATCTGACGTTAATCTTGCTGTTAACATGAATGCCATCTTCTA
CTTCGTAACTAGCCGCCGGTCTCCTACCACGCCATAGCTACAGCTTTGTGGACATAGATGT
AAAGATATGTATCTATTTTCAGTCGCAGGATCTTGTGTTCCCTTATGCAGGACAGTATTCT
TGAATGCTTCTGGTTTTTATCCGTCCGACGAAGTGGTGCTGCTGCAGCTACTGTCTATCAT
CGATCGTCTCGATGATACACGGATCCATCCCGAACTATATGCGTTGCCAAGAATTGGCCA
GGAGCTCGCAG

CB23 ***Bacillus sp.***

TGGGGGGTAAGAATGGAATGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCTA
ACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTT
ACCAGGTCTTGACATCCTCTGACAACTCTAGAGATAGAGCGTTCCCCTTCGGGGGACAGA
GTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGC
CGGTGACAAACCGGAGGAAGGTGGAGGATGACGTAAAA

CB24 ***Streptomyces sp.***

CTCACCTTTTTACTTTGGGGACTAGGTGTTGGCGACATTCCACGGTCGGTCGGTGCCGCAG
CTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATT
GACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACC
TTACCAAGGCTTGACATATACCGGAAAGCATTAGAGATAGTGCCCCCCTTGTGGTCGGTA
TACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTTGTCCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGG
AGACCGCCGGGGTCAACTCGGAGGAAGGTGGCCATGACGATGCGCTGACCCCCCCTGCC
GTCCAACAAATATGCTCTTTCTCGTTGCTCTCTGGCCAACCTGAGGAACTACTCGGATGCA
GGGACCGCTCAAGTGCCTGTTCAGATATACCGGATAGCGAATATTTGCACCTTTGATTG

Bacteria isolated from BOA treated soil (BB)

BB1 ***Pseudomonas sp.***

GGGCCCTAAGATGTCAACTAAGCCGTTGGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAAC
GCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CAGGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGA
CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACG
AGCGCAACCCTTGTCCTTAGTTACCAGCACGTCATGGTGGGCACTCTAAGGAGACTGCCG
GTGACAAACCGGAGGAAAGTGGGGATGACGTAAA

BB2 ***Pseudarthrobacter sp.***

GGGATGGTACGTGGGATAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGC
ATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCA
AGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCGGG
GTCAACTCGGAGGAAGGTGGGGATGACGTAGTTTTTTTCTAATAGACCGAAATCGGCAAA
ATCCCGTGAAAATGCTGTCAAGATCTTGAGAATCCGTTTTTGTGTTGATTCGAGGTG

BB3 ***Paenarthrobacter sp.***

GAGGGGTACGTGGGCCTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGC
ATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCA
AGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTCGTTCTATGTTGCCAGCGCGTTATGGCGGGGACTCATAGGAGACTGCCGGG
GTCAACTCGGAGGAAGGTGGGGATGACGTTATTTTTTTGCCTATTTGCCTTAATCGTAGAA
GTCCCTTGTAAGTCCGAGGAGGAGACCGAGATTGGGGTGAATGCAT

BB4 ***Pseudomonas sp.***

GGGGGCGTTACGATGTCACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACG
CATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGG
GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC
AGGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGAC
AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGA
GCGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGG
TGACAAACCGGAGGAAGATGGGGATGACGTAA

BB5 ***Rhodococcus sp.***

GGCCCTACGAGAAACTAAGCTGCTGGGGTGCATGGCATTTCAGTGGCGCAGCTAACGCAT
TAAGTTATCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGG
CCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAACG
TTTGACATCCCTATCGCGGATCGTGGAGACACTTTCCTTCAGTTCGGCTGGATAGGTGACA
GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTCGACTTTAGTTGCCATCATTTAGTTGGGTACTCTAAAGTAACCGCCGGTGAT
AAGCCGGAGGAAAGTGGGGATGACGTAAT

BB6 ***Cupriavidus sp.***

CGCCCCTTACGATGTCACTAGTTGTTGGGGATTCATTTCTTCAGTAACGTAGCTAACGCGT
GAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGA
CCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTACC
CTTGACATGCCACTAACGAAGCAGAGATGCATTAGGTGCCCGAAAGGGAAAGTGGACAC
AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTGTCTCTAGTTGCTACGAAAGGGCACTCTAGAGAGACTGCCGGTGACAAA
CCGGAGGAAGGTGGGAAAGACGTAA

BB7 ***Pseudomonas sp.***

GGACGGTACGAATGTCACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGC
ATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACA
GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAG
CGCAACCCTTGTCCTTAGTTACCAGCACGTCATGGTGGGCACTCTAAGGAGACTGCCGGT
GACAAACCGGAGGAAGGTGGGGATGACGTAATATACCTTCCAGACGACTAATCTGGCCA
GTGTTAGAAGCCGGATTTCTGGAGAAGCTGACGACTTTAGGTACC

BB8 ***Paenarthrobacter sp.***

GAGTCCTTTACGTGGGCCTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAAC
GCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTAC
CAAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGAGCATGGGTGTCGCCAATTCCAGTCATGAAATAATGGGTTAACCCCCGTCAAGA
ATGCAAGCCTCCCCGTATGTTCCCAGTGGTTCAGTGGGGGACTTATGAGCGATTGACGTT
GCTAACACAGAAGGAAAATTGATGTTGAAAGGGGGT

BB9 ***Paenarthrobacter sp.***

GAGGTCGTACGTGGGCCTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCGGG
GTCAACTCGGAGGAAGGAAAAAATAACGAAAACA

BB10 ***Pseudomonas sp.***

GGCCCCTAAGTGTCAACTAAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGC
ATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACA
GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAG
CGCAACCCTTGTCCTTAGTTACCAGCACGTCATGGTGGGCACTCTAAGGAGACTGCCGGT
GACAAACCGGAGGAAAGAGAAAAAAAAAAAAAAAGCTAACTCAGGTATGAGGTAGCTC
GACTTTCAGACCCAGCTCTTATCCTTGTAAGCTCGTTAATCGATTTCGCCTAGGATGCAGG
TACATCAGAGCCCAGCTTTTTTGCGTCTAATTTGCTAGTACGGCGTGGGGCCGCGCCTTTT
CCTTTGTCAGGAGGGAGACGAACGAGATGAGATACGACTACACAGAATCGAACATCTAT
GTGTTGGCCTCTTTCGGCAGATGCAACAGATGCAAGATCGGAGGGAAAGTCGCGGTGCC
GGGATGTTAGTGTCCGGAAATATATGGCGGGCTGCTCGACACCCCAGGAGGTGTATGTGG
TAAAACGGGCAACTGAATCGCACGGAACGCGTTTC

BB11 ***Paenarthrobacter sp.***

GGGGCGTACGTTGGGCCTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCGGG
GTCAACTCGGAGGAAGGTGGGGATGACGTAAC

BB12 ***Paenarthrobacter sp.***

GGGGCGAAGTTGGGCCTAAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCGCGTTATGGCGGGGACTCATAGGAGACTGCCGG
GGTCAACTCGGAGGAGAGAGAGGATGACAAAAAACATTGCCTACTAGTGCGCGTTTCAC
GGATCCCCAGGACCTATCACTGTTCATTATAGCATGAATAGACACTCTACCTGAGGGAGT
CGCGGATGCTCGCTAGCATTTATGTTGTGAACTACTACTCCCGCGAGCTCGGAATCTATGT
ATGCGGAGTTCGCATCTCCGTTTATGCTGCCAGATAAAAGTCCGCATCTCATAGTTCGTTT
ACTGAAGAGAAGCGAAGTGCAGGATTTTCATTTGAACGTCAGTTCTAGTTTTCTTCCGAA
ACAAAGATAGAGAACTGAGATCGGGTGAGGAGTGGCGCTAATTCGGGGGAGTTCTCTGA
AGTGTGCGTA

BB13 ***Pseudarthrobacter sp.***

GAGACGTACGTGGGGATAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGC
ATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCA
AGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCGGG
GTCAACTCGGAGGAAAGTGAAAAATAAAAAAAG

BB14 ***Paenarthrobacter sp.***

GGGATCAGTACGTGGGATAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCCTGAGATGTTGGGTTAAGTCCCGCAACAA
GCGCAACCCCCGTTCTATGTTGCCAGCGCGTGATGGTGGGCACTCATAGGACACTGCCGG
GGTCAAATCGGAGGAAAGTGAGAAAGAAATAACTTCCAGATCACCGCTGGGCGCGAATC
TGGATGAGCTGGGCCGGTT

