

Tryptophan metabolism and bacterial root commensals
synergistically control fungal abundance in roots to
promote *Arabidopsis thaliana* health

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List of Abbreviations

%	Percentage
1M-I3G	1-methoxy indolyl-3-methyl glucosinolate
35S	35S cauliflower mosaic virus promoter
4M-I3G	4-methoxy indolyl-3-methyl glucosinolate
µg	Microgram
µl	Microliter
<i>A. thaliana</i>	<i>Arabidopsis thaliana</i>
ABA	abscisic acid
ANOVA	Analysis of variance
AOP2	ALKENYL HYDROXALKYL PRODUCING 2
AOP3	ALKENYL HYDROXALKYL PRODUCING 3
ASV	Amplicon Sequence Variant
ATP	adenosine triphosphate
B	bacterial (community)
BAK1	BRI1-ASSOCIATED RECEPTOR KINASE
BKK1	BAK1-LIKE
BRI1	BRASSINOSTEROID INSENSITIVE 1
CAS	Cologne Agricultural Soil
CERK1	CHITIN ELICITOR RECEPTOR KINASE
cm	centimeter
CPCoA	Constrained Principal Component Analysis
Cys-IAN	Cysteine-indole-3-acetonitrile
DAMP	danger-associated molecular patterns

<i>deps</i>	<i>dde2/ein2/pad4/sid2</i>
DNA	deoxyribonucleic acid
EFR1	EF-Tu RECEPTOR
EF-Tu	elongation receptor Tu
eNAD(P)+	extracellular Nicotinamide Adenine Dinucleotide+ (Phosphate)
F	fungal (community)
FDR	false discovery rate
FLS2	FLAGELLIN-SENSITIVE 2
FOX1	FAD-LINKED OXIDOREDUCTASE 1
GLM	Generalized linear model
GSHPs	glucosinolate hydrolysis products
IAA	indole-3-acetic acid
IAD	indole-3-acetaldehyde
IAM	indole-3-acetamide
IAN	indole-3-acetonitrile
IAOx	indole-3-acetaldoxime
ICA	indole-3-carboxylic acid
ICN	indole carbonyl nitrile
IPA	indole-3-pyruvic acid
ITCs	isothiocyanates
JA	jasmonic acid
KAI2	KARRIKIN-INSENSITIVE 2
log	logarithm
LRR	leucine-rich repeat

LRR-RK	LRR-receptor kinase
LRR-RLK	LRR-receptor-like kinase
LUX	luminous flux per unit area
LYK4	LysM-CONTAINING RECEPTOR KINASE 4
LYK5	LysM-CONTAINING RECEPTOR KINASE 5
LysM	lysine motifs
MAMP	microbe-associated molecular patterns
MAX2	MORE AXILARY GROWTH 2
mg	miligram
min	minutes
ml	mililiter
mM	milimolar
mm	milimeter
MPK3	Mitogen-activated protein kinase 3
MPK6	Mitogen-activated protein kinase 6
MS	Murashige and Skoog
O	oomycetes (community)
OD	optical density
PAMPs	pathogen associated molecular patterns
PCR	polymeraqs chain reaction
PERMANOVA	Permutational multivariate analysis of variance
PGA	potato glucose agar
PRR	pattern recognition receptor
PTI	pattern-triggered immunity

qPCR	quantitative polymerase chain reaction
<i>quadruple</i>	<i>myb34/myb52/myb122/cyp71a13</i>
RLK	receptor-like kinase
RLP	receptor-like protein
RNA	ribonucleic acid
rpm	revolutions per minute
s	seconds
SA	salicylic acid
SAR	Systemic Acquired Resistance
Supp.	Supplementary
SynCom	synthetic community
Trp	Tryptophan
TSA	tryptic soy agar
TSB	tryptic soy borth

Abstract

The roots of healthy and asymptomatic plants are colonized by a staggering diversity of microbes, including bacteria, fungi, and oomycetes (i.e. the root microbiota), and yet plants have evolved a complex, multi-layer, immune system that detects microbial invasion and discriminate self from non-self. Although plant innate immunity has been extensively studied under laboratory settings between one specific microbe and one specific plant, our understanding of this complex machinery in a natural (i.e. community) context remains sparse, especially in plant roots. Recent studies indicate that certain sectors of plant immune system, namely phytohormones and tryptophan-derived (Trp-derived) secondary metabolites have an important role in the establishment of the plant microbiota. It is still unknown which pathways are required for a controlled accommodation of commensal microbes, which in return results in plant growth promotion. A major hypothesis is that colonization by both pathogenic and beneficial microbes acts as a selective force on the function of plant innate immunity, forcing task division among different immunity pathways.

Using experiments in a natural soil, combined with microbiota reconstitution experiments in a gnotobiotic system with a multi-kingdom synthetic community and a set of immunocompromised plants, I tested the extent to which different plant immune sectors are needed for commensal-induced plant growth promotion. I provide novel evidence for the importance of interaction between bacterial commensals and Trp-derived secondary metabolites biosynthesis pathway and co-receptors BAK1 and BKK1 in beneficial plant-microbiota interactions, especially in mediating beneficial growth-promotion effect in *Arabidopsis thaliana* (*A. thaliana*). In this thesis I show that not only growth during vegetative stage is affected in Trp-metabolism and co-receptor mutants, but the mutations affect the plants during their reproductive stage. Particularly, I showed that bacterial commensals and host Trp-derived secondary metabolites act in concert to prevent fungal overgrowth in plant roots and promote host-microbial homeostasis. Additionally, I have developed a working gnotobiotic system which allows accommodation of *A. thaliana* plants in their reproductive stage, which has potential to highly facilitate future research on the effect of microbiota on plants fitness, giving higher biological relevance of obtained results.

Zusammenfassung

Die Wurzeln gesunder und asymptomatischer Pflanzen werden von einer erstaunlichen Vielfalt von Mikroben besiedelt, darunter Bakterien, Pilze und Oomyceten (dh die Wurzelmikrobiota), und dennoch haben Pflanzen ein komplexes, mehrschichtiges Immunsystem entwickelt, das die Invasion von Mikroben erkennt und Selbst von Nicht-Selbst unterscheidet. Obwohl die angeborene Immunität von Pflanzen unter Laborbedingungen zwischen einer bestimmten Mikrobe und einer bestimmten Pflanze eingehend untersucht wurde, bleibt unser Verständnis dieser komplexen Maschinerie in einem natürlichen (d. h. gemeinschaftlichen) Kontext, insbesondere bei Pflanzenwurzeln, spärlich. Jüngste Studien zeigen, dass bestimmte Sektoren des pflanzlichen Immunsystems, nämlich Phytohormone und von Tryptophan abgeleitete (Trp-abgeleitete) Sekundärmetaboliten, eine wichtige Rolle bei der Etablierung der pflanzlichen Mikrobiota spielen. Es ist noch nicht bekannt, welche Wege für eine kontrollierte Akkommodation von kommensalen Mikroben erforderlich sind, was wiederum zu einer Förderung des Pflanzenwachstums führt. Eine wichtige Hypothese ist, dass die Besiedlung durch pathogene und nützliche Mikroben als Selektionsdruck auf die angeborene Immunität von Pflanzen wirkt und die Aufgabenteilung zwischen verschiedenen Immunitätswegen erzwingt.

Anhand von Experimenten in einem natürlichen Erde, kombiniert mit Mikrobiota-Rekonstitutionsexperimenten in einem gnotobiotischen System mit einer synthetischen Gemeinschaft mit mehreren biologischen Königreichen und einer Reihe von immungeschwächten Pflanzen, testete ich, inwieweit verschiedene Pflanzenimmensektoren für die durch Kommensal induzierte Förderung des Pflanzenwachstums erforderlich sind. Ich liefere neue Beweise für die Bedeutung der Wechselwirkung zwischen bakteriellen Kommensalen und Trp-abgeleiteten Biosynthesewegen der Sekundärmetaboliten und den Co-Rezeptoren BAK1 und BKK1 bei vorteilhaften Wechselwirkungen zwischen Pflanzen und Mikrobiota, insbesondere bei der Vermittlung der vorteilhaften wachstumsfördernden Wirkung von *Arabidopsis thaliana* (*A. thaliana*). In dieser Arbeit zeige ich, dass nicht nur das Wachstum im vegetativen Stadium in Trp-Metabolismus und Corezeptor Mutanten beeinflusst wird, sondern dass die Mutationen die Pflanzen während ihres Fortpflanzungsstadiums beeinflussen. Insbesondere zeigte ich, dass bakterielle Kommensalen und von Trp-abgeleitete

Sekundärmetaboliten des Wirts zusammenwirken, um das Überwachsen von Pilzen in Pflanzenwurzeln zu verhindern und so die Homöostase zwischen Wirt und Mikroben zu fördern. Zusätzlich habe ich ein funktionierendes gnotobiotisches System entwickelt, das die Unterbringung von *A. thaliana*-Pflanzen in ihrem Fortpflanzungsstadium ermöglicht und das Potenzial hat, zukünftige Forschungen über die Wirkung von Mikrobiota auf die Fitness von Pflanzen in hohem Maße zu erleichtern, was eine höhere biologische Relevanz der erhaltenen Ergebnisse ergibt.

Preamble

Part of this thesis is in preparation to be submitted as a manuscript. Figures that will be used in said publication were marked in their figure legends. Some paragraphs will also be used with slight modifications. Most of the experiments and analysis described here were done by myself. Contributions to experiments and analysis by other people are indicated in “Contribution” section (page 106).

Introduction

Plant-microorganism interactions

The roots of healthy and asymptomatic plants are colonized by a staggering diversity of microbes, including bacteria, fungi, and oomycetes (i.e. the root microbiota), forming complex multi-kingdom microbial communities that affect plant productivity (Bulgarelli et al., 2013). Paradoxically, plants have evolved a complex, multi-layer, immune system that detects microbial invasion and discriminate self from non-self. The first line of defence for the plant against pathogens is innate immune system, which is mainly comprised of the receptor and co-receptor layer responding to Microbe-/Danger- Associated Molecular Patterns (MAMPs and DAMPs) (X. Yu et al., 2017). Recognition of MAMPs and DAMPs triggers downstream responses (Pattern-Triggered Immunity, PTI) (Irieda et al., 2019) through various pathways and results in the increase of phytohormones (Berens et al., 2019) or plant secondary metabolites biosynthesis. These defence outputs include the phytohormones SA, JA, or brassinosteroids (M.-H. Yu et al., 2018; Peres et al., 2019), as well as Trp-derived secondary metabolites such as glucosinolates (Malka & Cheng, 2017) and camalexins (Jeandet et al., 2014), that restrict pathogen growth *in planta*. Through this complex network of interconnected pathways, the innate immune system is responding to microbial threats and protects the plant from diseases (X. Yu et al., 2017).

Plant-pathogen interactions

Three important pathogen groups that are studied for disease resistance and tolerance in plants are bacteria, fungi and oomycetes (Jelenska et al., 2010; Barah et al., 2013; Yang et al., 2015; Fawke et al., 2015; Berens et al., 2017; Wolinska & Berens, 2019). All three pathogen groups comprise of several agriculturally relevant pathogens, that cause severe losses in either pre- (Oerke, 2006) or post-harvest (Kader, 2005) phase during crop production. Up until now, various studies found different key components required for resistance to their respective pathogens. Taken together, these studies outline the complexity of gene networks that are required for plants immune system to function properly. So far, the studies determined several main cell receptors responsible for recognising pathogens by recognising Pathogen and Microbe Associated Molecular Patterns (PAMPs and MAMPs), such as FLAGELLIN-

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SENSITIVE 2 (FLS2, AT5G46330) (Gómez-Gómez & Boller, 2000), EF-TU RECEPTOR (EFR, AT5G20480.1) (Zipfel et al., 2006) for bacteria and CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1, AT3G21630) (Miya et al., 2007) and LysM-CONTAINING RECEPTOR KINASE 5 (LYK5, AT2G33580.1) (Cao et al., 2014) for fungi (all described in more detail in later sections of the Introduction). Plant cell receptors recognising oomycetes PAMPs were not studied to the same extent as bacterial and fungal ones, nevertheless some receptors were also identified (Judelson & Ah-Fong, 2019). Except for the receptors that recognise the pathogen directly, there are also receptors that are specialized in recognising molecules released by the plant itself after detecting an (un-) identified threat or being damaged. In this scenario elicitors are called Danger/Damage Associated Molecular Patterns (DAMPs) and are recognised by plasma membrane-localized receptors (Hou et al., 2019).

In addition to the relevance of immune system itself, one cannot forget about the commonly accepted theory of plant growth/defence trade-off (Huot et al., 2014; Wolinska & Berens, 2019), which is a crucial aspect from plant breeder's point of view. While disease resistance is crucial for plants to survive in their environment, it would be disadvantageous from the breeder's point of view if such plants had inferior yield in comparison to other varieties (Wu et al., 2020). However, in the view of the recent change in the plant-microorganisms interaction where more focus is being put on plant-microbiome research, improving plant immunity no longer focuses solely on the plant itself. In my thesis I dissect the role of plant innate immunity in shaping beneficial root-associated microbiota, which could ultimately help breeders in selecting for specific plant traits that not only improve the overall biotic tolerance, but also help in the establishment of beneficial microbiota, which in turn increases plants health and yield.

Plant-microbial community interactions

Although plant innate immunity has been extensively studied under controlled laboratory conditions between one specific microbe and one specific plant (Jelenska et al., 2010; Barah et al., 2013; Yang et al., 2015), our understanding of this complex machinery in a natural (i.e. community) context remains sparse, especially in plant roots (Millet et al., 2010; Hacquard et al., 2017; Rich-Griffin et al., 2020). Recent studies indicate that, on one hand certain sectors of plant immune system, namely phytohormones and Trp-derived secondary metabolites have

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an important role in the establishment of plant microbiota members (Lahrmann et al., 2015; Lebeis et al., 2015; Hiruma et al., 2016; Vishwanathan et al., 2020). On the other hand, single bacteria strains can also elevate the plant growth under unfavourable conditions and affect their rhizosphere composition (Luo et al., 2019). Due to the high complexity of plant-microbe interactions, it became clear that pathogenic microbes defined based on one-to-one relationship under laboratory conditions might not induce any disease symptoms in a community context or can even become beneficial for plant host growth under specific conditions (Vayssier-Taussat et al., 2014; Durán et al., 2018). Although it is known that certain neutral and/or beneficial microbiota members are able to dampen the immune responses, this is not the case for all the members, indicating a further control mechanism that allows these microbes to interact with the host without inducing host's immune response (Hacquard et al., 2017).

Microbe-microbe interactions

Microbe-microbe interactions, while less studied than plant-microorganism interactions in regard to plant's health, are another key regulatory component of plant performance. Microbe-microbe interactions within the same kingdom were shown to be indispensable for plant growth promotion and increased resistance to pathogens (Berendsen et al., 2018). Also, interactions between different microbial kingdoms are of high importance, for example when bacterial root commensals were shown to rescue *A. thaliana* growth from fungal root community, which was turning detrimental in absence of bacterial community (Durán et al., 2018). Another study presented that specific shifts in bacterial community can increase plants tolerance to a root pathogen (Carrión et al., 2019), suggesting a crucial role of microbe-microbe interaction in plant-pathogen tolerance. Taken together, a current hypothesis is that microbial homeostasis in roots is tightly controlled by a two-layered regulatory network that involves host-microbe and microbe-microbe interactions (Vannier et al., 2019).

Beneficial effect of microbiota on plants growth

Although the beneficial effect on plants performance of certain microorganisms, such as mycorrhizal fungi, was already known for years (Harrier, 2001), only in recent years the researcher's attention was drawn towards the beneficial effects of plant microbiota. Several studies reported a link between the presence of microbiota and increase in plants yield (Trivedi

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et al., 2017), plants growth (Berendsen et al., 2018), nutrient acquisition (Harrier, 2001; van der Heijden et al., 2016; Matilla & Krell, 2018) or biotic (Berendsen et al., 2018; Matilla & Krell, 2018) and abiotic (Hussain et al., 2018; Mishra et al., 2018) stress tolerance. Despite this field of research being in its early stages, the potential of utilizing microorganisms in increasing global food production is tremendous, and more desperately needed (Trivedi et al., 2017). Understanding the molecular mechanisms driving beneficial plant-microbiota associations is a critical part of fundamental research needed for uncovering the general principles, which will likely be of critical importance for developing alternative strategies to improve crop production.