BB15 ***Paenarthrobacter sp.***

GGGGGGAAATTGGGCCTAAGGTGTGGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAAC
GCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTAC
CAAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCGGG
GTCAACTCGGAGGAAGGTGGGGATGACGATAAGG

BB16 ***Pseudomonas sp.***

GTACCCGTTACGATGTCACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACG
CATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGG
GGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACC
AGGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAGCATTGAGAC
AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGA
GCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGG
TGACAAACCGGAGGAAGAAAGGAAATGACGTA

BB17 ***Streptomyces sp.***

GTTCCGTTCGTGGGACTACGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACGC
ATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCA
AGGCTTGACATACACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTGTACAGG
TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGGAGACCG
CCGGGGTCAACTCGGAGGAAGGTGGGGATGACGAAG

BB18 ***Arthrobacter sp.***

GGGCATTACGTGGCTACGTGTGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGCATT
AAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAAGG
CTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTACAGGTG
GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCGGGGTCA
ACTCGGAGGAAGGTGGGGATGACGTTGTCTACGCAGTAGCGGTGACACCTATTTTTTGCC
ACCTCCGTTTTCCCACCAAACCAACTATTGCGAGCCACCTGGGGACAT

BB19 ***Arthrobacter sp.***

GCAATTACGATGAGACATACGTGTGGGTACATTCCACGTTTTCCGCGCCGTAGCTAACGC
ATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCA
AGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCGGG
GTCAACTCGGAGGAAGGTGGGGATGACGTAGAAACCTGAATGCGAAGTGAAAATGATAA
AGGCCGCCCCGGCTATCCCTAATAAACCCGCTGTAGCGATCCTTGTGGGCCCCATTGCAT
TGGATAAACTGTGATCACTTGGCTGTTTGACAAAAAAAGGCTTATCTCGCGGGATACCAC
AGCATGGCCATCACTGAATTCTTCTTGAATGATTATCCTGATACAAAAAAGCTGTTTAAA
ATTCTTGGTGATTTGACAGAAGAACTGGAAAAGCTTGCTAGTCCCTCTATAAACGGGCTG
ATTTTGCCAA

BB20 ***Pseudomonas sp.***

GTACAGTTACGATGTCGACTAGCCGCTGGGATGCTTGAGATCTCAGTGGCGCAGCTAACG
CATTAAGTTAGTACCGCCTGGGGAGTACGGCCGCAAGATTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCAAACA
CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACG
AGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCG
GTGACAAACCGGAGGAAGGTGGGGATGACGTATTAGACAACTAAACCTGGGTCAAAATA
AAAACCTCCTTACAATTATCTTCCGCCAAAAACTTATTTAAATCATAGCATGCCAAGGATT
AG

BB21 ***Variovorax sp.***

GGCCCCAAGAGTCAACTGGGTTGTTGGGTCTTCCTGACTCAGTAACGAAGCTAACGCGTG
AAGTTGACCGCCTGGGCTCGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGA
CCCGCACAAGCGGTGGATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACC
TTTGACATGTACGGAATTCGCCAGAGATGGCTTAGTGCTCGAAAGAGAACCGTAACACAG
GTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTGTCATTAGTTGCTACATTTAGTTGGGCACTCTAATGAGACTGCCGGTGACA
AACCGGAGGAAGGTGGGGATGACGTATAGTCCACGCCCTAATGATGTCACTGGTTGTTGG
GTCTTCCTGACTAATTACGAATCTAAGCGCGAAGTTGACCGGCTGGGAAGTACGGACGCT
AGCTGAAACTCAAGGAATTGTGCGGAACCGCACAGGGATGGATGATCTTGATGATCTCG
ATCACGTGCATAGCACACCGGTTGGGCATCAACAACGAACTACGTGCCAAGCGCTGACG
CCAAATCTGTCCGAAGCTGACAGCACTGTATGAGATCAAAATGATAAC

BB22 ***Pseudomonas sp.***

GGCCGAAGAGTCAACTAAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCAT
TAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGG
CCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGG
CCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACAGG
TGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCG
CAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTGA
CAAACCGGAGGAAGGTGGGGATGACGTAAA

BB23 ***Paenarthrobacter sp.***

GGGGGGGAAGTTGGGCCTAAGGTGTGGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAA
CGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTA
CCAAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTA
CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCGG
GGTCAACTCGGAGGAAGGTGGGGATGACGATAAGG

BB24 ***Pseudomonas sp.***

GGCCGGAAGATGGTCAACTAAGCCGTTGGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAAC
GCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CAGGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGA
CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACG
AGCGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCG
GTGACAAACCGGAGGAAGGTGGGGGATGACGTAAA

BB25 ***Arthrobacter sp.***

GTGCAATACGTGGTACTATGTGTGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACGCA
TTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGG
GCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCAA
GGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTACAG
GTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCGGGG
TCAACTCGGAGGAAAGTGGGGATGACGTAA

BB26 ***Bacillus sp.***

GGGGGGGAAGAGAATGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGC
ATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGTCTTGACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTGA
CAAACCGGAGGAAGGAGGTGAAATGACGTAAAG

BB27 ***Streptomyces sp.***

GGCCGGAAGTTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTAACG
CATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACC
AAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTATACAG
GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGGAGACT
GCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTAAA

BB28 ***Streptomyces sp.***

GGCCGGAAAGTGGGAACCTTAGGTTGGTGGGCAACATTGCCACGTTGTCCGTGCCGCATC
TAACGCATTAAGTGCCCCGTCTGGGAGAGTACGGCCGCAAGGCTAACACTCAAAGGAAT
TGACGGGGGCCCGCACAAGCGGCGGAGCGTGTGGCTTAATTCGACGCAACGCGAAGAAC
CTTACCAAGGCTTGACATACACCGGAAACGTCCAGAGATGGGCGCCCCCTTGTGGTCGGT
GTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTTGTCCCGTGTTGCCATCACGCCCTTGTGGTGCTGGGGACTCACGGG
AGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGAGATGACATAAACTAAACCAAAGAA

BB29 ***Pseudomonas sp.***

CTAAATTACGATGTCACTAGCGTTGGGAGCTTGAGCTCTTAGTGGCGCAGCTAACGCATT
AAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGC
CTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACAGGT
GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGC
AACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTGAC
AAACCGGAGGAAGGTGGGGATGACGTTACTGCTAACTTAAACCTGAATGCGCTCTATAG
AGCATTAACTACGTTTATTAAACTAACGGATTGGGCACGCGCGCCGGGTCTAG

BB30 ***Arthrobacter sp.***

GGGGGGAAGTTGGGCCTAAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCGG
GGTCAACTCGGAGGAAGGTGGGGATGACGATAAA

BB31 ***Paenarthrobacter sp.***

GTGGAATAAACCGTTGGATACGTGTGGGGACATTCCACGTTTTTCCGCGCCGTAGCTTAA
CGCATTTAAGTGCCCCGCCAGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
ACCAAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTT
ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCG
GGGTCAACTCGGAGGAAGGTGGGAAATGACGTAGCCATGTATAACGTGTGATCAAATAT
TCTTTTTTCACCCTCCGTTTTTACCGCGTAATACCCCCCTCGGGAACACCTGGCAAAGGAC
T

BB32 ***Rhizobium sp.***

TGGCGTTCGATGATGTTAGCCGTCGGGCAGTATACTGTTCGGTGGCGCAGCTAACGCATT
AAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGC
CCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCC
TTGACATGCCCGGCTACTTGCAGAGATGCAAGGTTCCCTTCGGGGACCGGGACACAGGTG
CTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCTCGCCCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAG
CCGAGAGGAAGGTGGGGATGACGTAGGCGGCGCTTGGACCGGGGTTGTTATCTCCCCGT
ATCCTCTGAGTACCGTTTGAGCTAAGATTGTCAACTGCCACAACATAAGATGGTTGCCAG
ATTAAAACTAATGTGCTGGGCGTACCGGGACCGAGG