Possible host pathways regulating the beneficial microbiota

Understanding the composition and beneficial functions of microbial communities that colonize plant roots is only one part of the whole picture. Another important aspect is to also gain additional knowledge regarding plant pathways, which drive and/or facilitate plants colonization by microbiota members. Evidence is slowly accumulating, pointing to the active role of plants in attracting beneficial microorganisms (Rudrappa et al., 2008; Yuan et al., 2018; Friman et al., 2020), however there is also a strong possibility of involvement of other parties, like microorganisms themselves, that attract or repel other microorganisms through microbe-microbe interactions (Hacquard et al., 2017). Whether the process is active or passive, there is emerging evidence showing that disruption of certain genes and/or full pathways affects microbial community composition. As an example, disruption of phosphate starvation response pathway (Finkel et al., 2019), general nutritional status of a plant (Carvalhais et al., 2013) or salicylic acid (SA) pathway (Lebeis et al., 2015) lead to specific microbial community shifts. Changes in root exudation can also affect surrounding microbiota, e.g. changes in exudation of phenolic compounds, benzoxazinoids or triterpenes (Pascale et al., 2020).

Plant innate immunity and its relationship with plant root microbiota is the focus of this thesis. Innate immunity evolved to protect the plants from macro- (Pineda et al., 2017, 2019; Howard et al., 2020) and micro-pathogens (Berendsen et al., 2018; Yuan et al., 2018) and my working hypothesis was that a specialized defence pathway could be, at least to some extent, involved in the regulation of plant microbial community composition and not only in defence against pathogens. One argument supporting this hypothesis is the fact that vast majority of MAMPs

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recognized by plants immune system (being an indication of a pathogen being present in the direct surroundings of the plant) are shared by a wide range of microorganisms (Felix et al., 1999). Even if the plants' response to beneficial microorganisms is different than the one to pathogens, the receptor layer is most probably activated, and immune response dampened afterwards (Hacquard et al., 2017).

Plant innate immune system as microbial management system

During my PhD I investigated the role of different innate immunity pathways in the accommodation of beneficial microbiota. In the next paragraphs, layers and pathways that have been selected for further investigation in this thesis are introduced in more detail (see also a schematic representation on Figure 1A).

Receptor/co-receptor layer

Receptor/co-receptor layer is considered to be the first point of contact when it comes to detecting pathogens through MAMPs recognition (Tang et al., 2017). As such it was considered one of the most likely parts of innate immunity involved in detection and accommodation of beneficial microbiota. The receptor layer, which is considered as the first layer of immunity consists of two major parts. First, Pattern Recognition Receptors (PRRs) can be divided in two groups: receptor-like kinases (RLK) and receptor-like proteins (RLPs) (Jones & Dangl, 2006), which are localized at the cell surface. A large number of RLKs and RLPs has been identified as part of the plant defence, but only few have been characterized in detail, including their ligands (Tang et al., 2017). Three PRRs that are investigated in more detail in this thesis are FLS2, EFR and CERK1. FLS2 is a receptor belonging to a leucine-rich repeat (LRR)-containing PRRs and it is recognising a N-terminal, 22-long amino acid sequence of bacterial flagellin, commonly known as flg22 (Gómez-Gómez & Boller, 2000; Tang et al., 2017). As flg22 is a main target of FLS2, on one hand it gives a fitness advantage for bacteria to accumulate mutations which allow a detection evasion (Sun et al., 2006). On the other hand, fast-evolving MAMPs' coding sequences are causing an increased selection pressure on plant receptor genes, which caused several gene families to expand over time, including for instance the LRR-XII subfamily of RLKs, where FLS2 belongs to (Shiu et al., 2004). EFR is a receptor which can detect the N-terminal part of the bacterial elongation factor Tu (EF-Tu) (Zipfel et

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al., 2006; Tang et al., 2017). EFR is believed to restrict the transformation efficiency of *Agrobacterium tumefaciens* as well as growth of some *Pseudomonas syringae* pv. *tomato* DC3000 strains and additionally, EFR activation by EF-Tu was up to date only found in Brassicaceae species (Nicaise et al., 2009). CERK1 belongs to a subfamily of PRRs-containing lysine motifs (LysM) called LysM-RLKs. CERK1 and other two receptors belonging to the same group, LysM-CONTAINING RECEPTOR KINASE4 (LYK4) and LYK5 are required for chitin binding and signalling (Miya et al., 2007; Wan et al., 2008; Cao et al., 2014). CERK1 and LYK5 are two representative receptors for chitin binding. LYK5 is a receptor in chitin sensing in *Arabidopsis*, and the chitin sensing response mediated by CERK1 is only activated when LYK5 is present (Cao et al., 2014; Tang et al., 2017). Previous studies have shown that both are forming a chitin-recognition complex, however in *Arabidopsis* LYK5 has a greater binding affinity than CERK1 (Cao et al., 2014). There is also a growing evidence that CERK1 may be involved in bacterial PAMP detection (Gimenez-Ibanez et al., 2009), expanding on its importance as a plant innate defence receptor.

In addition to immune receptors, certain co-receptors have also been analysed at in this thesis, namely BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) and BAK1-LIKE 1 (BKK1). BAK1 is an LRR-RLK and it acts as co-receptor of several receptors, e.g. FLS2 or EFR (Tang et al., 2017). Fully functional BAK1 co-receptor is needed for PTI induction, interaction with brassinosteroids pathway and it is involved in cell death regulation (Chinchilla et al., 2007; Zhou et al., 2019). BAK1 and another receptor-like kinase, BKK1 are both required for proper activation of Systemic Acquired Resistance (SAR), eNAD(P)⁺ signalling (C. Wang et al., 2019) as well as modulation of SA-dependent cell death pathway (Y. Gao et al., 2017). Another, newly discovered, LRR-receptor kinases (LRR-RKs) are so-called APEX LRR-RKs, which were identified as important nodes in LRR-RK network and a disruption of APEX genes affects BRASSINOSTEROID INSENSITIVE 1 (BRI1, see description in the next paragraph) and FLS2 genes function in plants (Smakowska-Luzan et al., 2018).

Brassinosteroids

Brassinosteroids are originally known as one of the later discovered plant hormones group, with the main role in plant growth and development (Peres et al., 2019). Later in time brassinosteroids were also found to be regulators of growth/immunity trade-off (Lozano-Durán

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& Zipfel, 2015). Brassinosteroids are recognised by an LRR-RK called BRI1, which then cooperates with its co-receptor BAK1 and subsequent steps cause an activation of BRI1 kinase activity and downstream response (Lozano-Durán & Zipfel, 2015). An overexpression of BRI1 has led to an increased susceptibility of *Arabidopsis* to *Pseudomonas syringae* pv. *tomato* as well as *Hyaloperonospora arabidopsis* (Belkhadir et al., 2012) indicating an importance of this gene in plant-pathogen interactions. Considering the role of brassinosteroids in plant-pathogen interaction, I decided to evaluate whether the disruption of brassinosteroid sensing through BRI1 mutation can also affect the root microbiota assembly.

WRKY transcription factors

WRKY transcription factors include a large number of genes encoding transcription factors involved in a wide range of plant's processes (Jiang et al., 2017). Several WRKY transcription factors are known for their role in plant immunity (Birkenbihl et al., 2017) and two of them were chosen to be analysed in this thesis, namely WRKY33 and WRKY40. WRKY33 is involved in the regulation of biotic stress tolerance, for example against the necrotrophic pathogen *Botrytis cinerea* (Birkenbihl et al., 2012). WRKY33 is also a negative regulator of ABA, which strengthens its role in biotic stress tolerance by lowering ABA hormone levels (Liu et al., 2015), has a role in SAR triggered by local MAPK-activation (Y. Wang et al., 2018) and its involvement in MPK3/MPK6-downstream pathogen-induced camalexin biosynthesis activation (Mao et al., 2011). WRKY40, together with WRKY18 is involved in negative regulation of flg22-induced immunity (Birkenbihl et al., 2017) as well as negative regulation of resistance toward hemibiotrophic fungi (Pandey & Roccaro, 2010).

Phytohormones

Very important plant secondary metabolites involved in pathogen defence are phytohormones. SA, jasmonic acid (JA) and ethylene have a long-standing trail of evidence as being an essential branch of plant innate immunity (Berens et al., 2017, 2019). SA is most commonly known for its role in plants resistance against biotrophic and hemi-biotrophic pathogens as well as activation for SAR, while JA and ethylene are mostly known for resistance against necrotrophic pathogens and herbivorous insects. Interaction between SA and JA are mainly reported as antagonistic, however their interactions are often more complicated and show some synergism (Bari & Jones, 2009). In recent years the involvement of phytohormones in plant-

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microbiota interactions was also investigated, and the role of SA in shaping root (Lebeis et al., 2015) and leaf (Berens et al., 2019) microbiota was determined.

Glucosinolates

Glucosinolates are plant secondary metabolites, which are divided into three groups, namely aliphatic, aromatic and indolic glucosinolates (Halkier & Gershenzon, 2006). Mutations in *CYP79B2* and *CYP79B3* cause a loss of Trp-derived indole glucosinolates and camalexin accumulation as well as a decrease in aliphatic glucosinolates (Halkier & Gershenzon, 2006) and are controlled by three MYB transcription factors, namely MYB34, MYB51 and MYB122 (Frerigmann et al., 2015). *CYP79B2* and *CYP79B3* genes were found to be crucial for establishing beneficial relationship with fungal species, e.g. in *cyp79b2/b3* double knock-out mutant a beneficial *Colletotrichum tofieldiae* turned detrimental (Hiruma et al., 2016). For more detailed information about Trp-derived biosynthesis pathway of glucosinolates and related compounds see Figure 1B. Biosynthesis of glucosinolates increases under treatment with JA (R. Guo et al., 2013; Falk et al., 2016), indicating an interaction between both pathways, that may have a role in plant-pathogen interactions. Glucosinolates themselves are not reported as active compounds in defence against pathogens, but their hydrolysis products are (Rask et al., 2000; Poveda, 2020). Myrosinases are a group of catalytic compounds catalysing the hydrolysis of glucosinolates into their active defensive forms, overall called glucosinolate hydrolysis products (GSHPs). GSHPs together with camalexins have been reported to have a role in tolerance to a wide range of fungal (Buxdorf et al., 2013; Sotelo et al., 2015), bacterial (Velasco et al., 2013; Sotelo et al., 2015) and oomycetes pathogens (Schlaeppi et al., 2010; Prince et al., 2017), as well as herbivores (Falk et al., 2016) and abiotic stresses (del Carmen Martínez-Ballesta et al., 2013). Glucosinolates were also found to directly affect microbial community composition, when applied to soil (Siebers et al., 2018). Taken together glucosinolates show promise for further disentanglement of plant interaction with beneficial microbial community and investigated in detail in this thesis.

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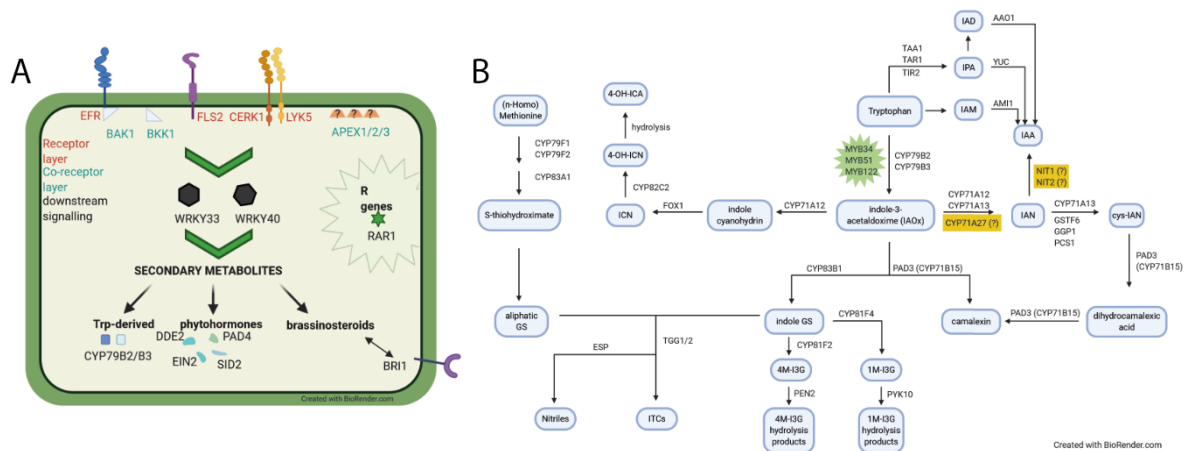


Figure 1: Schematic representation of investigated genes (A) and glucosinolates pathway with a focus on Trp-derived metabolites (B).

Panel B is based on Figure 1 from [Buxdorf et al., 2013](#), with additional information adapted from [Mano & Nemoto, 2012](#); [Frerigmann et al., 2015](#); [Rajniak et al., 2015](#); [Nakano et al., 2017](#) and [Koprivova et al., 2019](#). Black lettering indicates biosynthesis genes, blue boxes indicate chemical compounds, green background marks transcription factors and yellow background behind biosynthesis genes indicates a hypothetical role/place of a highlighted gene. 1M-I3G – 1-methoxy indolyl-3-methyl glucosinolate; 4M-I3G – 4-methoxy indolyl-3-methyl glucosinolate; Cys-IAN – Cysteine-indole-3-acetonitrile; IAA – indole-3-acetic acid; IAD – indole-3-acetaldehyde; IAM – indole-3-acetamide; IAN – indole-3-acetonitrile; ICA – indole-3-carboxylic acid; ICN – indole carbonyl nitrile; IPA – indole-3-pyruvic acid; ITCs – isothiocyanates. This figure will be a part of the manuscript in (un)changed form.

Plant systems for microbiome study

Studies involving plant-associated microbiota can be tackled from two main angles. The first approach is of a descriptive nature and involves field or greenhouse studies in complex environments. The second involves reductionist approaches in gnotobiotic plant systems that are better suited for testing causality and for understanding fundamental rules and mechanisms that drive microbiome assembly.

Natural soil as a complex environment

Field and greenhouse experiments allow the researchers to obtain a holistic picture of microbial diversity and of the major factors that drive community assembly in nature, however analysis of such complex systems can become very challenging due to too many co-variables, variable environmental conditions and overall environmental noise. Nevertheless, a definitive advantage of using natural soils over laboratory-grown microorganisms is the inclusion of a vast diversity of strains, species, or even whole microbial groups that cannot be cultured and

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maintained under laboratory conditions (Bai et al., 2015). Studies in natural soils provide a better overview of natural soil community's behaviour, however one has to always keep in mind a wide range of factors that can influence the results of microbiome studies, like temperature and humidity fluctuations (Araya et al., 2020), varying light conditions (especially important for phyllosphere microbiota (Carvalho & Castillo, 2018)), presence of other, unaccounted for, micro- and macroorganisms (Ourry et al., 2018) and, especially in field studies, an influence of the field's surroundings (Espenshade et al., 2019). For that reason, a more in-depth studies should probably be done in more controlled growth systems. The dilemma between a holistic and reductionist approach in microbiome studies is described in detail in the recent review (Tecon et al., 2019).

Gnotobiotic systems

In order to study the basic principles driving plant-microbiota interactions, there was an increasing need for development of laboratory systems, which would allow an in-detail microbiome studies in highly controlled, gnotobiotic environments.

Agar systems

One of the simplest gnotobiotic systems used for microbiological studies (and later including plant-microbe interactions) is the agar-based system (van Brussel et al., 1982; Hawes & Pueppke, 1989). Agar-based system refers to a wide range of setups, all having agar-based medium. Few examples used for plant-microbe interactions would be simple petri dishes filled with agar (Hiruma et al., 2016; Castrillo et al., 2017; J. Gao et al., 2019), small agar columns used for short-term or microscopic studies (van Brussel et al., 1982), 96-column format system used for a high-throughput screens (Burrell et al., 2017) or agar filled Magenta boxes, which allow a longer growth period by providing more space, while maintaining sterile conditions (Gourion et al., 2006). Agar-based systems are well suited for single plant-microbe or microbe-microbe interactions, allowing a deep insight for mechanistic studies, however their usefulness for studies focusing on complex microbial communities is very limited due to lack of soil-like porous structure which would be able to accommodate all the microorganisms and often reported, general problems with nutrient status of the agar-based media (Gruber et al., 2013) and light-exposure of roots (Xu et al., 2013).

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Calcined clay/vermiculite

In order to fill in the demand for a suitable gnotobiotic system for microbiota reconstitution experiments with germ-free plants, a calcined clay-based system was developed. Main advantage of calcined clay or vermiculite over agar is its porous structure, resembling the soil (Lebeis et al., 2015; Bai et al., 2015; Hiruma et al., 2016; Berens et al., 2019). Unfortunately, with time it became apparent, that eukaryotic microorganisms do not cope well with calcine clay as a matrix, probably due to low carbon availability. While bacteria do cope better and are able to proliferate, their growth is attenuated which may affect the results and final community profile (personal observation within the department).

FlowPots

The latest development in the field of gnotobiotic systems is a so-called FlowPot system, where peat (commonly used soil substrate in the greenhouses) supplemented with vermiculite is used as a soil matrix, allowing (up to date) the best conditions for microorganism's growth (Kremer et al., 2018). This set-up, where up to six small FlowPots with planted plants are placed together within one Microbox allows to sample several technical replicates within one Microbox, increasing the statistical power of the performed experiments. Another big advantage is that this system provides much better conditions for bacteria's and eukaryotic microorganisms growth (like fungi and oomycetes), giving an opportunity to study not only plant-microbe interactions but also multi-kingdom microorganism interactions with complex microbial synthetic communities (Durán et al., 2018).

Thesis aims

Despite extensive efforts made to characterize the plant immune system, it remains unclear to what extent this complex machinery affects diversity, structure, and abundance of microbial commensals colonizing plant tissues. More importantly, the relevance of the plant immune system for microbiota-mediated beneficial effects on plant health remains enigmatic. The main question that I seek to answer with my PhD project is which pathways of plant innate immune system are required for a controlled accommodation of beneficial commensal microbes. My main hypothesis is that the plant innate immune system has a key role in accommodating beneficial root-associated microbial commensals and its disruption might cause microbial imbalance (i.e. dysbiosis) in plant roots, with potential consequences on plant health. I aimed at identifying the most promising immune sectors that control beneficial plant-microbiota interactions through a wide screen of immunocompromised *A. thaliana* mutants grown in both natural (greenhouse) and laboratory-controlled (FlowPot gnotobiotic system) settings. In FlowPot gnotobiotic system I re-colonized germ-free *A. thaliana* with a complex multi-kingdom synthetic community (SynCom) comprising of bacterial (B), fungal (F) and oomycetes (O) communities, that are largely representative of the natural *A. thaliana* root microbiota. The aims of this thesis can be summarized in 5 points listed below:

- 1) Test the extent to which innate immune sectors impact root microbiota assembly in *A. thaliana* mutants grown in natural soil
- 2) Determine whether an intact innate immune system is needed for microbiota-induced plant growth promotion in a gnotobiotic system
- 3) Define whether immune sectors can prevent microbial dysbiosis by controlling microbial community composition and/or microbial abundance in plant roots.
- 4) Determine which microbial taxa must be kept at bay by the plant immune system to maintain host-microbe homeostasis and prevent dysbiosis
- 5) Test the extent to which the innate immune system alone is sufficient to maintain host-microbial homeostasis

Results

Interplay between innate immunity and root microbiota assembly in natural soil

Immunocompromised mutant's growth rate is time-dependent in natural soil

I first determined whether mutations in specific immune sectors affected plant development in natural soil. I evaluated the growth of 16 immunocompromised mutants (*bak1/bkk1*, *bak1/bkk1/cerk1*, *efr/fls2/cerk1*, *lyk5*, *apex1*, *apex2*, *apex3*, *wrky33/40*, *wrky33*, *wrky40*, *deps*, *pad4*, *cyp79b2/b3*, 35SBRI, *bri301* and *rar1*, see Materials & Methods for more details) in the greenhouse in Cologne Agricultural Soil (CAS) under short light conditions (8h) and their rosette's fresh weight (FW) was measured after 5 and 8 weeks of growth (Figure 2). At week 5, 8 mutants significantly differed in rosette's FW from WT (rosette's FW of *bak1/bkk1*, *cyp79b2/b3*, 35SBRI and *rar1* was significantly lower and rosette's FW of *bak1/bkk1/cerk1*, *apex3*, *wrky33* and *wrky40* was significantly higher than WT) and at week 8 only 3 mutants significantly different in size from WT (rosette's FW of *bak1/bkk1* and *deps* was significantly lower and rosette's FW of *wrky33* was significantly higher than WT). However, only two mutants had a consistently higher (*wrky33*) and lower (*bak1/bkk1*) rosette FW over the course of the whole experiment. This result highlights the importance of analysing several time points in regard to plant size and growth. With this screen I could identify that *bak1/bkk1/cerk1*, *apex3* and *wrky40* showed a faster growth in the first 5 weeks but did grow slower from week 5 until week 8, while *cyp79b2/b3*, 35SBRI and *rar1* showed an opposite trend. Although it remains difficult to disentangle the effect of mutation, microbial composition and environmental factors on plant growth in the greenhouse, I had performed a separate screen in a highly-controlled, gnotobiotic system FlowPot and confirmed that the 11 out of 14 of these mutants do not affect rosette's FW under sterile conditions, with only exception of *lyk5*, *apex1* and *pad4*, which had a significantly higher FW under sterile conditions in comparison to WT (Supp. Figure 1A). Altogether, it suggests that inactivation of several immune sectors alters plant development in natural soil.

Results

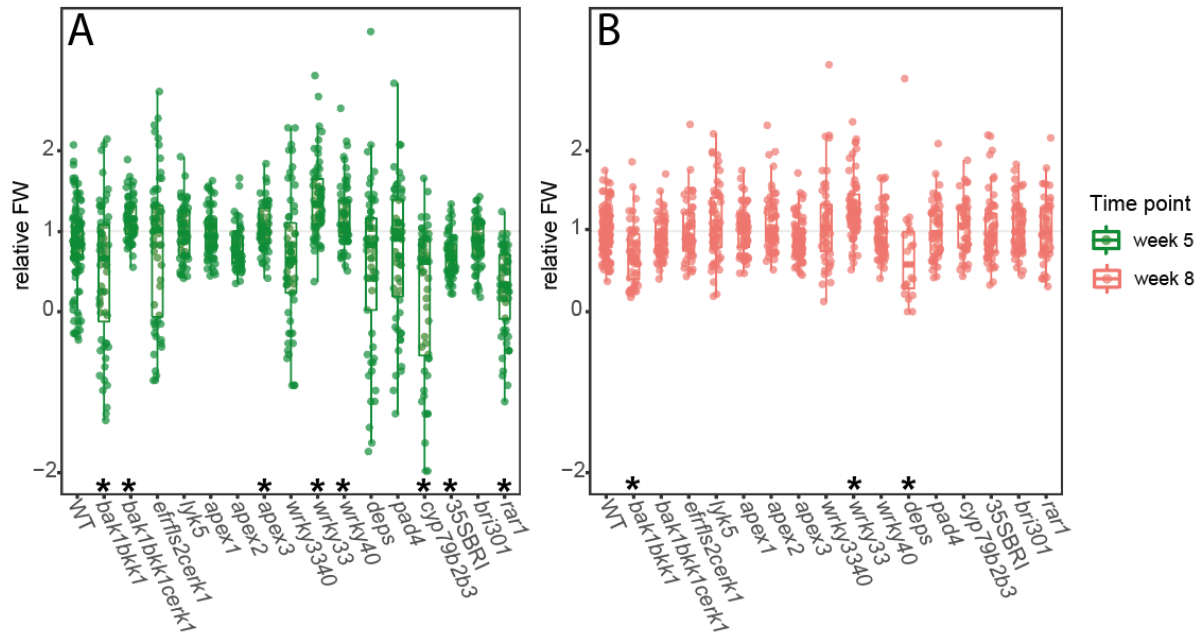


Figure 2: Rosette's FW of *A. thaliana* grown in natural soil at week 5 (A) and week 8 (B) normalized to WT rosette's FW within each biological replicate x time point combination.

Asterisks indicate genotypes significantly different from WT, calculated with Kruskal-Wallis and Dunn control test with Bonferroni-Hochberg correction ($p < 0.05$) with WT used as a control. Data points come from at least three independent biological replicates, with an exception of *deps* mutant in week 8 in which two biological replicates were lost. Plant number range: 53-114 (A) and 20-113 (B), median -0.117695 (A) and -0.03703073 (B) and mean -0.1617533 (A) and -0.08604307 (B). Colours indicate time point.

Influence of compartment and time point on root-endophytic microbial communities

Based on earlier reports (Lebeis et al., 2015; Durán et al., 2018), I hypothesized that compartment and time-points modulate root microbiota assemblages more extensively than gene mutations in different host immune sectors. To test this, I amplified V4-V7 region of bacterial 16S rRNA (799F and 1192R), fungal ITS1 region (ITS1F and ITS2) and oomycetes ITS1 region (ITS1-O and 5.8s-Rev-O) for bacteria, fungi and oomycetes respectively with use of previously published primers (Durán et al., 2018), Table S2 in said publication). Based on unconstrained Principal Component Analysis (PCoA) and independent PERMANOVA I observed that the compartment effect had a stronger effect on B-community composition (18.806%, $p = 0.001$, PERMANOVA) than on F- and O-communities (8.432%, $p = 0.001$ for F- and 11.791%, $p = 0.001$ for O-community, PERMANOVA) (Figure 3A-C, Table 1). This finding is in line with previously published research (Durán et al., 2018), where compartment was found to be a prominent force driving B-community structure in natural conditions. The

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Colours in panels A-C indicate compartment x time point (black for endophytic root fraction and brown for soil) and shapes indicate experimental round (all genotypes were divided into two groups, each group with its own respective WT. Results from WT are combined and analysed together). Each experimental round consists of three independent biological replicates. Shannon index of alpha diversity for bacterial (D), fungal (E) and oomycetes (F) community for each genotype/compartment x time point combination, colours indicate genotypes. Statistical analysis for Shannon index were done using Kruskal-Wallis and Dunn test ($p < 0.05$). Asterisks indicate significant difference from WT within a respective time point, while delta signs indicate significant difference within genotype, between two time points.

Table 1: Permutational analysis of variance (PERMANOVA) of compartment, genotype, time point and experiment/biological effects on root microbial community from natural soil greenhouse experiment.

Analysis done on Bray-Curtis distances with percentage of explained variance and P value with FDR correction. Top part of the table displays results for a full dataset and bottom part displays results for only endophytic root samples.

with soil	Bacteria				Fungi				Oomycetes			
	df	F	R2	P	df	F	R2	P	df	F	R2	P
Compartment	1	229.613	0.18806	0.001	1	55.627	0.08432	0.001	1	80.608	0.11791	0.001
Genotype	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Time point	1	67.588	0.05536	0.001	1	20.59	0.03121	0.001	1	16.002	0.02341	0.001
experiment/biological	5	37.404	0.15318	0.001	5	15.984	0.12115	0.001	5	11.009	0.08052	0.001
Residuals	342	NA	0.28011	NA	340	NA	0.51538	NA	360	NA	0.52659	NA
Total	453	NA	1	NA	456	NA	1	NA	476	NA	1	NA
without soil	df	F	R2	P	df	F	R2	P	df	F	R2	P
Compartment	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Genotype	16	2.298	0.04547	0.001	16	1.0325	0.03393	0.314	16	1.4378	0.0439	0.006
Time point	1	61.278	0.0758	0.001	1	19.1305	0.03929	0.001	1	19.1522	0.03655	0.001
experiment/biological	5	35.312	0.21841	0.001	5	13.5665	0.13932	0.001	5	11.17	0.10657	0.001
Residuals	281	NA	0.3476	NA	280	NA	0.57511	NA	299	NA	0.57055	NA
Total	381	NA	1	NA	385	NA	1	NA	404	NA	1	NA

Table 2: Percentage of variance explained by genotype x time point interaction effect of each genotype in comparison to WT samples in natural soil experiment.

Significant effects ($p < 0.05$) are highlighted in bold.

Results

genotype	bacteria		fungi		oomycetes	
	% of variance	p value	% of variance	p value	% of variance	p value
<i>bak1/bkk1</i>	6.72	0.001	5.00	0.33	5.48	0.13
<i>bak1/bkk1/cerk1</i>	7.47	0.001	4.93	0.46	4.5	0.71
<i>efr/fls2/cerk1</i>	8.11	0.001	4.11	0.92	5.96	0.1
<i>lyk5</i>	6.28	0.001	4.24	0.83	3.87	0.94
<i>apex1</i>	8.37	0.001	4.55	0.7	3.71	0.94
<i>apex2</i>	5.78	0.003	4.57	0.7	3.72	0.9
<i>apex3</i>	6.37	0.002	4.55	0.74	4.52	0.64
<i>wrky33</i>	7.07	0.001	4.76	0.55	5.66	0.18
<i>wrky33/40</i>	6.98	0.001	5.29	0.14	4.55	0.36
<i>wrky40</i>	7.26	0.001	4.25	0.82	3.86	0.91
<i>deps</i>	9.19	0.001	6.29	0.22	6.43	0.16
<i>pad4</i>	7.36	0.001	6.91	0.002	5.15	0.2
<i>cyp79b2/b3</i>	8.92	0.001	5.20	0.12	4.62	0.44
35SBRI	6.28	0.001	4.88	0.57	3.88	0.87
<i>bri301</i>	6.11	0.002	4.71	0.64	5.76	0.16
<i>rar1</i>	8.6	0.001	5.34	0.1	5.09	0.26

Influence of innate immunity on root-endophytic microbial community composition

Next to compartment and time point effect, the third factor analysed in this dataset was genotype effect (4.547%, p=0.001 for B-, 3.393%, p=0.314 for F- and 4.39%, p=0.006 for O-communities) (Table 1). Based on single-genotype comparisons 5 genotypes (*cyp79b2/b3*, *efr/fls2/cerk1*, *apex1*, *deps* and *rar1*) harboured a significantly different B-community than WT at both time points, with additional 2 genotypes (*wrky33/40* and *pad4*) and additional 5 genotypes (*bak1/bkk1*, *apex2*, *apex3*, 35SBRI1 and *bri301*) harbouring significantly different communities from WT at week 5 and week 8, respectively (Figure 4A and D). F-community at week 5 was significantly different in only three genotypes (*deps*, *rar1* and *pad4*) and *deps* was the only mutant harbouring a significantly different community at week 8 (Figure 4B and E). In O-community no genotype was found which would be consistently significantly different from WT, but in week 5, 4 genotypes were marked as significantly different (*rar1*, *bak1/bkk1*, *pad4* and *efr/fls2/cerk1*) and in week 8 there were 2 genotypes (*bri301* and *wrky33*) (Figure 4C and F). At the family level for B-community, relative abundance of Nocardiaceae was significantly altered in roots of *bak1/bkk1*, *bak1/bkk1/cerk1*, *efr/fls2/cerk1*, *lyk5*, *apex1*, *wrky33/40*, *wrky33*, *wrky40*, *pad4*, *cyp79b2/b3* and *rar1*. *apex1* was the genotype with the