BB33 ***Paenarthrobacter sp.***

GGGGGGAAATTGGGCCTAAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCGGG
GTCAACTCGGAGGAAGGTGGGGATGACGATAAG

BB34 ***Arthrobacter sp.***

GGGGGGGAAGTTGGGGCCTAAGGTGTGGGGGAATTCCACGTTTTCCGCGCCGTAGCTAAC
GCATTAAGTCCCCCTGGCGGAGCGTGCCGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTAC
CAAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCGG
GGTCAACTCGGAGGAAGGTGGGGATGACGATAAGG

BB35 ***Paenarthrobacter sp.***

GGGGGGAAGTTGGGCCTAGGTGTGGGGGGAACATTCCACGTTTTCCGCGCCGTAGCTAAC
GCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTAC
CAAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCGGG
GTCAACTCGGAGGAAGGTGGGGATGACGATAAGAA

BB36 ***Rhizobium sp.***

TTAAATACGATGATGTAGCGTCGGGCAGTATACTGTTCGGTGGCGCAGCTAACGCATTAA
ACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTT
GACATGCCCGGCTACTTGCAGAGATGCAAGGTTCCCTTCGGGGACCGGGACACAGGTGCT
GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAAC
CCTCGCCCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCC
GAGAGGAAGGTGGGGATGACGTAGCCCACGATGCATACCCGACCGCTAGAACTATAGCT
AGAATCCTGGGCGAACCAACGATGCTCTACTTCCAGAAAAGCGAAGATGGTAACCACTTC
ATCCCGGGTCAGCACCACCGGCAAGCGCCGCGACGGCCGAGGTCTTCCGATCTCCTGAAG
CCAGAGCATATCCCGTGCACAGCACCTTGTCGTAGAAAAACAACGAGGCCGCCAATGCCT
GACGATGCGTGCAGACCGAAACCTTGCGCTCGTCTCCATCCTTGACAAAAATGCCTCGAC
TTCCTGCTGCCCAAGTTGCCGGGTGACGCACACCTTGGAAACCGATGAATGCACCAACCC
ACTTGACATGAACCCTGTTCTATCGTATACTGTAATGCAATATCATATGCTGCTCACGCAA
CTAGGACCAAACCTTGACCGAATGCATCGGTTGGTAACGTCGCATTGCGGTTTTCATGTCT
TGTTATCACTGTTATTTTGTACAGTCTATGTCACGAGCAATCAAACAGCAAACCCGTTACG
TCGTGTGTCAATGTCTGATGGTATGGATCGGCAATCGATGTTCCGCAGCAGAACCAAAGT
TACGCAGCAAGGATAATCAACCTAGATCATTGTTAGGTTGCTCAGTATGGGTCATCATTC
GGCGACATGTAAGCATCGGGCCCTGACAAGTCGAATGCCATGCGAAGCT

BB37 ***Paenarthrobacter sp.***

GGGGGGGAAAGTTGGGCCTAAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAA
CGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTA
CCAAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTA
CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCGG
GGTCAACTCGGAGGAAGGTGGGGATGACGATAA

BB38 ***Nocardioides sp.***

GTAAAAGACCCGTTGGCGCTAGTGTGGGACCTATTCCATGGGTTCCGTGCCGTAGCTAAC
GCATTAAGCGCCCCGCGGGCGGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACCT
GGGTTTGACATACACGGGAAGCCTGCAGAGATGTGGGTCTCTTTGATACTCGTGTACAGG
TGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTCGTTCCATGTTGCCAGCGGGTTATGCCGGGGACTCATGGGAGACTGCCGGGGT
CAACTCGGAGGAAGGTGGGGATGACATGA

BB39 ***Streptomyces sp.***

GGGGGGAAATTGGGAATAGGTGTTGGCGAACATTCCACGTCGTCGGTGCCGCAGCTAAC
GCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTAC
CAAGGCTTGACATATACCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGTATACA
GGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGGAGAC
TGCCGGGGTCAACTCGGAGGAAGGTGGGGGATGACGTAAGGG

BB40 ***Arthrobacter sp.***

GGGGGGGAAGTTGGGCCTAAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAAC
GCATTAAGTGCCCCGCCTGTGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTA
CCAAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTA
CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCG
GGGTCAACTCGGAGGAAGGTGGGGATGACGATAA

BB41 ***Paenarthrobacter sp.***

GGAGGGGACAGTTGGGGCCTTAGGTGTGGGGGGAATTCCACGGTTTTCCGCCGCCGTAGC
TAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATT
GACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACC
TTACCAAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGT
TTACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGC
CGGGGTCAACTCGGAGGAAGGTGGGGGGATGACGTAAA

BB42 ***Pseudoduganella sp.***

GGCCCCAAAAGTTCAACTAAGTTGTTGGGTCTTAATTGACTTAGTAACGCAGCTAACGCG
TGAAGTAGACCGCCTGGCGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGG
ACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTAC
CCTTGACATGGCAGGAATCCCGGAGAGATTTGGGAGTGCTCGAAAGAGAACCTGCACAC
AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTTGTCATTAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGGTGACAAA
CCGGAGGAAGGTGGGGATGACGTAAG

BB43 ***Mycobacterium sp.***

GGCCCGGAAGGGGGGACTAAGGTGTGGGTTCCTTCCTTGGGATCCGTGCCGTAGCTAACG
CATTAAGTACCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACC
TGGGTTTGACATGCACAGGACGCTGGTAGAGATATCAGTTCCCTTGTGGCCTGTGTGCAG
GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCCTATCTTATGTTGCCAGCGCGTTATGGCGGGGACTCGTAAGAGACTGCCGGGG
TCAACTCGGAGGAAGGTGGGGATGACGTAACCAC

BB44 ***Pseudarthrobacter sp.***

TAAGAAATACGATTGGGCCTATGTGTGGGGGACATTCCACGTTTTTCGCGCCGTAGCTAA
CGCATTAAGTGCCCCGTGGGTGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTAC
CAAGGCTTGACATGAACCGGACCGACCTGGAAATGTGTTTCCCCCTTGGGGGCCGGTTCA
CGGGGGGGGATGGGTGGTCTCCACCCCGGGCCGGGAAAGGTGGGGTAAAGCCCCGCACC
AACCCCACCCCCCGTCCCTGGTGGCCACCCCTGATGGGGGGGAACTCAAGGAAAACGGC
CGGGTCCAACCCGAAGAAAGGGGGGAATAACTTAGCCAGATGCTTATCATTTTATTTCTC
TCCTTGATCTGTTAATATTCCTGAGTATACGTAGACAACCACTAGAACTATCGACTAGAGT
CCTGGGCGAACAAACGATGCGCACCTTCCAGAAAACCGAGGATGCGAAGCACTTCATAC
CGTGTCAGCACCGCCGGCAAGCGCCGCGACAGCCGAGGTCTTCCGATCTCCTGAAGCCAG
GGCAGATCCGAGCACAGTGCCTTGCCGTAAAATAACAATAAGGCCGTGAATGCCTGACG
ATGCGTGCAGACCGAAGCCTTGCGCTCGTTCGCCAGCCAGGACACAAATGCCTCGACTTC
GCTGCTGTCTCACGTTGCCGGTTGACCCACTTCCGTGGAAACAGCATGAACGCTCGAACC
CACGTGACATAAACACTGTTCGGGTCGCAAACTGTAACGCAATAGCGATATGCACTCACG
CACTGGTCCAGATCCTTTGACCCAACACAGCCTGATAACGGTTCAGTAGCAGTTCTCATG
GCCTGTTATGACTGTTTTTTGTACAGACTATTCCTTCGTACATCCAAGCAGTTATCATACT
AC

BB45 ***Phyllobacterium sp.***

TGCATTACATTGAGTAGCTAGCTGTCGGGCAGTATACTGTCTCGGTGGCGCAGCTAACGC
ATTAAGCTCTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCA
GCCCTTGACATCCCGATCGCGGTTACCAGAGATGGTATCCTTCAGTTAGGCTGGATCGGT
GACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGCCCTTAGTTGCCATCATTCAGTTGGGCACTCTAAGGGGACTGCCG
GTGATAAGCCGAGAGGAAGGTGGGGATGACGTACGCCTTCCGTTCCTATGAAATTTAAGC
TTCGGCACAACTGGCCGGTGGCGCAGAAACGCATTAAGCTCTCCGCCGGGAAGTAT

Bacteria isolated from gramine treated soil (GB)

GB1 ***Arthrobacter sp.***

GGGGTCCGTACGTTGGGCCTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAA
CGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTA
CCAAGGCTTGACATGAACCGGACCGCTGCAGAAATGTGGTTTCCCCTTTGGGGCTGGTTT
ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCC
GGGGTCAACTCGGAGGAAGGTGGGGATGACGTA