Results

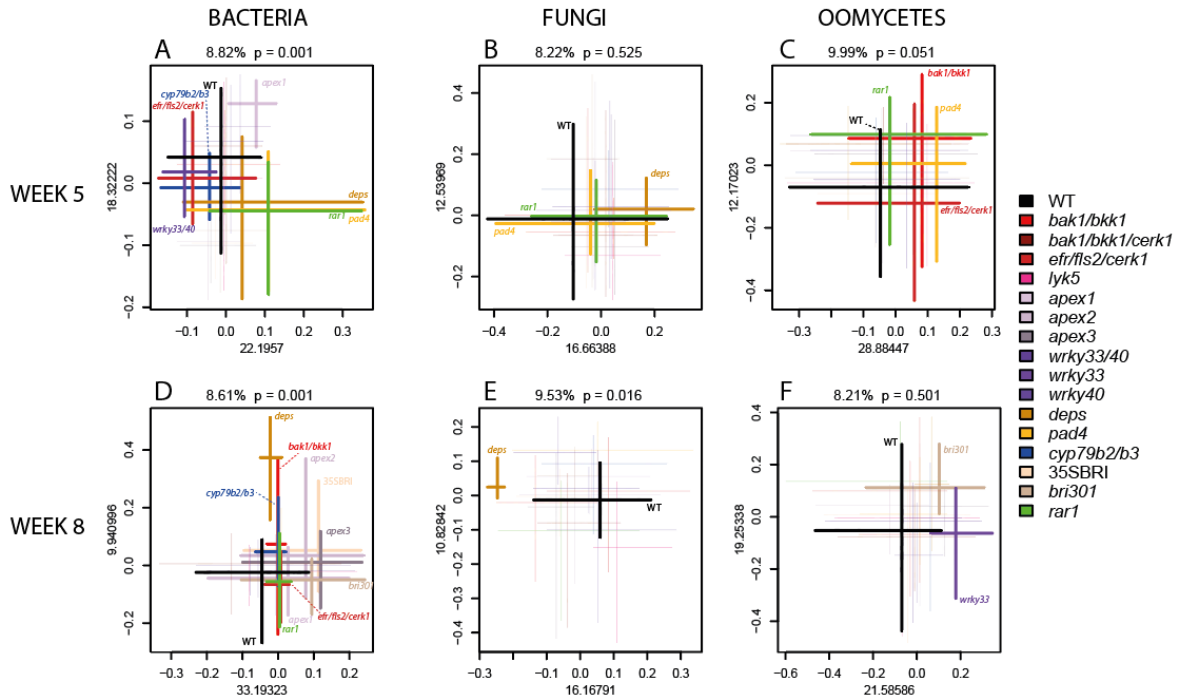
highest number of significantly different families in comparison to WT (Hyphomicrobiaceae, Nocardaceae, Nocardoidaceae, Sinobacteraceae and Spirochaetaceae). For F-community, relative abundances of Lulworthiaceae, Mortierellaceae and Saccharomycetaceae were significantly altered in four genotypes, but only *deps*, *pad4* and *rar1* were overlapping between Lulworthiaceae and Saccharomycetaceae family. *deps* mutant had the strongest genotype effect among tested mutants on family level, with relative abundance of 3 families being significantly different from WT (Lulworthiaceae, Nectriaceae and Saccharomycetaceae). In O-community relative abundance of Apodachlya family was significantly altered in roots of 5 mutants (*apex1*, *apex2*, *wrky33*, *wrky40* and *deps*). *bak1/bkk1*, *wrky40* and *apex1* harboured 2 significantly different and distinct family-level communities, as only Apodachlya family was overlapping between *apex1* and *wrky40* (Figure 5, Annex I). ASV-level (Amplicon Sequence Variant-level) analysis did not yield any conclusive results, with many individual ASVs being affected, but without a clear taxonomical signal. F-community from both time points and O-community from week 8 showed clustering on ASV-level, but only based on the experimental batch (Supp. Figure 3-5). All together it indicates that plant innate immunity pathways have only subtle effect on root microbial community and the effect is not limited by taxonomical assignment.

Influence of innate immunity on root-endophytic total microbial abundance

Beta diversity analyses are based on relative abundances of microbial strains in relation to one another, which does not provide information regarding absolute microbial abundance in root samples. This is why it is important to develop alternative methods to assess microbial abundance (X. Guo et al., 2020). Here I used a RT-qPCR method to assess microbial abundance relative to plant material with use of UBQ10 as a plant control (see Materials & Methods for more details). Quantification of fungal and oomycetes total abundance in plant roots across immunocompromised mutants and WT revealed no major differences in microbial abundance across genotypes, except for the *deps* mutant, for which a significant increase in oomycetes total abundance was observed (Figure 6). An additional technical limitation of RT-qPCR detection level did not allow me to detect total bacterial abundance in endophytic fraction. Cause is unknown but there is a possibility it is due to the combined effect of low B-community abundance in endophytic fraction and cross-amplification of plant DNA by 16S

Results

primers used for total bacterial abundance measurements. Overall, the data suggest that inactivation of different immune sectors mildly alters microbial community composition and eukaryotic microbial abundance in roots when plants are grown in natural soil under greenhouse conditions.



Results

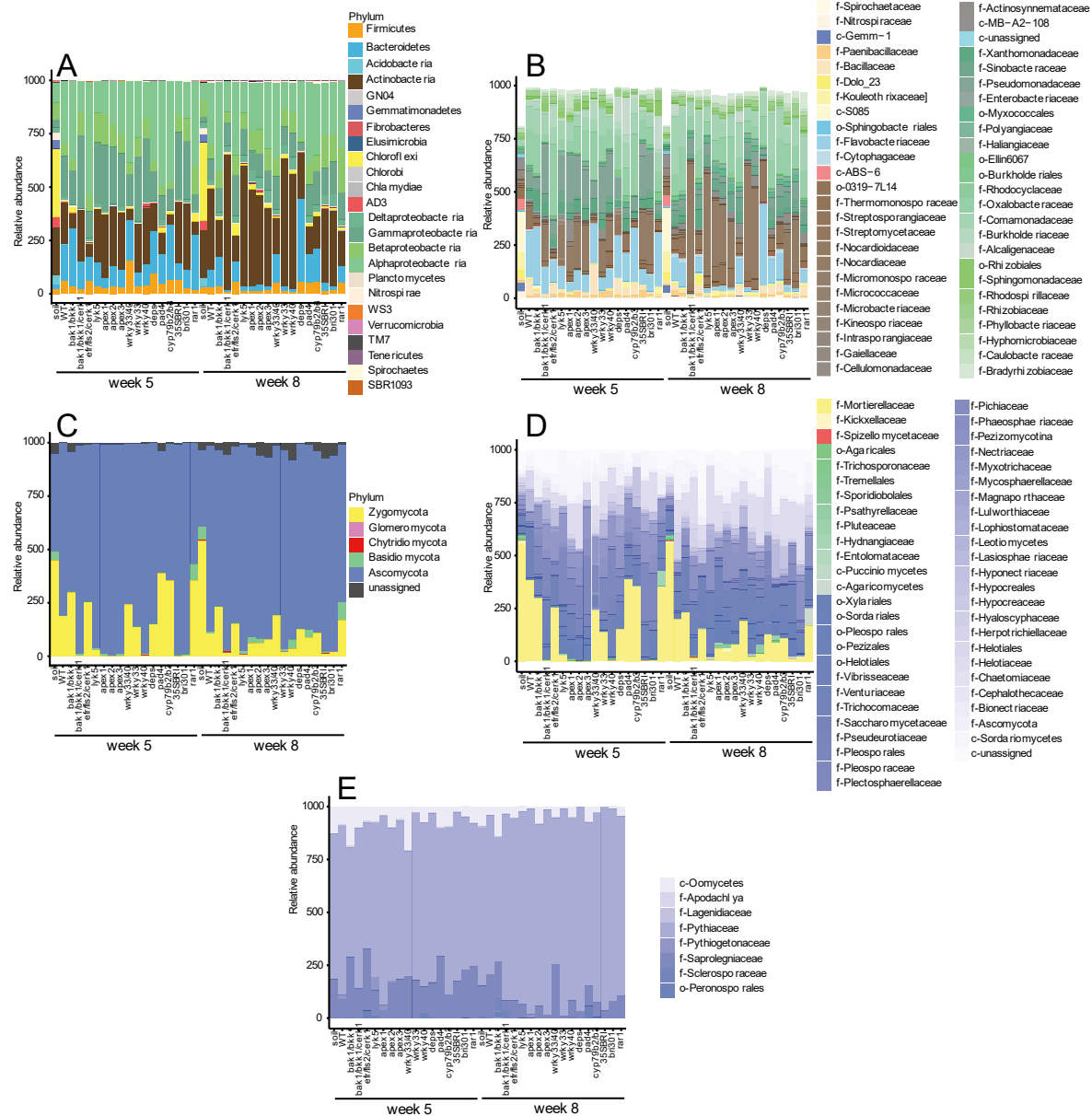


Figure 5: Relative abundance of microbial community in natural soil experiment on phylum/class (A and C for bacterial and fungal community, respectively) and class/family/order level (B, D and E for bacterial, fungal and oomycetes community, respectively).

Colours indicate the taxonomical assignment, first letter in class/family/order legend (in panels B, D and E) indicate taxonomical level assignment (c – class, f – family and o – order). The relative abundance in each sample was normalized to 1000 for between-sample comparison purposes. Statistical analyses were done with Kruskal-Wallis and Dunn test ($p < 0.05$) and the output of genotype/compartments effect can be found in Annex I.

Results

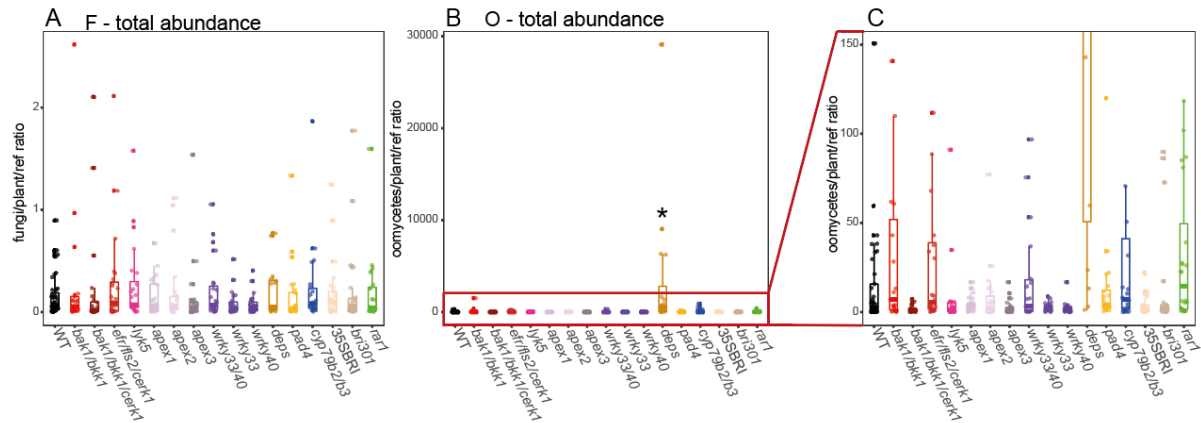


Figure 6: Total fungal (A) and oomycetes (B and C) abundance in root endosphere of *A. thaliana* grown in natural soil.

Panel C shows a subset of oomycetes samples without outliers above the ratio of 150 for clarity reasons. Statistical analyses were done using Kruskal-Wallis with Dunn control test with Bonferroni-Hochberg correction on full datasets ($p < 0.05$) and WT as a control. Asterisk indicates a genotype significantly different from WT.

Natural soil is a highly complex and unpredictable environment

As I confirmed that majority of the mutations do not have a direct effect on plants' aboveground biomass under sterile conditions (11 out of 14 tested, $p < 0.05$, Supp. Figure 1A), it became clear that the environment itself plays an important role in shaping plants phenotype. It is known that even in the greenhouse the environmental conditions can fluctuate and plants are exposed to several potential pathogens, coming from both, below- and aboveground. This can pose a problem when working with severely immunocompromised mutants. An observation of an extreme phenotype of *deps* mutant was made during the course of the experiment, where *deps* plants were performing very poorly in regard to germination and survival and two full biological replicates (2/3 of all cultivated plants) were lost during the eight' week of growth due to various environmental stressors with herbivorous insects being most likely the main cause of plants death (personal observation of insect bites on plant leftover). This observation, coupled with the possibility that complex natural soil environment may obscure subtle interplay between innate immunity and root microbiota, was my main reason to focus on more detailed analysis of the effect of dysfunctional innate immune system on beneficial plant-microbe interactions under strictly controlled laboratory conditions, with use of the FlowPot gnotobiotic system (Kremer et al., 2018).

Innate immune sectors prevent microbial dysbiosis in plant roots in a gnotobiotic system

FlowPot system limits environmental factor influence and simplifies microbial interactions

FlowPot gnotobiotic system (Kremer et al., 2018) allows a considerable reduction in ecosystem's complexity, both on microbial side by using a synthetic, yet representative microbial community consisting of 183 bacterial, 25 fungal and 6 oomycetes strains (Methods Figure 1, (Bai et al., 2015; Durán et al., 2018; Thiergart et al., 2020)), as well as on the side of environmental factors, by excluding undesired biotic stressors and limiting abiotic stressors. A crucial advantage is the possibility to break down complex ecological processes into testable hypotheses by controlling various variables in the system. By reducing inherent environmental noise, it becomes possible to test for causality and to identify first principles that govern microbiome assembly, which would be otherwise impossible to decipher using field experiments only. Benefits of using the FlowPot system for studying immunocompromised *A. thaliana* mutants were especially clear in case of *deps* mutant, which, as mentioned before, 2/3 of cultivated plants were lost in natural soil (Figure 2), while in microbiota reconstitution experiment in FlowPots the *deps* mutant was performing as well as WT in sterile conditions (no statistically significant difference between rosette FW of *deps* and WT under sterile treatment, Supp. Figure 1A). Another important advantage of a gnotobiotic system is the possibility to disentangle the effect of the mutation from the effect of microbiota on plant's growth by being able to grow plants in both gnotobiotic and axenic conditions. Using this FlowPot system I was able to show that only *lyk5*, *apex1* and *pad4* mutants do exhibit a rosette biomass changes due to their mutations, which was not possible in greenhouse settings.

Role of innate immune system in microbe-mediated plant growth promotion

I first tested the extent to which a complex BFO-SynCom promotes plant growth in the gnotobiotic FlowPot system in the WT context. 5-week old WT plants recolonized by the BFO-SynCom showed a significant increase in rosette's FW compared to sterile control plants (Figure 7A, $p < 0.05$). In a second step, I tested whether an intact innate immune system is needed for BFO-induced plant growth promotion. To test this hypothesis, I used the same

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mutants as describe above in natural soil experiment, with an exception of *wrky40* and *bri301* (for details on the use of *bri301* in reconstitution experiments, please see Materials & Methods, section “FlowPot preparation and growth conditions”) and performed the same recolonization experiments as for WT. Remarkably, majority of all tested immunocompromised mutants (9 out of 14) lost the ability to benefit from the presence of microbial community, having a significantly lower increase in FW than WT after addition of BFO-community to the system (Figure 7B). In order to ensure that the observed phenotype is caused by the interaction between living BFO-SynCom and plants I performed a test experiment where I grew WT plants in sterile, BFO and heat-killed conditions (for details see Materials & Methods, section “Heat-kill FlowPot control experiment) and validated that the growth promotion phenotype is only observed with a living (and not heat-killed) BFO-SynCom (Supp. Figure 6).

Complete lack of growth promotion in *pad4* mutant intuitively contradicts the result obtained for *deps*, as *deps* harbours a PAD4 mutation. Nevertheless, *deps* mutant is compromised in JA, SA and ethylene biosynthesis/signalling and most probably displays several pleiotropic effects, which could explain the initial discrepancy. Another two mutants that showed complete loss of plant growth promotion effect are two WRKY transcription factor mutants. WRKY transcription factors are known for their regulatory involvement in regulation of numerous pathways (Jiang et al., 2017), which makes it difficult to narrow down the cause behind lack of microbiota-mediated growth promotion loss. Next, both *bak1/bkk1* double and *bak1/bkk1/cerk1* triple mutant displayed a significant loss of microbiota-mediated growth-promotion effect, but not *efr/fls2/cerk1* mutant, indicating the main role of *BAK1* and *BKK1* co-receptor genes. Another important observation is lack (or even a significant decrease of mutant’s FW after addition of BFO community, Figure 7A) of microbiota-mediated plant growth promotion in *cyp79b2/b3* mutant, pointing to the conclusion that not only co-receptor layer, but also Trp-derived secondary metabolites is of high importance for beneficial plant-microbiota interactions. All together this reconstitution experiment revealed the importance of an intact immune system for microbiota-mediated plant growth promotion effect.

Results

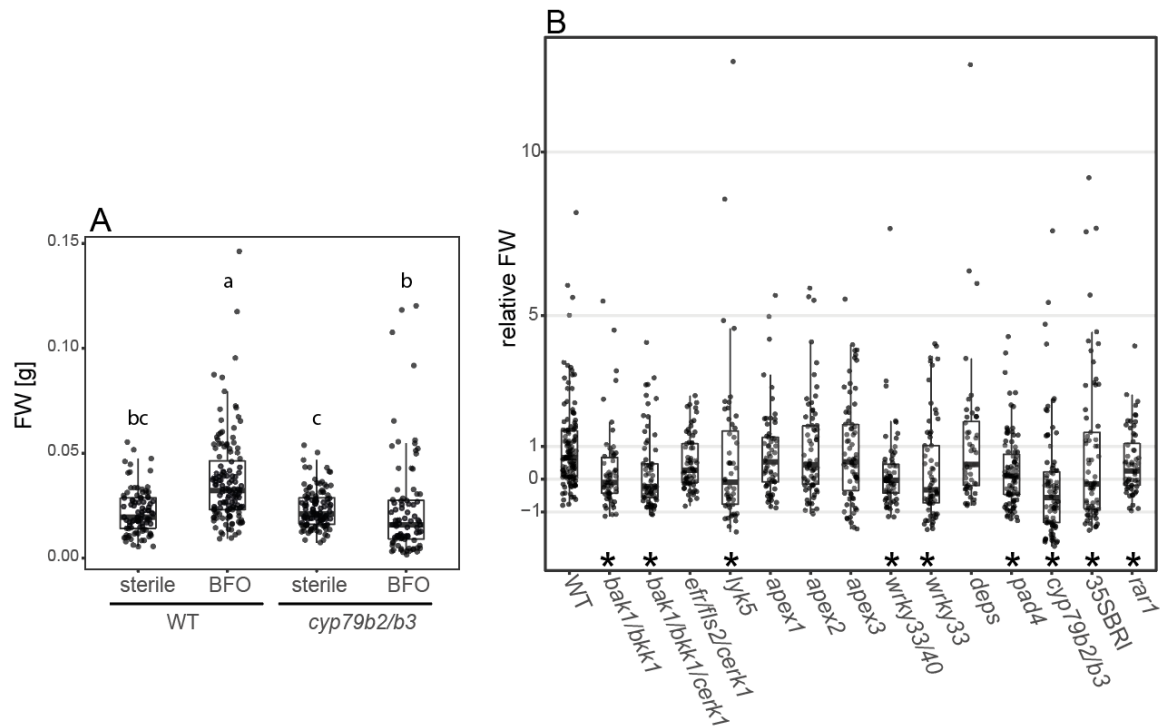


Figure 7: Intact plant innate immunity is needed for BFO-mediated plant growth promotion in FlowPot system. Rosette's FW at vegetative stage FlowPot experiment.

FW comparison between sterile and BFO-inoculated WT and *cyp79b2/b3* (A). Relative FW of all tested mutants (B) was calculated by subtracting the average sterile FW of each mutant from each BFO-treated mutant and later dividing it by the average difference between BFO-treated and sterile WT (respective WT for each mutant). Plant number = 132-20 with a median of 58.50 and mean of 56.72. Data comes from three independent biological replicates, with an exception for WT and *cyp79b2/b3* mutant, for which data from six biological replicates is available. Significant differences were calculated using Kruskal-Wallis with Dunn test with Bonferroni-Hochberg correction ($p < 0.05$) (panel A) and Dunn control test with Bonferroni-Hochberg correction ($p < 0.05$) with WT as a control, based on transformed FW data (panel B). This figure will be a part of the manuscript in (un)changed form.

Influence of innate immunity on structure and diversity of root-associated microbial communities

Given the earlier observation that several immune sectors are needed for BFO-induced plant growth promotion in the FlowPot system (Figure 7), I hypothesized that microbial community diversity and composition was likely altered in roots of immunocompromised mutants. I analysed community richness and composition of bacteria (16S V5-V7 region), fungi (ITS1) and oomycetes (oITS1). Interestingly, no dramatic changes in microbial alpha diversity nor composition were detected that could explain the striking phenotype differences across tested mutants (Figure 8, Supp. Figure 7). Several genotypes showed a significant shift in microbial composition, especially B-community, however genotypes with significant shifts in B-

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community composition did not overlap with the microbiota-mediated plant growth promotion phenotype (Table 3, Figure 7B) and regression analysis between microbial community composition and relative FW growth did not yield any significant results either ($p < 0.05$, ANOVA, Supp. Figure 8). I also analysed phylum and family level microbial shifts in search for smaller-scale effects (Figure 9 and Annex II). Relative abundance of three bacterial phyla/classes (Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria) and three bacterial families (Bradyrhizobiaceae, Flavobacteriaceae and Microbacteriaceae) were significantly affected within the roots of single genotypes, while no relative abundance of fungal phyla, families nor oomycetes OTUs showed a significant genotype effect.

The last level of analysis was at OTU-level resolution, where I analysed the results of individual OTU fold changes between mutants and the WT. Within bacterial commensals the fold change in comparison to WT of three OTUs (namely OTU_745, OTU_83 and OTU_236) was significantly affected by 6 different genotypes. In fungal community, abundance of strain 21 was the most affected (significant fold change in 6 genotypes), with abundance of other strains showing a significant fold change in 2-3 genotypes out of 15 tested. In summary certain individual strains abundances were indeed significantly affected by individual genotypes in comparison to WT. Irrespective of these subtle differences, no clear link between community composition clusters on OTU-level and microbiota-mediated growth promotion phenotype could be observed (Supp. Figure 9, Supp. Table 2).

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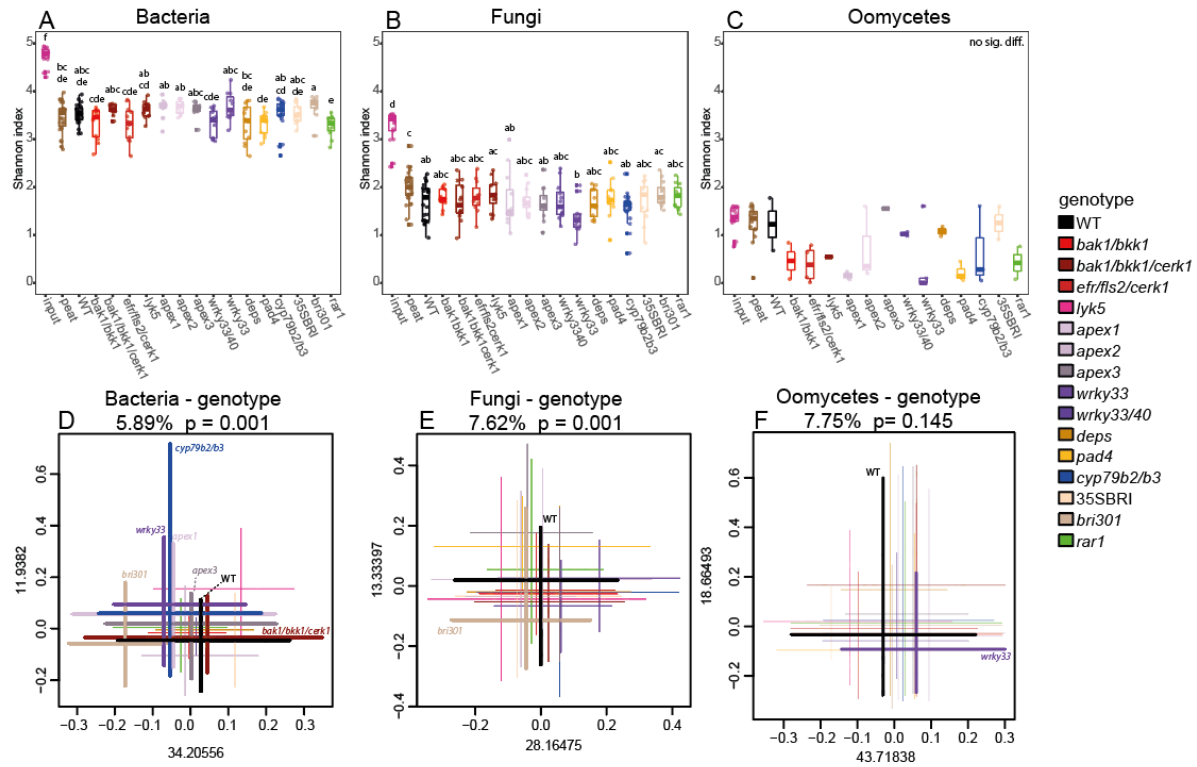


Figure 8: Root microbial diversity and composition in vegetative stage FlowPot experiment poorly explains lack of microbe-mediated growth promotion phenotype.

Alpha diversity Shannon index for bacterial (A), fungal (B) and oomycetes (C) community. “input” indicates initial microbial input, “peat” a sample originating from an unplanted FlowPot filled with peat. PCoA based on Bray-Curtis distances, constrained by genotype for bacterial (D), fungal (E) and oomycetes (F) communities. Each cross covers the minimal and maximal values per axis of the respective genotype. Percentage value above the graph represents the variance explained by genotype effect. Genotypes significantly different from WT (ANOVA, $p < 0.05$) are highlighted in bold and their respective genotypes names are added on the graphs. Exact percentage of variance and p values can be found in Table 3. This figure will be a part of the manuscript in (un)changed form.

Table 3: Exact percentage of variance of root community composition in vegetative FlowPot experiment for each genotype-WT comparison explained by genotype factor with a corresponding p value.

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Significant genotype effects ($p < 0.05$) are highlighted in bold. Genotypes are sorted alphabetically. This table will be a part of the manuscript in (un)changed form.

genotype	BACTERIA		FUNGI		OOMYCETES	
	% of variance	p value	% of variance	p value	% of variance	p value
35SBRI	1.91	0.177	3.21	0.101	2.73	0.371
apex1	4.2	0.001	0.97	0.983	1.77	0.723
apex2	2.52	0.155	2.53	0.285	3.23	0.347
apex3	2.74	0.016	2.79	0.117	4.09	0.122
bak1/bkk1	1.27	0.423	1.69	0.581	2.46	0.466
bak1/bkk1/cerk1	3.32	0.012	2.03	0.452	3.88	0.157
bri301	6.92	0.001	3.58	0.045	2.05	0.621
cyp79b2b3	2.25	0.005	1.56	0.461	1.8	0.509
deps	1.51	0.368	3.16	0.104	1.48	0.837
efr/fls2/cerk1	1.58	0.159	1.39	0.733	1.22	0.885
kai2	1.88	0.058	2.35	0.194	2.89	0.36
lyk5	2.31	0.052	2.47	0.292	2.5	0.425
pad4	1.66	0.184	2.88	0.14	2.06	0.652
quadruple	1.67	0.128	2.09	0.388	3.34	0.244
rar1	1.63	0.367	1.28	0.885	1.48	0.857
wrky33	4.44	0.001	2.45	0.223	5.81	0.026
wrky33/40	1.51	0.208	1.97	0.396	2.04	0.588

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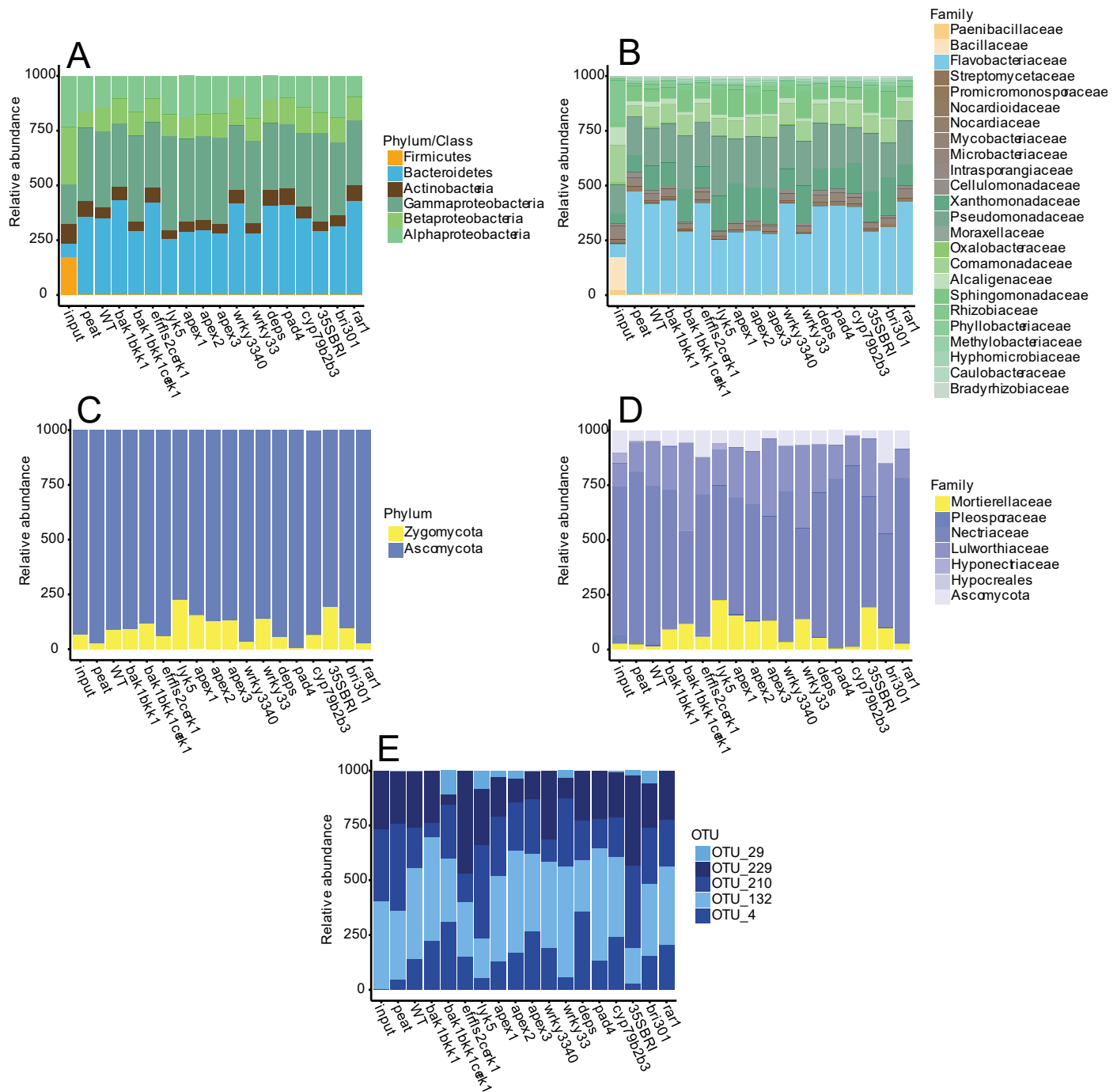


Figure 9: Relative abundance of microbial community in vegetative stage FlowPot experiment on phylum/class (A and C for bacterial and fungal community, respectively) and family/OTU level (B, D and E for bacterial, fungal and oomycetes community, respectively).

Colours indicate the taxonomical assignment. The relative abundance in each sample was normalized to 1000 for between-sample comparison purposes. Statistical analyses were done with Kruskal-Wallis and Dunn test ($p < 0.05$) and the output of genotype/compartments effect can be found in Annex II.