GB2 ***Pseudomonas sp.***

GGAAAGCCCGGGCTTTGGGCCTTCCTTTCCGAAGGCCTAGGTGGGTTGTTTGTTGTGGAG
TAAGGGTCTCCAAGCGAGGTTCGTAATTGTTTGAGAGATGTCAGTCCAACTGGACTGAGA
CACCGTCCAGACTCCTACGGAGGCAGCAGTGGGAATATGGACAATGGGCGAAAGCCTGA
TCCAGACATGCCGCGTGTGTGAAGAAGGTCTTCGGATGTAAAGCACTTTAAGTTGGAGGA
AGGGCATTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAAC
TCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAA
CTGCATTCAAAACTGACGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCG
GTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATA
CTGACACTGAGGTGCGAAAGCGTGGGGGCAAACAGGATTAGATACCCTGGTAGTCCACG
CCGTAAACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCA
TTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG
GCCTTGACATCCAATGAACTTTCCAGAGATGGATGGGTGCCTTCGGGAACATTGAGACAG
GTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGC
GCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTG
ACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTA
CACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCC
ACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGC
TAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC
GTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACCTTCGGGAGGACGGTT
ACCACGGGATCGGGCC

GB3 ***Pseudarthrobacter sp.***

AGACATTTCACATTTTTCGCGCTGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACG
GCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCG
GATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGAAAGACCT
GGAAACAGGTGCCCCGCTTGCGGTCGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGT
GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCA
CGTGATGGTGGGGACTCATAGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGA
CGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCCGGTACAA
AGGGTTGCGATACTGTGAGGTGGAGCTAATCCCAAAAAGCCGGTCTCAGTTCGGATTGGG
GTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCG
GTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAAAGTTGGTAACACC
CGAAGCCGGTGGCCTAACCCCTTGTGGAGGGAGCTGTTCGAAGGGGCGGGTCC

GB4 ***Pseudomonas sp.***

TTTTAAGATGAGGGGGTGAGCAATCTGCCGAGTGATATATTTCATTTGATTCAGCACGAA
GAACTTTACCTTCGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGA
ACATTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT
CCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGG
AGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTA
CGGCCTGGGCTACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGT
GGAGCTAATCCCACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGA
AGTCGGAATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTG
TACACACCGCCCGTCACACCATGGGAGTGGGTTGCACCAGAAGTAGCTAGTCTAACCTTC
GGGAGGACGGTTACCACGGGATCGGGCC

GB5 ***Pseudarthrobacter sp.***

GGGTCGGTACGATGGGGCCTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAA
CGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTA
CCAAGGCTTGACATGAACCGGACCGCTGCAGAAATGTGGTTTCCCCTTTGGGGCTGGTTT
ACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCC
GGGGTCAACTCGGAGGAAGGTGGGATGACGTA

GB6 ***Pseudomonas sp.***

GGGCGGTTACGATGTCACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGC
ATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACA
GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAG
CGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGT
GACAAACCGGAGGAAGGTGGGGATGACGTAA

GB7 ***Pseudarthrobacter sp.***

GGGGCGAAGTTGGGCCTAAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCGG
GGTCAACTCGGAGGAAGGTGGGGATGACGTAAA

GB8 ***Pseudomonas sp.***

GACCGTTACGATGTCACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCA
TTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG
GCCTTGACATCCAATGAACTTTCCAGAGATGGATGGGTGCCTTCGGGAACATTGAGACAG
GTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGC
GCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTG
ACAAACCGGAGGAAGGTGGGGATGACGTAA

GB9 ***Pseudarthrobacter sp.***

GGGGCGAAGTTGGGCCTAAGGTGTGGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAAC
GCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTAC
CAAGGCTTGACATGAACCGGACCGCTGCAGAAATGTGGTTTCCCCTTTGGGGCTGGTTTA
CAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCG
GGGTCAACTCGGAGGAAGGTGGGGATGACGTAG

GB10 ***Pseudomonas sp.***

CGGCCGGTACGATGTCACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGC
ATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACA
GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAG
CGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGT
GACAAACCGGAGGAAAGTGGGGATGACATAA

GB11 ***Pseudarthrobacter sp.***

GGGGCGAAGTTGGGCCTAAGGTGTGGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAAC
GCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTAC
CAAGGCTTGACATGAACCGGTAACGCCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGGACTCATAGGAGACTGCCGG
GGTCAACTCGGAGGAAGGTGGGGATGACGTAATGAAGAGAAAACTGAGAC

GB12 ***Pseudomonas sp.***

GGGCGTTACGATGTCACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCA
TTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG
GCCTTGACATCCAATGAACTTTCCAGAGATGGATGGGTGCCTTCGGGAACATTGAGACAG
GTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGC
GCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTG
ACAAACCGGAGGAAAAAAAAAAGAAGACAAAAA

GB13 ***Pseudomonas sp.***

AGGGCGTTACGATGTCACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGC
ATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCA
GGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACA
GGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAG
CGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGT
GACAAACCGGAGGAAGAAAAAAAAAGACGT

GB14 ***Paenarthrobacter sp.***

GGGGCTTAGTTGGGCCTAAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTGCCCCGCTTGCGGTCGGTTTAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCTATGTTGCCAGCGGTTCGGCCGGGGACTCATAGGAGACTGCCGGG
GTCAACTCAGAGGAAGGTGGGAGATGACTGTTAAT

GB15 ***Pseudomonas sp.***

GGCCCTAAGTGTCAACTAAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAACGCA
TTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGG
GCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG
GCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGACAG
GTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGC
GCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGACTGCCGGTG
ACAAACCGGAGGAAAGTGGAGATGACGTAAGGATTTTACGCT

GB16 ***Streptomyces sp.***

GGGGGGGTAAGGGGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCTA
ACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTT
ACCAAGGCTTGACATCGCCCGGAAAGCATCAGAGATGGTGCCCCCCTTGTGGTCGGGTGA
CAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTGTTCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGGAG
ACTGCCGGGGTCAAGGCGGAGGAAGGTGTCGGATGACGTTAA

GB17 ***Arthrobacter sp.***

CGCAAACACTTCGTTGGGCCTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTA
ACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
ACCAAGGCTTGACATGGACCGGACCGCCGCAGAAATGTGGTTTCCCCTTTGGGGCCGGTT
CACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGTTCCATGTTGCCAGCGCGTAATGGCGGGGACTCATGGGAGACTGC
CGGGGTCAACTCGGAGGAAGGTGTTTAATGACGTAT

GB18 ***Arthobacter sp.***

TTCCCCCCTACGTTGGGACTATGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAAC
GCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG
GGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTAC
CAAGGCTTGACATGGACCGGACCGCCGCAGAAATGTGGTTTCCCCTTTGGGGCCGGTTCA
CAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTCGTTCCATGTTGCCAGCGAGTAATGGCGGGGACTCATGGGAGACTGCCG
GGGCCATCTCGTACGAATGCGTCATGACGTAGCTCATCACGTCCACCCTCTCGACGTCCTT
CTCCATCCCTCTGTTCCAGCAGAGCGGTGTCTCGCCGGCGCCTACTATCCGCATACCTTCA
ATATACAACATTGTGCCAGAACGCCCCCCGATGATGCGCTCGCCCAGACACGTCCTCTCC
CTTCTTTCCGCTCCCTTTGACGAAGTCAGGCCTATAATCTCGAACGCTTCCTACGCTCAAT
TTCTGTGAAAATATGACATCAGAGATTGACTACCTCCTGTGTGTCTTCCCTCGATACCTCC
ACATGCTTGACTACCCCCCCGGTATGCGGGTGTCTCCGGACCTCCTGTCGCGTGTCGTGGT
GTGAAAGCAGCGTCTCATGACTAGACGCGGATCTGC

GB19 *Streptomyces sp.*

TCCCTTTACCAAAAATCCTTTTGCCACTAGGTGTGCGCGACATTCCACGTCGTCCGTGCCG
CAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGG
AATTGACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAG
AACCTTACCAAGGCTTGACATACACCGGTAAGCCGTAGAGATACGGCCCCCCTTGTGGTC
GGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC
GCAACGAGCGCAACCCCTGTTCTGTGTTGACAGCATGCCTTTCGATTTGACGGCGTCTCAC
TCCTCTTGTTCGCCATCTATGTCCAAATTTACTCCCGTTACCTGCACATTCCCGTAGTTTCT
TATCTTCTTCCTTTCCTTAATCT