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Influence of innate immunity sectors on microbial abundance in plant roots

Since no clear link could be found between lack of microbe-mediated growth promotion and microbial community composition in roots, I hypothesised, that the absolute microbial abundance in roots, rather than compositional shifts in the microbiota, is a key factor explaining microbe-mediated growth promotion phenotype. Similarly to the natural soil experiment, I quantified bacterial, fungal and oomycetes total abundance in relation to plant DNA marker gene using qRT-PCR, for details see Materials & Methods). In FlowPot experiment a distinction between endosphere and rhizoplane is impossible due to specificity of the system, which allowed me to have more starting material and quantify bacterial total abundance as well (in contrast to natural soil experiment where bacterial total abundance was not detected in endophytic root fraction with the available detection method). I detected significant genotype-specific variation for two microbial groups, namely bacteria and fungi (Figure 10A-C). *bak1/bkk1* harbours a significantly increased total B-community abundance in comparison to WT and *efr/fls2/cerk1*, *wrky33/40* and *cyp79b2/b3* harbour significantly increased total F-community abundance. In order to find out whether total microbial abundance can more precisely explain plant's phenotype, I used regression analysis of relative FW of BFO-inoculated plants (normalized by its respective sterile controls, data shown on Figure 7B) with bacterial, fungal and oomycetes total abundance read-outs (Figure 10D-F, respectively). Increase in bacterial abundance shows a non-significant correlation trend with lack of microbe-mediated growth promotion effect. Remarkably, a statistically significant association is observed for fungal total abundance, where increase in fungal abundance in roots is significantly correlated with a decrease in microbe-mediated growth promotion effect (Figure 10E). At the same time increase in fungal abundance is not significantly correlated with an increase in bacterial abundance (Figure 10G). This result suggests that modulation of total fungal abundance by the innate immune system, rather than modulation of fungal diversity and community composition is likely the key factor for beneficial BFO-community function.

cyp79b2/b3 and bak1/bkk1 display the most striking phenotype among tested mutants

Out of all tested genotypes, *cyp79b2/b3*, which is a mutant with a disrupted Trp-derived secondary metabolites pathway (indolic glucosinolates pathway), displayed the most striking

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phenotype. Not only did it completely lack beneficial microbe-mediated growth promotion (with a significant detrimental effect due to addition of BFO-community) (Figure 7), but it also contained one of the highest total fungal abundance read-outs (Figure 10B and E). As *CYP79B2* and *CYP79B3* genes are two key biosynthesis genes at the beginning of Trp-derived secondary metabolites biosynthesis I performed a literature search in order to find out more about currently known downstream pathways. Based on literature search, known pathways directly linked to *CYP79B2* and *CYP79B3* biosynthesis genes are indolic glucosinolates and their hydrolysis products, camalexins, indole-3-acetic acid (IAA) and indole cyanohydrin (Figure 1B). Therefore, I devised a new hypothesis that one of the downstream *CYP79B2* and *CYP79B3*-mediated Trp-derived secondary metabolites pathways is the key component explaining *cyp79b2/b3* phenotype.

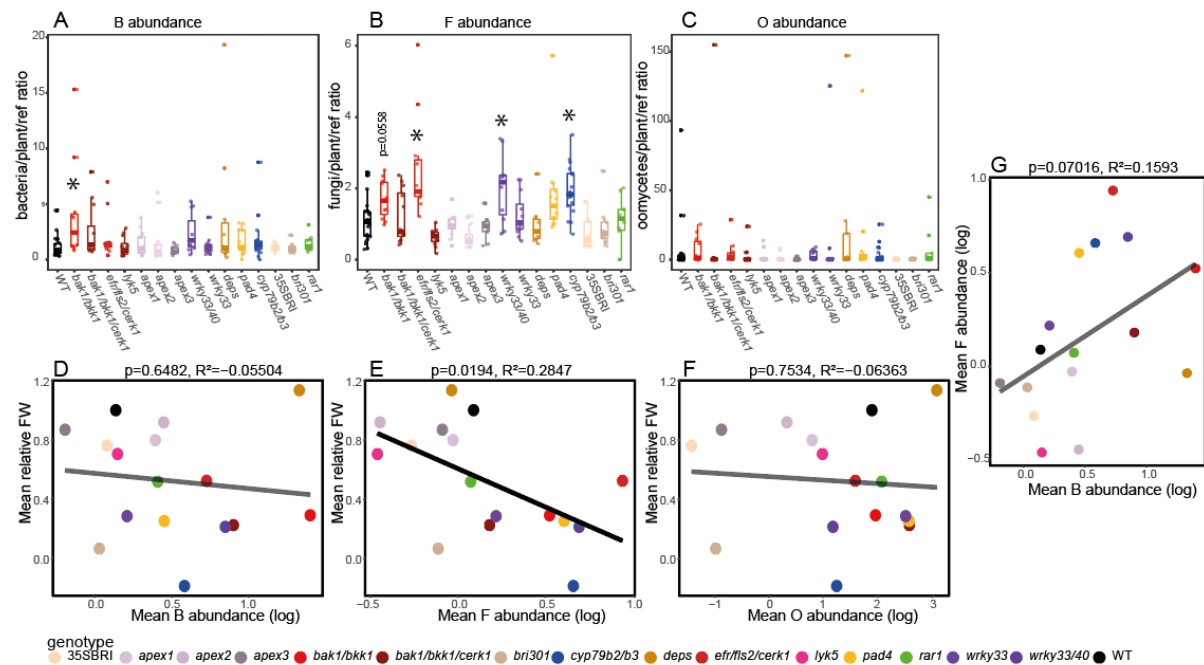


Figure 10: Fungal abundance is a likely candidate to explain microbe-mediated plant growth promotion. Total bacterial (A), fungal (B) and oomycetes (C) abundance in plant root samples, calculated based on RT-qPCR data relative to plant UBQ10 reads. Asterisks indicate genotypes that are significantly different from WT. Significant differences were calculated using Kruskal-Wallis and Dunn control test with Bonferroni-Hochberg correction ($p < 0.05$) and WT as a control. Linear regression between mean total bacterial (D), fungal (E) and oomycetes (F) abundance and mean plant relative FW, p-value and R^2 were obtained from ANOVA. Panel G presents a regression between bacterial and fungal total abundance, p-value and R^2 were obtained from ANOVA. This figure will be a part of the manuscript in (un)changed form.

Second most interesting mutant identified alongside *cyp79b2/b3* is *bak1/bkk1*. First of all, mutations in *BAK1* and *BKK1* genes showed a strong reduction in microbe-mediated growth-

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promotion effect in both *bak1/bkk1* and *bak1/bkk1/cerk1* mutant, while *efr/fls2/cerk1* which shared *CERK1* mutation did not show as severe phenotype, indicating the importance of mutations in *BAK1* and *BKK1* genes (Figure 7B). *bak1/bkk1* mutant was the only analysed mutant that showed a significant increase in bacterial abundance and elevated (although not significant) fungal and oomycetes abundance in roots (Figure 10A and B). Taken together it puts *bak1/bkk1* in the group of mutants with a strong FW – microbial abundance correlation (Figure 10D and E). The reason why it was chosen over other mutants with lack of growth-promotion effect is that it shows a strong FW phenotype (in contrast to *lyk5*, 35SBRI and *rar1*) and both *BAK1* and *BKK1* are protein-coding genes and not transcription factors (like *wrky33* and *wrky33/40*), which gives a higher chance for low-level perturbations in overall functioning of the plant. Additionally, *PAD4* mutation is involved in camalexin biosynthesis pathway and since this pathway will be investigated in more detail alongside *cyp79b2/b3*, it was deemed unnecessary to follow up on *pad4* mutant specifically.

Role of Trp-derived secondary metabolites in beneficial plant-microbiota interaction

In order to further disentangle the striking effect seen in *cyp79b2/b3* mutant, I tested 9 mutants with disruption in different parts of Trp-derived secondary metabolites pathway (*quadruple*, *pen2/cyp71a12/a13*, *cyp71a12/a13*, *pen2/pad3*, *pen2*, *pad3*, *myb34/51/122*, *pyk/bglu*, *cyp71a27*) and I used *cyp79b2/b3* as my negative control (Methods Table 1, Figure 1B). Tested glucosinolates mutants cover as many sub-pathways of Trp-derived secondary metabolites that relay on functioning *CYP79B2* and *CYP79B3* genes as possible. Analysis of the phenotype of all gathered knock-out mutants should lay solid foundation for future biochemical analysis that would allow to draw conclusions as to which chemical compounds from Trp-derived secondary metabolites are the most crucial elements behind the observed *cyp79b2/b3* phenotype.

Trp-derived glucosinolates poorly explain cyp79b2/b3 mutant phenotype

FW analysis revealed high biological variation within this experiment, reducing statistical power of the analysis. Nevertheless, all the mutants displayed WT-like rosette's FW in sterile conditions (Supp. Figure 1B) and the negative control *cyp79b2/b3* still showed a clear trend

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for lack of microbe-mediated growth promotion phenotype (Figure 11A). At first glance *pen2/cyp71a12/a13* mutant looked like a promising candidate for narrowing down the cause of *cyp79b2/b3* phenotype. However, this mutant line was very unstable in sterile conditions, where despite high germination score (average over three biological replicates 84.7% for sterile and 89.6% for BFO-inoculated treatments of germinated/alive seedlings 2 weeks after sowing), two full, independent, sterile biological replicates were lost due to premature plant's death. *pen2/cyp71a12/a13* plants did seem to be rescued by presence of BFO-community, however due to unstable phenotype this mutant was excluded from further analysis. FW analysis of the remaining glucosinolates mutants showed intermediate phenotype, with no clear candidates recapitulating the *cyp79b2/b3* phenotype. Additional analysis of bacterial, fungal and oomycetes total abundance (Figure 11B-D respectively) also did not help in identifying a promising candidate to explain lack of microbiota-mediated growth promotion phenotype. Only two mutants (apart from negative control *cyp79b2/b3*) that showed a significant increase in total fungal abundance were *quadruple* and *pen2/cyp71a12/a13* mutants (mediocre FW phenotype and unstable phenotype respectively). Nevertheless, especially based on *quadruple* mutant results, it is very interesting to see that increased total abundance can be a cause or effect of lack of microbiota-mediated growth promotion phenotype but these two factors are not fully coupled. The comparison between *quadruple* and *cyp79b2/b3* mutant could help explain which pathways are responsible for controlling fungal growth in the roots. Taken together the data suggest that components of the indole glucosinolates branch of the Trp-derived secondary metabolites pathway tested in this experiment, poorly explain the reversion from beneficial-to-detrimental effect of the BFO-SynCom observed in the *cyp79b2/b3* mutant, but are to some extent involved in the control of fungal proliferation in roots. It suggests that other, yet uncharacterized molecules regulated by *CYP79B2* and *CYP79B3* genes prevent fungal dysbiosis in *A. thaliana* roots.

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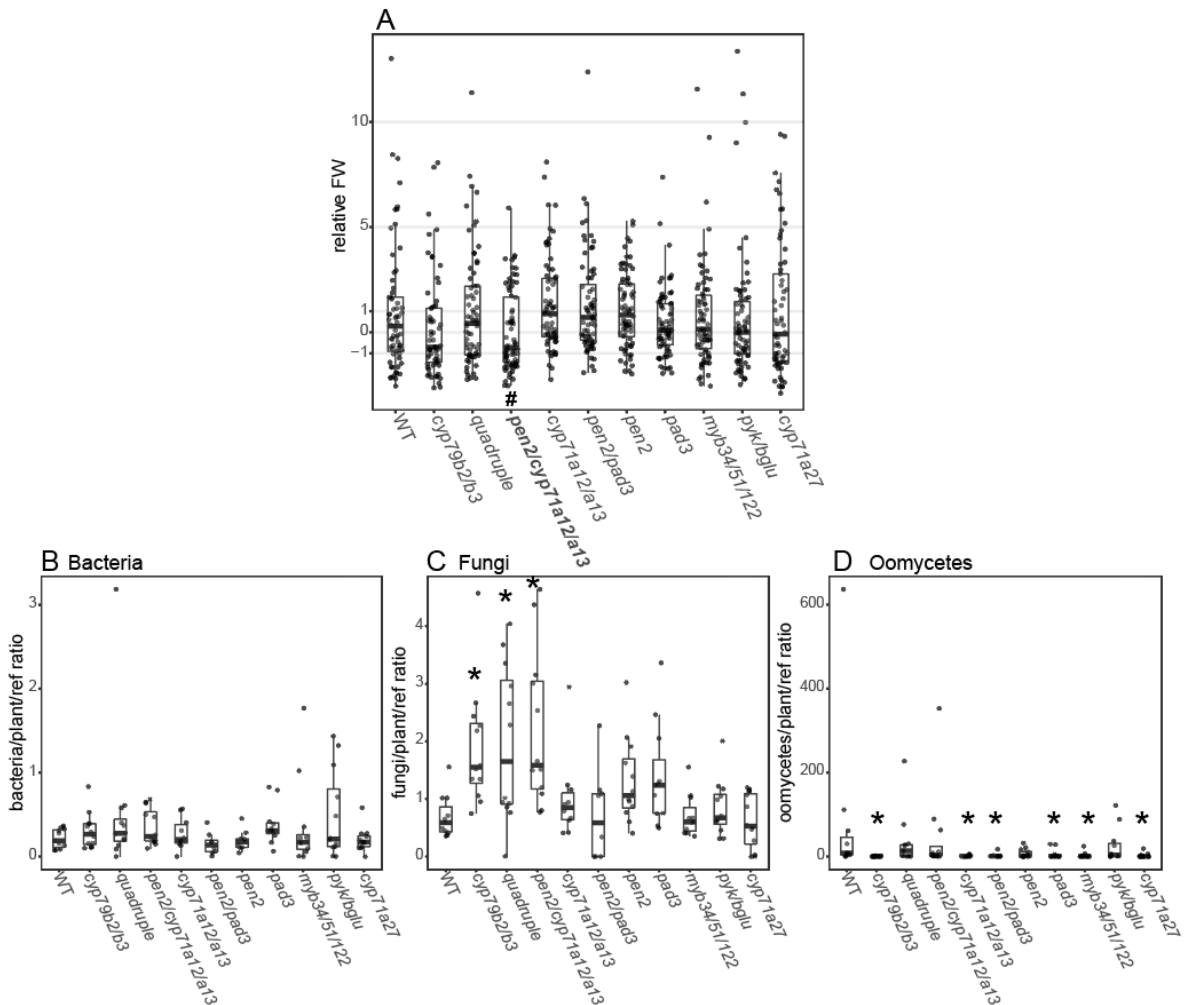


Figure 11: Trp-derived glucosinolates pathway does not explain the striking *cyp79b2/b3* FW phenotype.

Relative FW of all tested glucosinolates mutants (A) and the bacterial (B), fungal (C) and oomycetes (D) total abundance in their root samples. For details behind the analysis see Figure 7B (relative FW) and Figure 10A-C (total abundance). Significant differences between relative FW (A) and total microbial abundances (B-D) were calculated using Kruskal-Wallis with Dunn control test with Bonferroni-Hochberg correction ($p < 0.05$). Plant number on panel A ranges from 63 to 72, with median of 0.229071 and mean of 0.7943009. Asterisks indicate genotypes that are significantly different from WT. Mutant marked with # (*pen2/cyp71a12/a13*) showed premature seedling mortality in 2/3 of the sterile biological replicates. This figure will be a part of the manuscript in (un)changed form.

Influence of innate immunity on plant's reproductive stage

Intact immune system and balanced root-microbial community are both essential for maintaining plant's performance

As the dissection analysis of Trp-derived metabolites pathway did not yield decisive results, I decided to take a step back and focus once again on *cyp79b2/b3* mutant itself, including *bak1/bkk1* in further experiments as well. With data accumulated so far, I could clearly show

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an important role of these two mutants in beneficial plant-microbe interactions during the vegetative growth stage. Nevertheless, I had no information about plant-microbe relationship during reproductive stage and how does *cyp79b2/b3* and *bak1/bkk1* mutation affects this relationship in terms of fitness proxy. I hypothesised that the interaction between an intact immune system and root-microbial community is indispensable for maintaining plants performance in reproductive stage. Firstly, after testing several setups, I optimized the FlowPot system for a long-term fitness proxy experiment, including sufficient space for plant's inflorescence (for details see Materials & Methods). This adjustment allowed me to grow both WT and mutant plants under sterile or gnotobiotic conditions from day 1 till the very end of the experiment, reaching reproductive stage.

I measured a wide range of phenotypic traits including rosette's and stem's FW and dry weight (DW), number of days until bolting, flowering and siliques formation, number of inflorescences stems and branching points, main stem length and chlorophyll content (Figure 12 and Supp. Figure 10). The most striking phenotype was that despite a high germination rate (Supp. Figure 10I), a 100% death rate of *cyp79b2/b3* plants in all treatments lacking B-community was observed at the end of the experiment. These survival results further strengthened my hypothesis that the relationship between *cyp79b2/b3* and fungal strains in their roots is crucial for plant fitness and survival. Additionally, FW and DW measurements of plant's rosette showed an increased susceptibility of *cyp79b2/b3* to the presence of F-community, that could not be fully rescued by the presence of B-community, as in WT (Figure 12A, Supp. Figure 10D). Rosette's water content showed a similar trend, with *cyp79b2/b3* rosettes tended to have a lower water content at 9 weeks of growth in treatments including F-community (Figure 12B). Surprisingly the same trend was not visible in stem, which FW, DW and water content were much less affected by either the genotype or treatment (Figure 12D and E and Supp. Figure 10E). WT phenotype of siliques number is in line with a generally accepted hypothesis that plants under stress falling within their tolerance levels will invest into seed production (Figure 12C) (Zandt et al., 2020). In this case stressful environment is most likely created by treatments lacking B-community. *bak1/bkk1* mutant showed signs of stress under any microbial treatment, especially visible in decrease of rosette's FW in comparison to sterile conditions (Supp. Figure 10D), but it did not lose the increase in siliques number in treatments lacking B-community in comparison to sterile conditions. *cyp79b2/b3* again

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showed the most striking phenotype with a lack of increase in siliques number despite a clear evidence of stress based on rosette's DW in e.g. BF treatment (Figure 12A), pointing to the conclusion that the stress associated with presence of microbes in this mutant prevents an increase in investment into siliques production. At the same time, I did not detect any substantial changes in the length of different growth phases (bolting, opening the first flower or setting the first silique) during reproductive stage (Supp. Figure 10A-C) or other plant parameters, like an average number of inflorescences stems per plant, branching point indicating the complexity of the inflorescence, the longest stem's length or germination rate (Supp. Figure 10F-I) due to plant's genotype or treatment. An additional interesting, although puzzling, observation was made based on chlorophyll content measurements (Chlorophyll Content Index, CCI) where the presence of filamentous microorganisms tends to lower the chlorophyll content in WT's leaves. In *bak1/bkk1* an overall chlorophyll content was lower than in WT in sterile and B-inoculated plants, but the decrease due to filamentous microorganisms was absent from this mutant. In *cyp79b2/b3* the WT-like decrease in chlorophyll content observed in the presence of filamentous microorganisms was present, but additionally just the presence of B-community tended to lower chlorophyll content of *cyp79b2/b3* leaves, indicating once again a general susceptibility of *cyp79b2/b3* and its lack of established beneficial plant-microbe interactions (Supp. Figure 10J). However, it has to be kept in mind that chlorophyll content measurements are based on very few datapoints (for details see Materials & Methods section on harvesting) and as such did not give a strong basis for statistical analysis.

As an additional test to confirm the hypothesis according to which the high susceptibility of *cyp79b2/b3* is directly caused by fungal presence, I grew *cyp79b2/b3* for 5 weeks (in order to evaluate vegetative stage of the plant), under the same microbial treatments as in the reproductive stage experiment. Obtained FW data confirmed observed phenotype and susceptibility of *cyp79b2/b3* to fungi, with 100% mortality in F and FO treatments (in O treatment only 4 plants out of 72 sowed seeds survived until harvesting date). In addition, a significant increase in *cyp79b2/b3* FW of B-treated plants in comparison to all treatments including F-community (BFO and BF) was also present at the vegetative stage, indicating that this result is robust across both vegetative and reproductive stages. Another important finding from the vegetative stage experiment was the increased susceptibility of *bak1/bkk1* to

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oomycetes. In reproductive stage experiment plant's DW (and FW) was highly decreased in O treatment (Figure 12A and D and Supp. Figure 10D and E) and I observed a similar trend in vegetative stage experiment, where in O treatment *bak1/bkk1* plants had a significantly lower FW than WT (Figure 13).

Effect of innate immunity and microbe-microbe interactions on total microbial abundance in plant's roots

Following the phenotypic trait analysis, I focused on determination of total microbial abundance in roots of flowering plants, in order to see whether the significant increase in total fungal abundance observed in roots of *cyp79b2/b3* mutant in vegetative stage is retained in reproductive stage. Indeed, under BFO treatment *cyp79b2/b3* maintains its high fungal abundance in roots in comparison to WT in reproductive stage (Figure 12G). Additionally, I show that total fungal and oomycetes abundance in WT and *bak1/bkk1* roots decreases significantly when B-community is added to the system (Figure 12G and H). Interestingly oomycetes abundance was also decreasing when F-community was added, regardless whether B-community was present or not (Figure 12H), overall indicating the importance of microbe-microbe interactions in modulating total microbial abundance. Further analysis of oomycetes abundance revealed a significantly higher oomycetes abundance in *bak1/bkk1* BFO treatment in comparison to WT BFO. Within *bak1/bkk1* mutant, oomycetes abundance in single O treatment was much higher in comparison to oomycetes abundance from any other treatment (Figure 12H), indicating a possibility that *bak1/bkk1* mutant has either an increased susceptibility to oomycetes and/or is unable to properly restrict their growth without the presence of other microbial groups in its roots. These results indicate that plant innate immunity has a role in modulating total microbial abundance in plant roots. Finally, an intriguing effect was observed for B-community, where the increase in overall community complexity caused an increase in total bacterial abundance (significant increase from BF to BFO in WT and from B to BFO in both tested mutants) (Figure 12F). Overall these analyses give more insight into total microbial abundance status in plant roots during plant's reproductive stage and highlight the importance of microbe-microbe interactions in plant roots.

Results

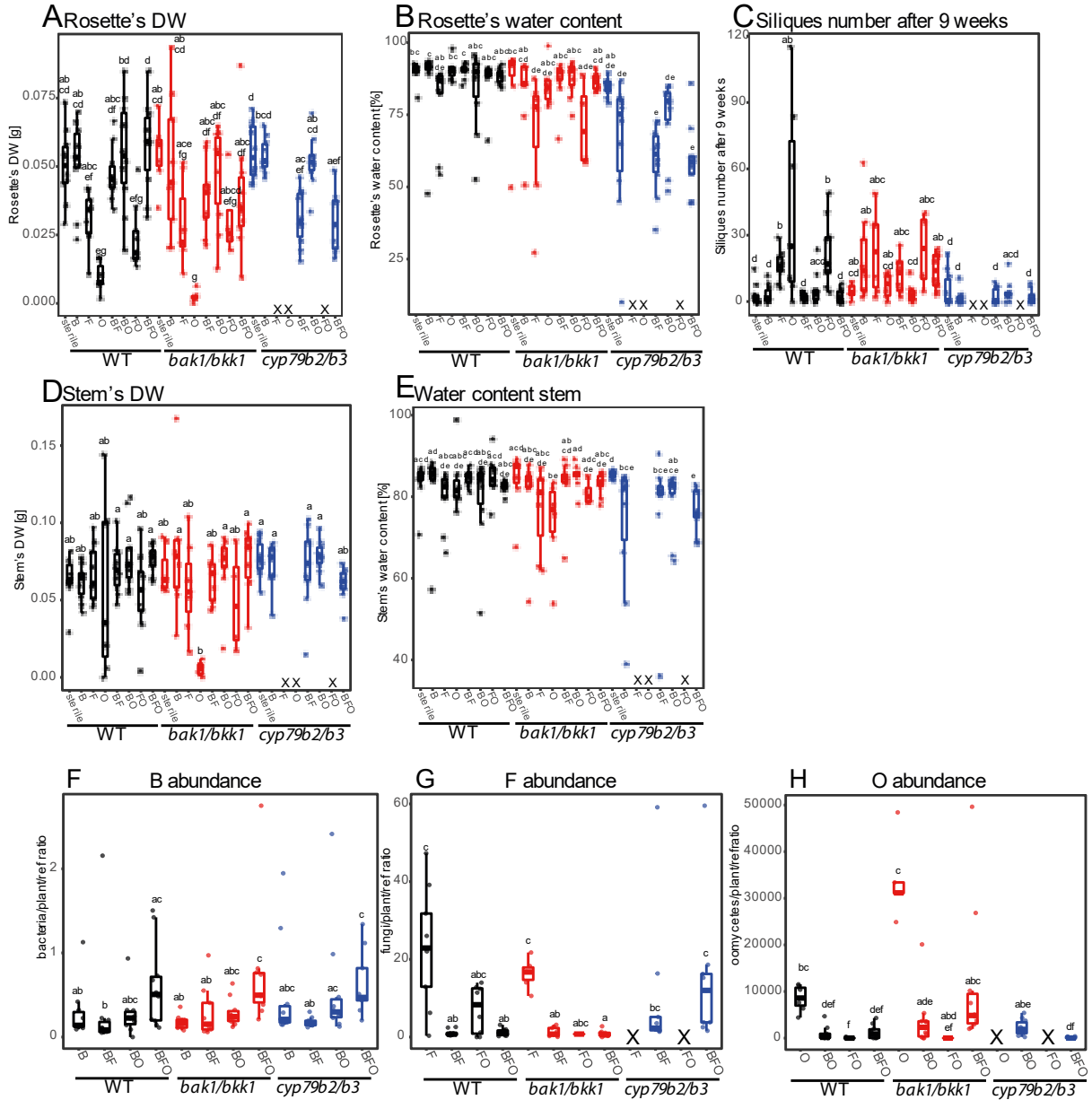


Figure 12: Presence of bacteria and an intact innate immune system is required for plants homeostasis and preservation of fitness traits.

Panels A-E show several phenotypic traits measured from 9-week old plants. Panels F-H show total bacterial (F), fungal (G) and oomycetes (H) abundance in the roots of 9-week old plants. Statistical analysis of rosette's DW (plant number: 0-10, median: 0.04395, mean: 0.04174086) (A) were done with ANOVA and Tukey post-hoc test ($p=0.05$). Statistical analysis for rosette's water content (plant number: 0-10, median: 86.73, mean: 80.96) (B), siliques number after 9 weeks (plant number: 0-10, median: 4.0, mean: 9.489) (C), stem's DW (plant number: 0-10, median: 0.0689, mean: 0.06692097) (D), stem's water content (plant number: 0-10, median: 83.67, mean: 81.22) (E), and total microbial abundance (plant number: 11-15 [B]; 0-15 [F]; 0-15 [O], median: 0.2296030 [B]; 1.5547 [F]; 369.50 [O], mean: 0.3747296 [B]; 6.3662 [F]; 4621.74 [O]) (F-H, respectively) were done using Kruskal-Wallis and Dunn test with Bonferroni-Hochberg correction ($p<0.05$). This figure will be a part of the manuscript in (un)changed form.

Results

Effect of genotype on root-associated microbial communities in reproductive stage

Microbial community analysis of root samples from vegetative stage FlowPot experiment showed only subtle microbial community shifts caused by plant's genotype. Nevertheless, based on previously published research (Durán et al., 2018), microbe-microbe interactions can have a substantial effect on microbial community composition. I hypothesised that mutations in plant innate immunity will magnify the microbe-microbe interaction effect on root community composition. I sequenced microbial community from root samples of 9-week old plants in the same manner as for vegetative stage FlowPot experiment and analysed an impact of genotype and treatment on the community composition. Due to very low number of high-quality reads from oomycetes, they were removed from any of the analysis. Plant's genotype x treatment interaction had a significant effect on B-community composition (7.903%, $p=0.002$, PERMANOVA) (Figure 14A-D, Table 4), but no substantial changes were observed for alpha-diversity (Supp. Figure 11). More detailed analysis of treatment effect revealed that microbial communities are more strongly affected in unplanted peat (30.4%, $p=0.002$ and 48.8%, $p=0.001$ for B- and F- community, respectively) than when associated with plant roots for either B- (13.9%, $p=0.002$; 13.9%, $p=0.001$ and 14.8%, $p=0.002$ for WT, *bak1/bkk1* and *cyp79b2/b3*, respectively) or F- (28.2%, $p=0.001$; 25.7%, $p=0.001$ and 3.38, $p=0.58$ for WT, *bak1/bkk1* and *cyp79b2/b3*, respectively) community (Supp. Figure 12A-H). Overall certain significant genotype x treatment effects are present in B-community, which is especially strongly affected by F- and O-treatments in both mutants, and to a lesser extent in WT (Supp. Table 3).

Based on PERMANOVA analysis, WT B-community shifts are significantly affected by the addition of F- and O-community (Supp. Table 5), presence of fungi also causes a treatment cluster separation in a graphical representation with Constrained Principal Component Analysis (CPCoA) (Supp. Figure 12B). In *bak1/bkk1* presence of oomycetes is an important factor and in *cyp79b2/b3* any filamentous community causes a shift in overall B-community composition (Supp. Table 5). On family level analysis, relative abundance of Flavobacteriaceae family is significantly affected by *bak1/bkk1* genotype in BFO treatment in comparison to WT, while relative abundance of Phyllobacteriaceae family is significantly

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affected by both *bak1/bkk1* and *cyp79b2/b3* mutations in B treatment in comparison to WT. Additionally a comparison of BO and BFO treatments in *cyp79b2/b3* yielded one bacterial family (Promicromonosporaceae), which relative abundance was significantly affected by the addition of F-community.

F-community in both WT and *bak1/bkk1* seem to be most strongly affected by the presence of B-community in the system (Supp. Figure 12E-H, Supp. Table 4 and 5) with relative abundance of 5 fungal families (Ascomycota, Hyponectriaceae, Nectriaceae, Plectosphaerellaceae and Pleosporaceae) being significantly affected in root samples by an addition of B-community, especially in WT roots (Figure 15 and Annex III). Due to 100% mortality of *cyp79b2/b3* plants in treatments lacking bacteria, similar analysis could not be performed for the said mutant. The only clear conclusion for F-community in *cyp79b2/b3* mutant is that it is not substantially affected by an addition of O-community in the presence of B-community, which is in line with the observed WT and *bak1/bkk1* phenotype.

Overall genotype effect within the treatments showed a stronger influence on microbial community composition than what I observed in vegetative stage experiment (Figure 8D-F, Supp. Figure 13), especially in case of B-community, where genotype effect in both vegetative and reproductive stage FlowPot experiment was significant when *cyp79b2/b3* and *bak1/bkk1* was compared to WT (overall genotype effect of 17.1%, $p=0.001$ and 2.65%, $p=0.01$ for reproductive and vegetative stage, respectively) (Figure 12H and Supp. Figure 13A). It is pointing to the conclusion that the importance of genotype in shaping root microbial communities increases in reproductive stage. Family level analysis strengthen this conclusion even further, as in reproductive stage FlowPot experiment, two bacterial families were affected by *bak1/bkk1* and one of them was also affected by *cyp79b2/b3* (Annex III), while in vegetative stage experiment only one family (Bradyrhizobiaceae) was affected by *cyp79b2/b3* and no significant effect of *bak1/bkk1* was detected (Annex II).