GB20 *Arthrobacter sp.*

AAACCAAAAAAAGTTGCGGACTAAGTGTGAAGCACCTTCTCGACATCCGCGTGGAGGAA
TGATTAATGGCCCCGCCTGGAGAGTACTGCCGGAAGGGTAAAACTCCAGGAATTGACGG
CCGCCCGCGAAGCGCGGAGCTTGCGGATTAATTAGATGCATCGCGAACAACCTTACCAAG
GCTTGGCATGGACCGGACCGCCGCAGAAATGTGGTTTCCCCTTTGGGGCCGGTTCACAGG
TGGTGCATGGTTGTCGCCAGCTCGTGTCGTGAGATGCTGGGTTAAGTCCCGCAACGAGCG
CAACCCTCGTTCCTTGTTGCCTGCGCGTAATGGAGGTGATTCATGGGAGACTGCCGGGGT
CAGCTCGGAGGAAGGCGGGAATGACGTAATCTTCATAGCTTCTACTGTCTTAACGTTGAT
TCCTGGCGTCCTCGTGAGGTCTCATGAACGGAATCCAGACTCGAACAGTATATCGTCTTC
CCCAAGATTACAGGAATGCGCTAGGATGCCAACTTTGAAGATGCTTCTTTAATGAAGACT
ACTGTAATTTATTTACCTACTGAGATGATCATGTTCATTCTCACTTCCAGAGCAGAGGAGC
TTTGCCAGATAAATCAGTCACTTGATGCTATGCTCCGTATGATTGCGTTGCCTTTAGCAGA
GTTGCTCCTTTGCACTTTCTTGTAACCCAACTTGCCTTAACAAACGAAAT

GB21 *Pseudarthrobacter sp.*

ATAAAACAATTTCCGTTTGGCCTTAGGTGTGGAGAATTCCACGTTTTCCGCGCCGTAGCTA
ACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTT
ACCAAGGCTTGACATGGACCGGACCGCCGCAGAAATGTGGTTTCCCCTTTGGGGCCGGTT
CACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAA
CGAGCGCAACCCTCGTTCCATGTTGCCAGCGCGTAATGGCGGGGACTCATGGGAGACTGC
CGGGGTCAGGATAGGAGGAAGGTGTCAGATGACGTAACT

Bacteria isolated from quercetin treated soil (QB)

QB1 Arthrobacter sp.

TTAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGG
ACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGACCGCTGCAGA
AATGTGGTTTCCCCTTTGGGGCTGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGT
GATGGTGGGACTCATAGAGACGCACGAC

QB2 *Pseudarthrobacter sp.*

TTAACTGTATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGG
ACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGAAAGACCTGG
AAACAGGTCCCCCACTTGTGGTCGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGT
GATGGTGGGACTCATAGAGACGCCCGG

QB3 ***Pseudarthrobacter sp.***

TTAACAGGATAAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGG
ACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGAAAGACCTGG
AAACAGGTCCCCCACTTGTGGTCGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGT
GATGGTGGGACTCATAGAGACGCAGGTG

QB4 ***Arthrobacter sp.***

TTAACAGGATAAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGG
ACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGAAAGACCTGG
AAACAGGTCCCCCACTTGTGGTCGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGT
GATGGTGGGACTCATAGAGACTGCCCAGCG

QB5 ***Arthrobacter sp.***

CGGAACCTCCCCCGCGGGCATATGTGCGTAGGTATTGCCGACATATAAGAACGTCCCCCA
AAACTCGTGCTCCAAGGCTAGGCCAGGACCGAACAGGATTAGATACCCTGGTAGTCCATG
CCGTAAACGTTGGGCACTAGGTGTGGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGAACCGGAAAGACCTGGAAACAGGTCCCCCACTTGTGGTCGGTTTACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG
CGCAACCCTCGTTCTATGTTGCCAGCACGTGATGGTGGGACTCATAGAGATGCCTGC

QB6 ***Pseudarthrobacter sp.***

CGTTAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGG
GGACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACG
GCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCG
GATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGAAAGACCT
GGAAACAGGTCCCCCACTTGTGGTCGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGT
GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCA
CGTGATGGTGGGACTTCATAGAGACGCCGCCC

QB7 ***Arthrobacter sp.***

TTAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGG
ACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGAAAGACCTGG
AAACAGGTCCCCCACTTGTGGTCGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGT
GATGGTGGGACTCATAGAGACTCCCTGT

QB8 ***Novosphingobium sp.***

TTAACAGGATTAGTTACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCCGGG
TACTTGGTACTTGGGTGGCGCAGCTAACGCATTAAGTTATCCGCCTGGGGAGTACGGTCG
CAAGATTAAAACTCAAAGGAATTGACGGGGGCCTGCACAAGCGGTGGAGCATGTGGTTT
AATTCGAAGCAACGCGCAGAACCTTACCAGCGTTTGACATGCCGGTCGCGGATTTGGGAG
ACCATTTCCTTCAGTTCGGCTGGACCGTGCACAGGTGCTGCATGGCTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTCCTTAGTTGCCAGCAT
TTAGTGGGCACTCTAAGGATCGCCAGTGAC

QB9 ***Pseudarthrobacter sp.***

TTAAACAGGATAAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGG
GACATTCCACGTTTTCCGCGCCGTAGTTAACGCATTAAGTGCCCCGCCTGGGGAGTACGG
CCGCAAGGTTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGG
ATTAATTGGATGCAACGGGAAGAACCTTACCAAGGCTTGACATGGACTGGAAATACCTG
GAAACAGGTGCCCCGCTTGCGGCCGGTTCACAGGTGGTGCATGGTTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCGC
GTTATGGCGGGGACTCAAGGAGACGCGTCGG

QB10 ***Nocardioides sp.***

TTAACAGGATTAGATACCCTGGTAGTCCACACCGTAAACGTTGGGCGCTAGGTGTGGGGC
TCATTCCACGAGTTCCGTGCCGCAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCTGGGTTTGACATATGCCGGAAAGCATCAGA
GATGGTGCCCCTTTTTGTCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTCTTATGTTGCCAGCACGTAATG
GTGGGACTTCATTAAGAGACGCCGGCC

QB11 ***Novosphingobium sp.***

TTTAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCCGG
GTACTTGGTACTTGGGTGGCGCAGCTAACGCATTAAGTTATCCGCCTGGGGAGTACGGTC
GCAAGATTAAAACTCAAAGGAATTGACGGGGGCCTGCACAAGCGGTGGAGCATGTGGTT
TAATTCGAAGCAACGCGCAGAACCTTACCAGCGTTTGACATGCCGGTCGCGGATTTGGGA
GACCATTTCCTTCAGTTCGGCTGGACCGTGCACAGGTGCTGCATGGCTGTCGTCAGCTCGT
GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTCCTTAGTTGCCAGCA
TTGAGTGGGCACTCTAAGGATCGCCACGCC

QB12 ***Arthrobacter sp.***

TTAACAGGATATTGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGG
GACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGG
CCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGG
ATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGAAAGACCTG
GAAACAGGTCCCCCACTTGTGGTCGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCAC
GTGATGGTGGGACTCATAGAGACGCCCGT

QB13 ***Pseudarthrobacter sp.***

TTAACAGATTAAGATACTCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGG
ACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGAAAGACCTGG
AAACAGGTCCCCCACTTGTGGTCGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGT
GATGGTGGGACTCATAGAGACGCCTGCG

QB14 ***Pseudarthrobacter sp.***

TAACAGGATTAGTTACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGGA
CATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGCC
GCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGAT
TAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGGACTGGAAATACCTGGA
AACAGGTGCCCCGCTTGCGGCCGGTTCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCGCGT
TATGGCGGGACTCATAGAGATTCCATCG

QB15 ***Novosphingobium sp.***

TATATAGGTAATAATTACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCCGG
GTACTTGGTACTTGGGTGGCGCAGCTAACGCATTAAGTTATCCGCCTGGGGAGTACGGTC
GCAAGATTAAAACTCAAAGGAATTGACGGGGGCCTGCACAAGCGGTGGAGCATGTGGTT
TAATTCGAAGCAACGCGCAGAACCTTACCAGCGTTTGACATGCCGGTCGCGGATTTGGGA
GACCATTTCCTTCAGTTCGGCTGGACCGTGCACAGGTGCTGCATGGCTGTCGTCAGCTCGT
GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTCCTTAGTTGCCAGCA
TTTAGTGGGCACTCTAAGGAACGCACGTG

QB16 ***Pseudomonas sp.***

TTAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGG
TCCTTGAGACTTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCG
CAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT
AATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCTAGAGAT
AGATTGGTGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCG
TGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTT
ATGGTGGGCACTCTAAGAGACGCCATTCTC

QB17 ***Arthrobacter sp.***

TTAACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGG
ACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGAACCGGACCGCTGCAGA
AATGTGGTTTCCCCTTTGGGGCTGGTTTACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCACGT
GATGGTGGGACTCATAGAGACGCCTGCC

QB18 ***Arthrobacter sp.***

TTAACAGGATTATATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGG
ACATTCCACGTTTTCCGCGCCGTAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGGACTGGAAATACCTGGA
AACAGGTGCCCCGCTTGCGGCCGGTTCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTC
GTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCTATGTTGCCAGCGCGT
GATGGCGGGACTCATAGAGACGCATGCC

QB19 ***Novosphingobium sp.***

CTTTAACAGTTTTAATATACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCCG
GGTACTTGGTACTTGGGTGGCGCAGCTAACGCATTAAGTTATCCGCCTGGGGAGTACGGT
CGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCTGCACAAGCGGTGGAGCATGTGGT
TTAATTCGAAGCAACGCGCAGAACCTTACCAGCGTTTGACATGCCGGTCGCGGATTTGGG
AGACCATTTCCTTCAGTTCGGCTGGACCGTGCACAGGTGCTGCATGGCTGTCGTCAGCTC
GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTCCTTAGTTGCCAG
CATTTAGTGGGCACTCTAAGGAAACGCACTG

QB20 ***Novosphingobium sp.***

CGAACGTTACGATGATACTAGCTGTCGGGTACTTGGTACTTGGGTGGCGCAGCTAACGCA
TTAAGTTATCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGG
GCCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGC
GTTTGACATGCCGGTCGCGGATTTGGGAGACCATTTCCTTCAGTTCGGCTGGACCGTGCA
CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTCGTCCTTAGTTGCCAGCATTGAGTTGGGCACTCTAAGGAAACTGCCGGT
GATAAGCCGGAGGAAGATGGGGATGACGTA

QB21 ***Pseudomonas sp.***

TGGGGGGTAAGATGTCAACTAAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAAC
GCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG
GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTAC
CAGGCCTTGACATCCAATGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACATTGAGA
CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACG
AGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGATACTGCCG
GTGACAAAAGGGAGAGGAAAGTGGAGATGACGTAGA

QB22 ***Arthrobacter sp.***

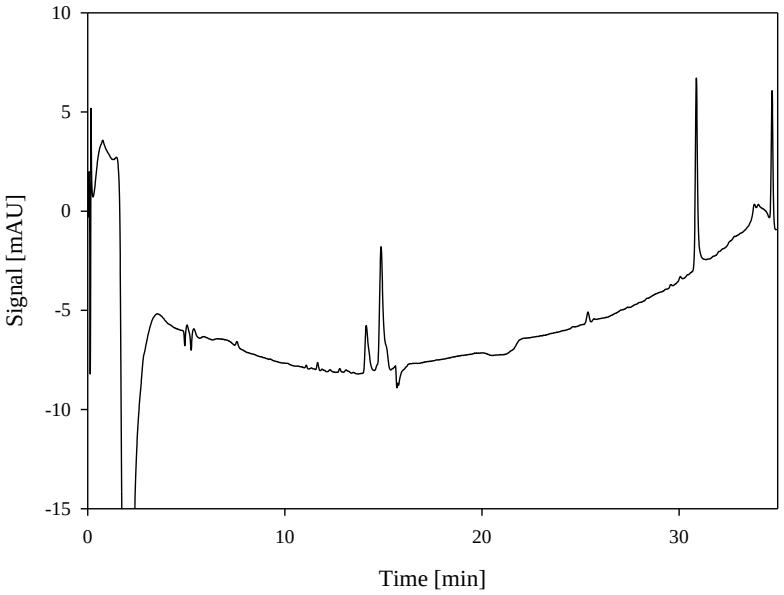
TTCTACCCTTAACGTTGGCCTAGTGTGGGGACATTCCACGTTTTCCGCGCCGTAGCTAACG
CATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCTTACC
AAGGCTTGACATGGACCGGACCGCCGCAGAAATGTGGTTTCCCCTTTGGGGCCGGTTCAC
AGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCTCGTTCCATGTTGCCAGCGCGTAATGGCGGGGACTCATGGGAGACTGCCGG
GGTCAACTCCCAGGAAGGTGAGATGACGAAACTGCCATTCCATTGCTTTAGCTTTCCCAT
TATAACCTGCCATTCTCAATTTAACGCTTCCCATTGCACTCATCTTATGTTTCCTACTCCAC
TCTTCATGTGGGTTGTTTTTCTCCTCCTATATT

QB23 ***Arthrobacter sp.***

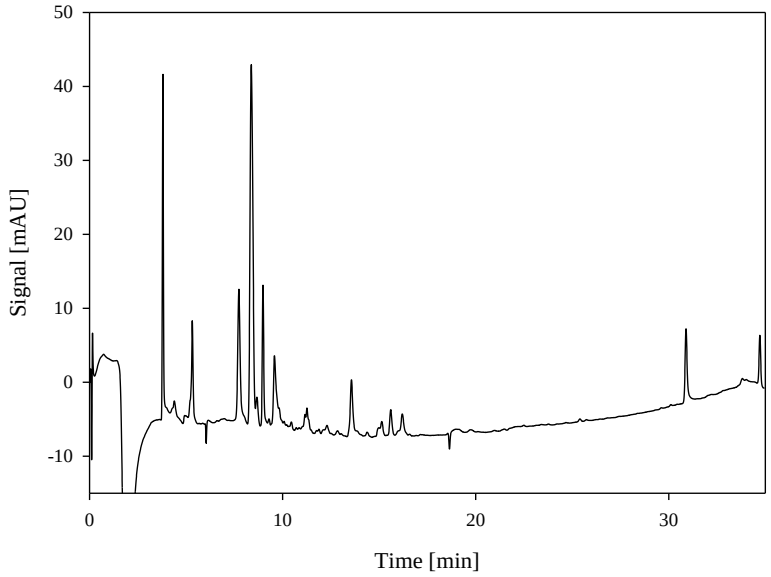
TACCTTAACTTCCGTTTTGGCACTAAGTGTGTGGACATTCCACGTTTTCCGCGCCGTAGCT
AACGCATTAAGTGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAACGCGAAGAACCT
TACCAAGGCTTGACATGGACCGGACCGCCGCAGAAATGTGGTTTCCCCTTTGGGGCCGGT
TCACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA
ACGAGGGCAACCCTCGTTCCATGATGCCAGCGCATGATGGCGGGCACTCATGCCCAAACT
GCCGGGGTCGGGGCGGAGGGATGTGTGGATCACGATA

8.4. Screening for Degradation Products of BOA, Gramine, and Quercetin by HPLC-DAD

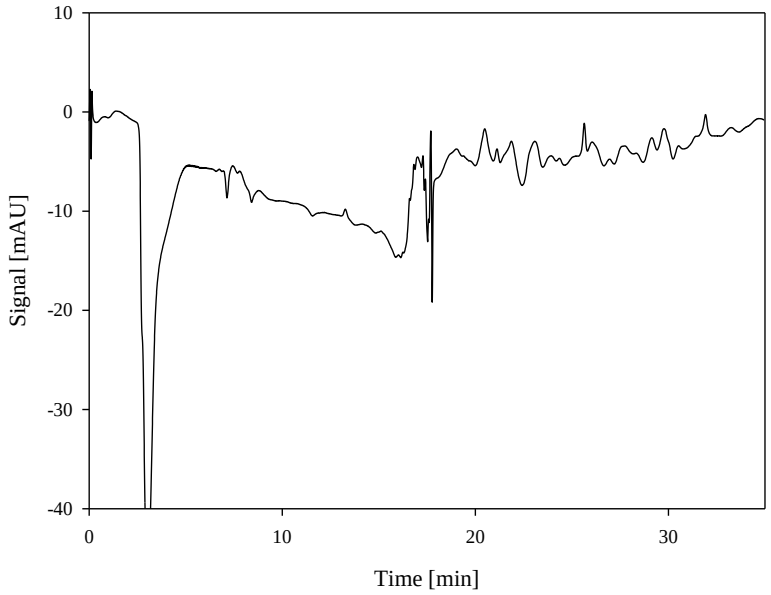
Controls of the organic and aqueous phases after extraction from liquid cultures of *Arthrobacter* sp. GB1 and CB4 and *Pseudomonas* sp. CB3 and MPI9 showed no accumulation of substances related to BOA, gramine, or quercetin (Supplementary Figure 1 – 8). Samples taken after 72 h of incubation are shown which are representative for all organisms.



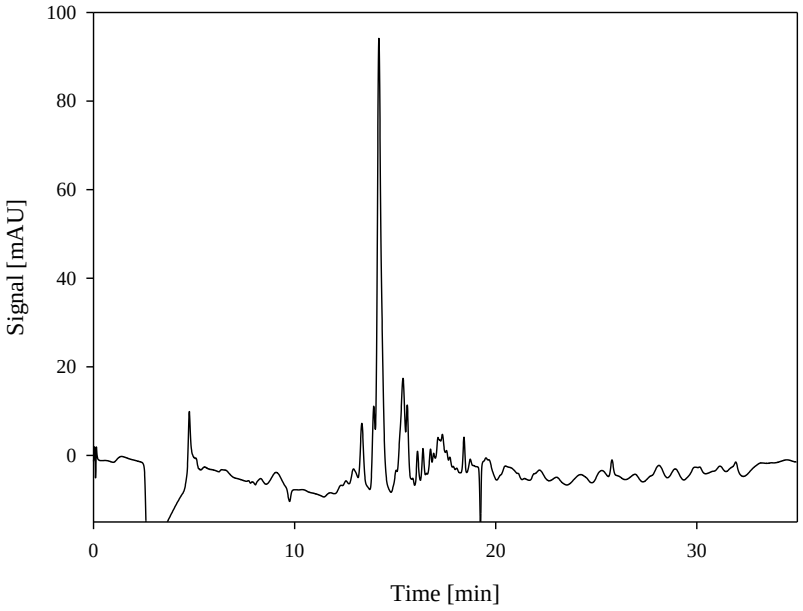
Supplementary Figure 1: HPLC-DAD chromatogram of an organic extract of an *Arthrobacter sp.* GB1 control culture.
HPLC-DAD analysis at 270 nm of an organic phase extracts from control culture taken after 72 h of cultivation of *Arthrobacter sp. GB*1 in liquid media containing DMSO.



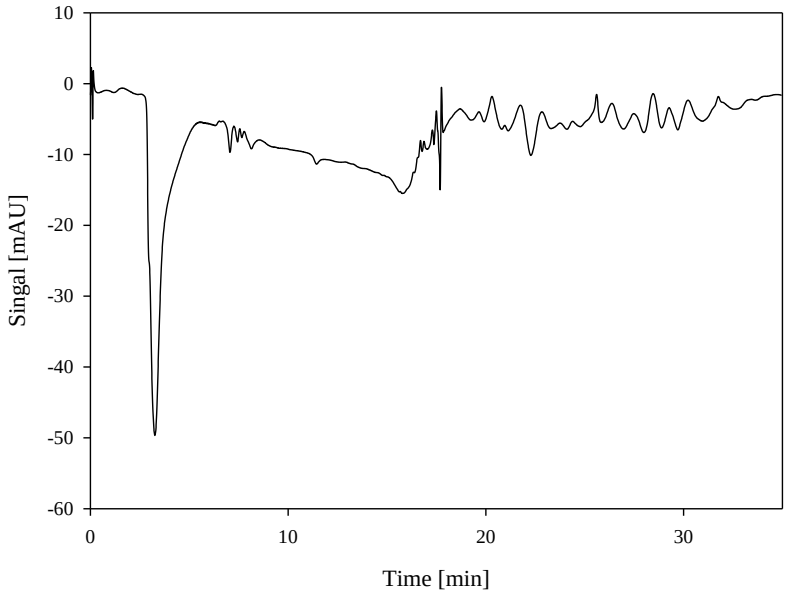
Supplementary Figure 2: HPLC-DAD chromatogram of an aqueous extract of an *Arthrobacter sp.* GB1 control culture.
HPLC-DAD analysis at 270 nm of aqueous phase extracts from control culture taken after 72 h of cultivation of *Arthrobacter sp.* GB1 in liquid media containing DMSO.



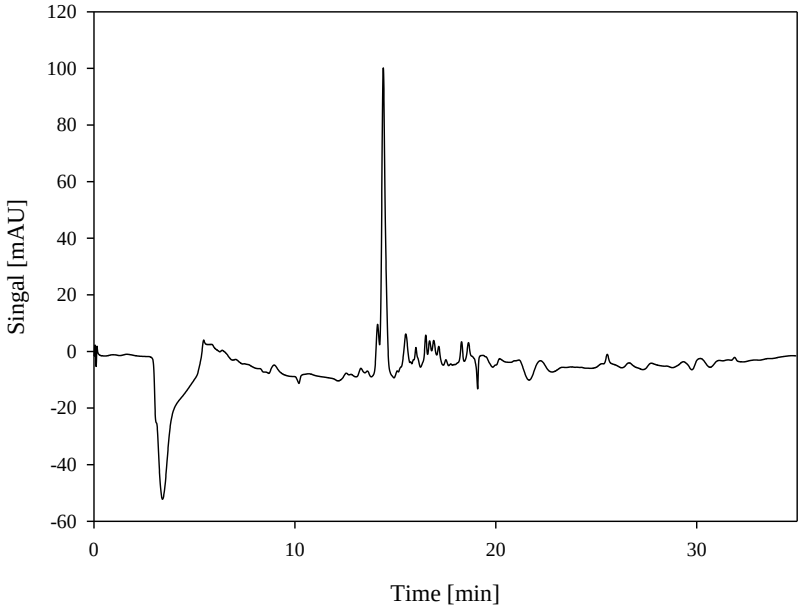
Supplementary Figure 3: HPLC-DAD chromatogram of an organic extract of an *Arthrobacter sp.* CB4 control culture.
HPLC-DAD analysis at 270 nm of organic extract from a control culture taken after 72 h of cultivation of *Arthrobacter sp.* CB4 in liquid media containing DMSO.



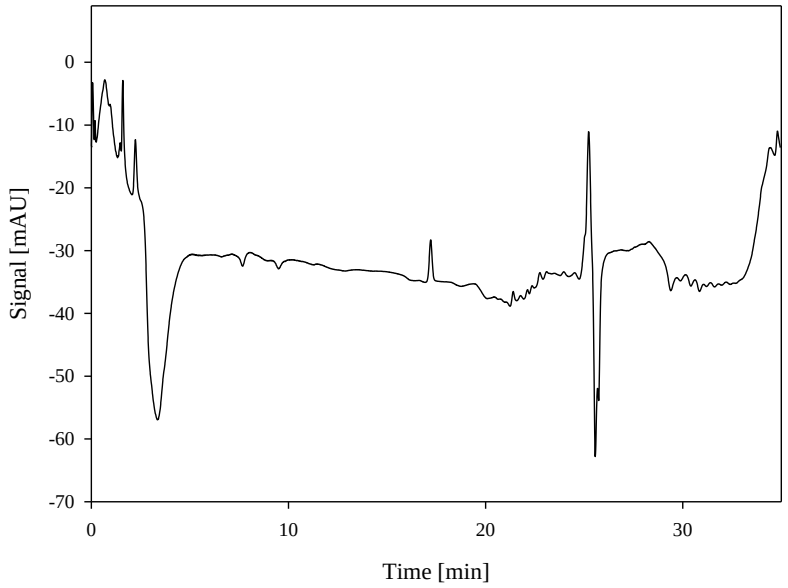
Supplementary Figure 4: HPLC-DAD chromatogram of an aqueous extract of an *Arthrobacter sp.* CB4 control culture.
HPLC-DAD analysis at 270 nm of an aqueous extract taken of control culture after 72 h of cultivation of *Arthrobacter sp.* CB4 in liquid media containing DMSO.



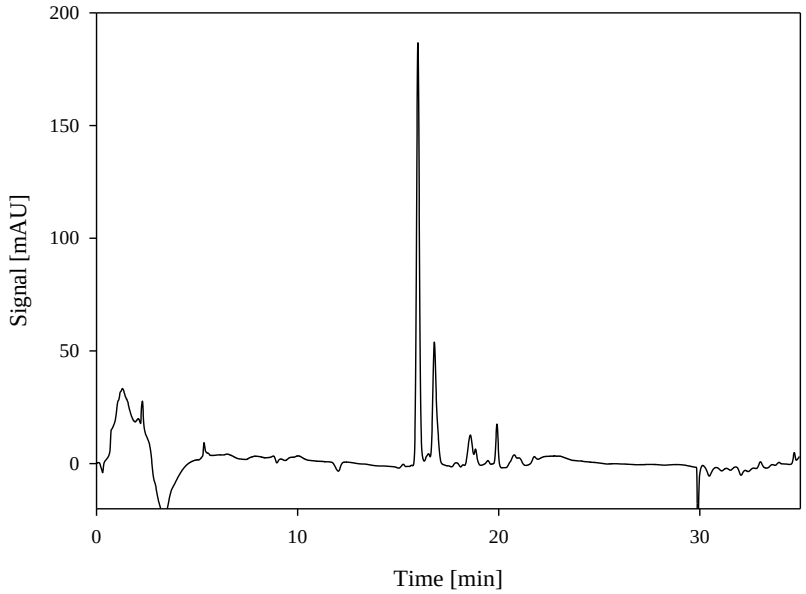
Supplementary Figure 5: HPLC-DAD chromatogram of an organic extract of a *Pseudomonas sp.* CB3 control culture.
HPLC-DAD analysis at 270 nm of organic phase control extracts taken after 72 h of cultivation of *Pseudomonas sp.* CB4 in liquid media containing DMSO.



Supplementary Figure 6: HPLC-DAD chromatogram of an aqueous extract of a *Pseudomonas sp.* CB3 control culture.
HPLC-DAD analysis at 270 nm of aqueous phase control extracts taken after 72 h of cultivation of *Pseudomonas sp.* CB4 in liquid media containing DMSO.



Supplementary Figure 7: HPLC-DAD chromatogram of an organic extract of a *Pseudomonas sp.* MPI9 control culture.
HPLC-DAD analysis at 270 nm of organic phase control extracts taken after 72 h of cultivation of *Pseudomonas sp.* MPI9 in liquid media containing DMSO.



Supplementary Figure 8: HPLC-DAD chromatogram of an aqueous extract of a *Pseudomonas sp.* MPI9 control culture.
HPLC-DAD analysis at 270 nm of aqueous phase control extracts taken after 72 h of cultivation of P*seudomonas sp.* MPI9 in liquid media containing DMSO.

9. Publications

Voloshchuk, N., **Schütz, V.**, Laschke, L., Gryganskyi, A. P., and Schulz, M. (2020). The *Trichoderma viride* F-00612 consortium tolerates 2-amino-3H-phenoxazin-3-one and degrades nitrated benzo[d]oxazol-2(3H)-one. *Chemoecology* 30, 79–88. doi: 10.1007/s00049-020-00300-w

Schütz, V., Bigler, L., Girel, S., Laschke, L., Sicker, D., and Schulz, M. (2019). Conversions of benzoxazinoids and downstream metabolites by soil microorganisms. *Front. Ecol. Evol.* 7. doi: 10.3389/fevo.2019.00238

Schulz, M., Hofmann, D., Sicker, D., Hennig, L., **Schütz, V.**, Preusche, M., et al. (2018). *Pantoea ananatis* converts MBOA to 6-methoxy-4-nitro-benzoxazolin-2(3H)-one (NMBOA) for cooperative degradation with its native root colonizing microbial consortium. *Nat. Prod. Commun.* 13, 1934578X1801301. doi: 10.1177/1934578X1801301010

Siebers, M., Rohr, T., Ventura, M., **Schütz, V.**, Thies, S., Kovacic, F., et al. (2018). Disruption of microbial community composition and identification of plant growth promoting microorganisms after exposure of soil to rapeseed-derived glucosinolates. *PLoS One* 13, e0200160. doi: 10.1371/journal.pone.0200160

Parts of this work were published in:

Laschke, L.[1], **Schütz, V.**[1], Schackow, O., Sicker, D., Hennig, L., Hofmann, D., et al. (2022). Survival of plants during short-term BOA-OH exposure: ROS related gene expression and detoxification reactions are accompanied with fast membrane lipid repair in root tips. *J. Chem. Ecol.* 48, 219–239. doi: 10.1007/s10886-021-01337-z
[1]Autrhos contributed equally.

Schütz, V., Frindte, K., Cui, J., Zhang, P., Hacquard, S., Schulze-Lefert, P., et al. (2021). Differential impact of plant secondary metabolites on the soil microbiota. *Front. Microbiol.* 12, 666010. doi: 10.3389/fmicb.2021.666010

10. Acknowledgments

An dieser Stelle möchte ich mich natürlich auch herzlich bei allen bedanken, die mir mit Rat und Tat zur Seite standen und es mir ermöglicht haben, diese Arbeit anzufertigen.

Zunächst möchte ich mich hierfür bei Prof. Dr. Peter Dörmann bedanken. Danke, dass du es mir ermöglicht hast, in diesem Thema zu promovieren und Teil des IMBIOs zu sein. Seit meiner Ankunft in Bonn war deine Tür immer offen und dein großes Interesse an der Arbeit ermöglichte es mir, an diesem Punkt in meinem Leben zu stehen.

PD Dr. Margot Schulz, bei dir möchte ich mich erstmal dafür bedanken, dass du mich an diesen Forschungsbereich herangeführt hast. Unsere Zusammenarbeit auf diesem Gebiet hat bereits mit meiner Masterarbeit begonnen und dauert bis heute noch fort. Danke für dein großes Interesse an diesem Projekt und deine aktive Betreuung. Auch möchte ich mich bei dir bedanken, dass du mich an die Hand genommen und durch diesen Lebensabschnitt geführt hast. Ich freue mich, auch in Zukunft mit dir über neue Entdeckungen und Ideen zu sprechen! Danke für deine immer offene Tür und dein immer offenes Ohr.

Bei Prof. Dr. Raoul-Martin Memmesheimer und Prof. Dr. Gabriel Schaaf möchte ich mich für ihre Zeit und dafür bedanken, dass sie sich bereit erklärt haben, Teil meiner Prüfungskommission zu sein.

Dr. Katharina Frindte und Prof. Dr. Claudia Knief möchte ich für die Berechnung und Auswertung der NGS Daten danken.

Mein Dank gilt auch Pegfan Zhang, Dr. Stéphan Hacquard und Prof. Dr. Paul Schulze-Lefert des MPI für Pflanzenzüchtungsforschung für die Bereitstellung der Erde, die NGS Messung sowie die Verarbeitung der Rohdaten.

Dr. Diana Hofmann und Dr. Björn Thiele aus dem Forschungszentrum Jülich danke ich für die Messung und Identifizierung der Stoffe. Auch bei Sergey Girel und Prof. Dr. Laurent Bigler von der Universität Zürich möchte ich mich für die Strukturaufklärung und Synthese von Substanzen bedanken.

Meinen Studenten danke ich für ihr Interesse und ihre Hilfe. Insbesondere David Jacobs und Jiaxin Cui möchte ich für die Zeit danken. Ich bin mir sicher, dass wir auch in Zukunft noch viel zusammen lachen werden.

10. Acknowledgments

Natürlich bin ich auch dem IMBIO-Team dankbar für die gesamte Zeit und die Unterstützung. Ich wünsche euch allen weiterhin viel Erfolg und werde eure Arbeit verfolgen.

Yannic, Andreas und Nina. Am besten fasse ich mich hier mal kurz (Abwechslung muss sein). Ich bin froh, in dieser Zeit so gute Freunde gefunden zu haben. Eine Zeit ohne euch kann, will und muss ich mir zum Glück nicht ausmalen.

Und dann wäre da noch meine Familie. Danke für eure Unterstützung! Ohne euch wäre das alles nicht möglich gewesen. Ihr habt mir nicht nur die Freiheit gegeben, meinen eigenen Weg zu gehen, sondern habt mich bedingungslos unterstützt. Ihr wart nicht nur da, um mit mir geglückte Versuche zu feiern, sondern habt auch den Unmut weniger erfolgreicher Experimente abgefangen. Ja, auch du Sofia. Danke für alles!

Es ist nun tatsächlich geschafft. Oder wie Oma sagen würde: „Ach geh. Das glaub ich net!“.