Results

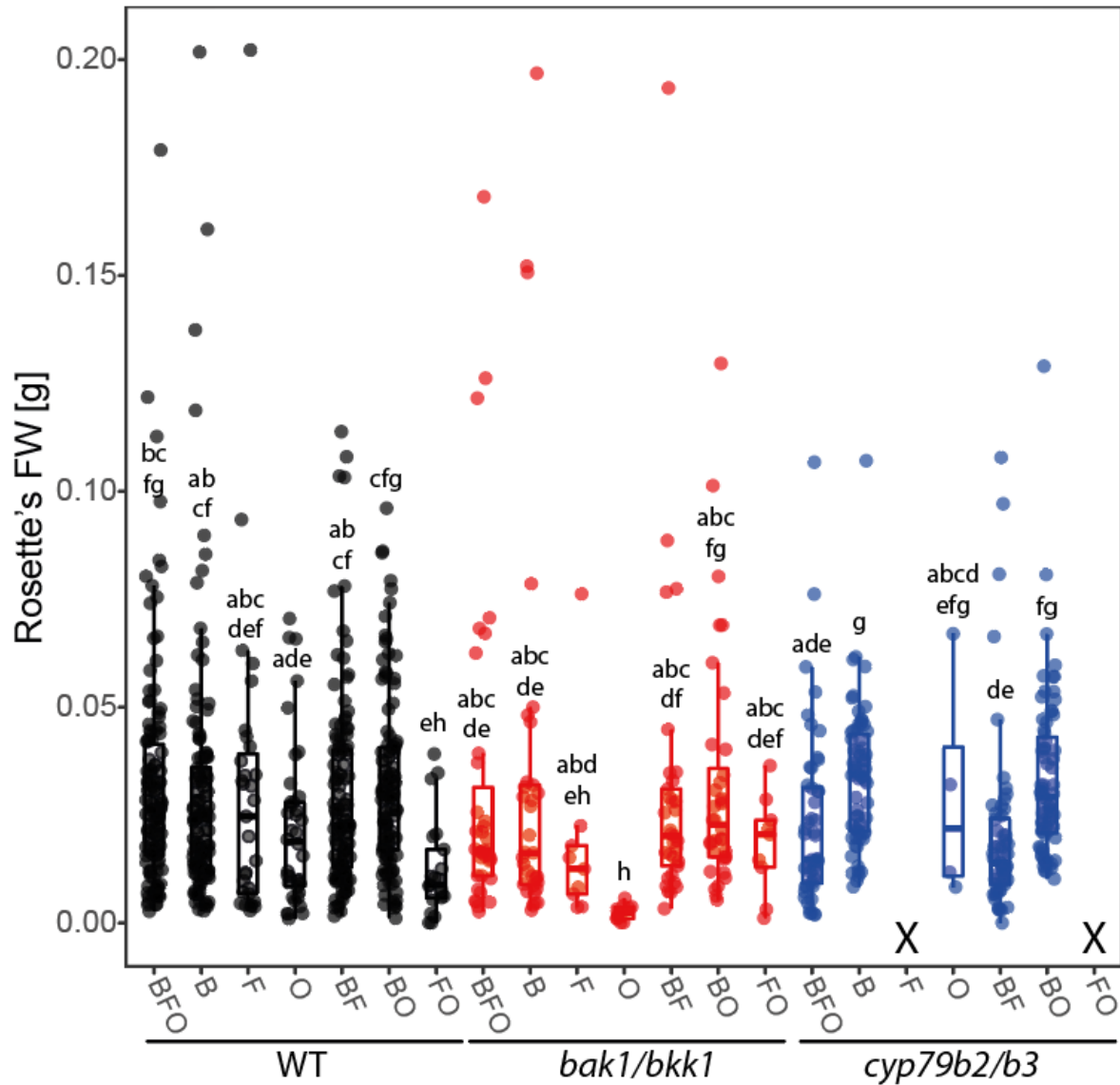


Figure 13: *cyp79b2/b3* displays a strong fungi-sensitive and *bak1/bkk1* strong oomycetes-sensitive FW phenotype.

FW data of 5-week old plants grown in vegetative stage FlowPot system, inoculated with varying microbial communities. B – bacterial, F – fungal, O – oomycetes synthetic community. Statistical analyses were done with Kruskal-Wallis and Dunn test with Bonferroni-Hochberg correction ($p < 0.05$). This figure will be a part of the manuscript in (un)changed form.

Table 4: Permutational analysis of variance (PERMANOVA) of genotype and treatment effects on root microbial community from reproductive stage FlowPot experiment.

Analysis done on Bray-Curtis distances with percentage of explained variance and P value with FDR correction.

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	Bacteria				Fungi			
	<i>df</i>	<i>F</i>	<i>R2</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>R2</i>	<i>P</i>
Genotype	2	4.0673	0.06263	0.001	2	4.2288	0.13275	0.004
Treatment	3	3.8261	0.08838	0.001	1	1.2659	0.01987	0.272
Genotype:Treatment	6	1.7108	0.07903	0.002	2	0.993	0.03117	0.4
Residuals	100	NA	0.76996	NA	52	NA	0.8162	NA
Total	111	NA	1	NA	57	NA	1	NA

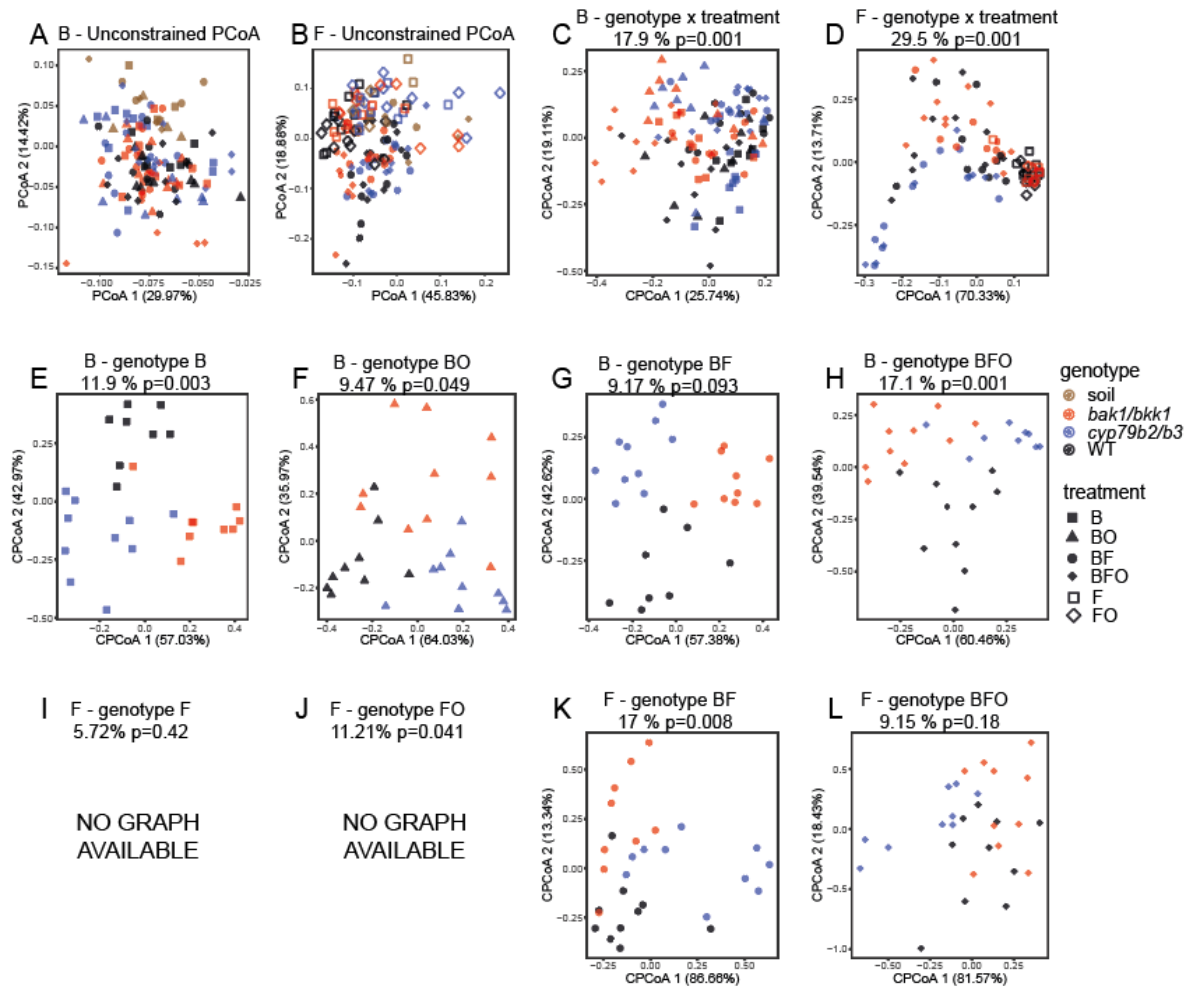


Figure 14: Root microbiome composition of 9-week old *A. thaliana* plants is affected by genotype and treatment.

Unconstrained (A and B) and Constrained (C-L) PCoA plots based on Bray-Curtis distances, constrained by genotype x treatment interaction for bacteria (C) and fungi (D) and constrained by genotype for bacterial community in B, BO, BF and BFO treatments (E-H respectively) and for fungal community in F, FO, BF and BFO treatments (I-L respectively). Colours indicate genotypes/peat samples and shapes indicate treatments. Panels I and J do not contain graphs due to too low number of treatment groups (*cyp79b2/b3* plants from F and FO treatments did not survive and percentage of variance was only calculated from the remaining two). This figure will be a part of the manuscript in (un)changed form.

Results

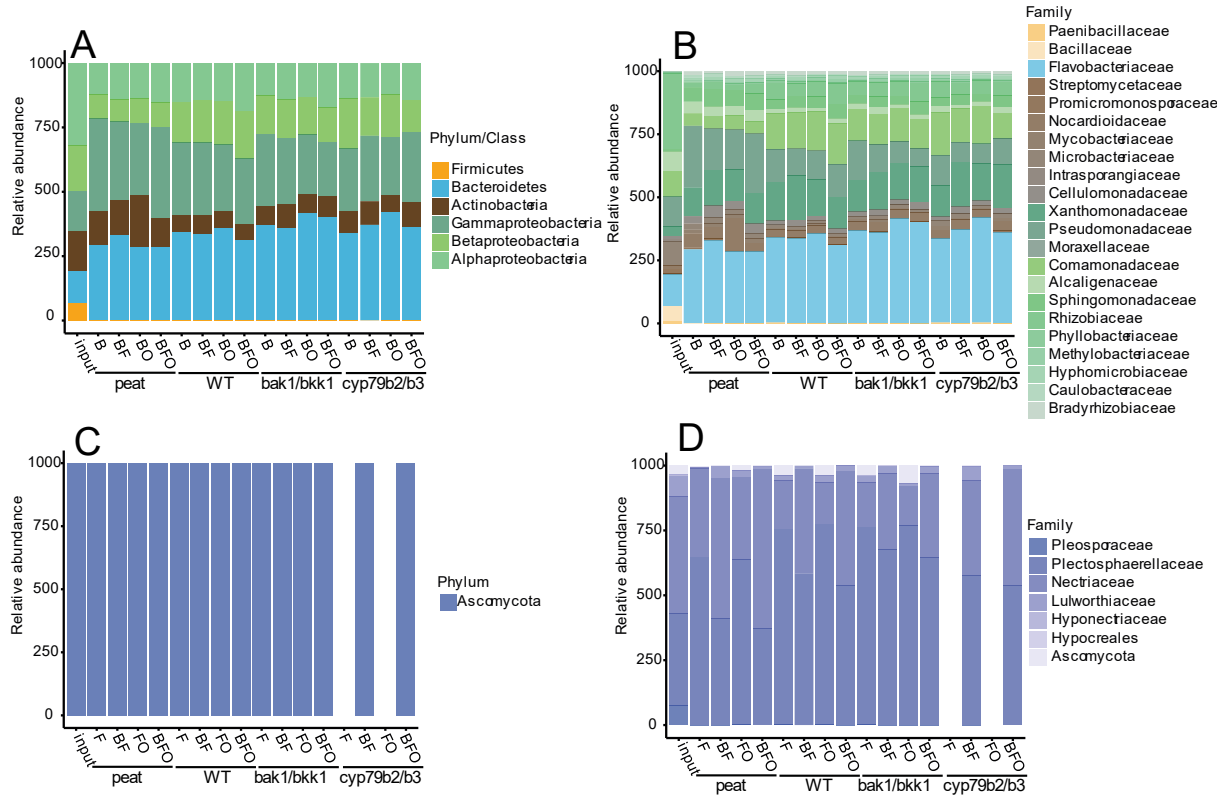


Figure 15: Relative abundance of microbial community reproductive stage FlowPot experiment on phylum/class (A and C for bacterial and fungal community, respectively) and family level (B and D for bacterial, and fungal community, respectively).

Colors indicate the taxonomical assignment, relative abundance in each sample was normalized to 1000 for between-sample comparison purposes. Statistical analyses were done with Kruskal-Wallis and Dunn test ($p < 0.05$) and the output of genotype/compartments/treatment effect can be found in Annex III.

Discussion

Arabidopsis thaliana growth dynamics in natural soil is altered in immunocompromised mutants

Plants grown in the greenhouse conditions in natural soil face a very complex environment and a wide range of light- and medium-stressors. The best example presented in this thesis would be a loss of two full biological replicates of *deps* mutant between week 5 and week 8. Further investigation indicated the insect feeding on plant rosettes was most likely the reason behind it, as leftover stems and leaves residues carried insect bite marks (personal observation). This observation is supported by generally known susceptibility of JA and SA mutants to insect feeding (Thaler et al., 2012). Despite several limitations arising from working with immunocompromised mutants in natural soil, I could nonetheless observe that the growth of *A. thaliana* mutants is time dependent, with some mutants growing at a faster (*wrky33*) or slower (*bak1/bkk1*) rate than WT (Figure 2). This result highlights the importance of analysing several subsequent time-points before drawing conclusions whether a given mutation(s) affect(s) plant's growth and/or biomass in comparison to WT. As shown in this experiment, after 5 weeks of growth *bak1/bkk1/cerk1*, *apex3* and *wrky40* had all a significantly higher rosette's FW than WT, however after additional 3 weeks of growth their increase in rosette's FW was slower than the one of WT, resulting in WT-like phenotype at week 8. The exact opposite trend was found for *cyp79b2/b3*, *35SBRI1* and *rar1* mutant, which had a significantly lower rosette's FW at week 5 but reached WT-like level at week 8 through an increase in their growth rate. Similar growth rate fluctuations were described in detail in Tessmer et al., (2013), where the authors evaluated new phenotyping imaging system by comparing growth rates of WT and ATPase family protein knock-out mutant. Both show the same growth rate under constant light, but light fluctuations can significantly change the growth rate between WT and the mutant, indicating different mutations can affect plant's ability to grow and cause time-dependent growth retardations.

Genotype influence is difficult to assess in greenhouse settings

Another important finding from the natural soil experiment, is that the genotype's influence on plants phenotype and root microbiota is at least partially masked by an abundance of different

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stressors. Despite finding several mutants which displayed a difference in rosette's FW in comparison to WT, based on natural soil results alone it is impossible to conclude whether the observed phenotype comes from the knock-out mutation or its interaction with environment. Fortunately, I was able to confirm that vast majority of these mutants do not exhibit differential growth in comparison to WT in sterile conditions, with the exception of *lyk5*, *apex1* and *pad4*, whose rosette's FW was even significantly higher than the one of WT (Supp. Figure 1A). Considering the phenotype from axenic conditions, it is very likely that the phenotype observed in natural soil of all other mutants that were tested in sterile conditions is due to specific genotype x environment interactions. The reduced growth of the mutants *bak1/bkk1*, 35SBRI, *rar1*, *deps* and *cyp79b2/b3* compared to WT observed in natural soil is potentially linked to a change in pathogen pressure, as plants with weakened immune system would be more susceptible than WT plants and could repeatedly enter growth retardation phases due to increased (a)biotic stress pressure over their growth period (Albrecht & Argueso, 2016).

Nevertheless, it was unexpected to see that overall none of the knock-out mutants displayed a drastically changed root microbiota composition when grown in natural soil. Although all the mutants that displayed an impaired growth in comparison to WT in at least one time point (*bak1/bkk1* at both time points, 35SBRI, *rar1* and *cyp79b2/b3* at week 5 and *deps* at week 8) did at the same time display a significant shift in microbial community composition, the shifts mostly do not overlap well with their FW-phenotype. For example, *bak1/bkk1* showed growth impairment at both time points, while B- (week 8) and O- (week 5) community shifts were significant at only one time point. 35SBRI displayed growth impairment at week 5 but the only significant B-community shift was observed in week 8. *rar1* harboured significantly different BFO community in comparison to WT at week 5, and only B-community at week 8. *cyp79b2/b3* harboured significantly different B-community at both time points, but displayed an impaired phenotype only at week 5. *deps* mutant showed growth impairment only at week 8, while both B- and F-community was significantly different from WT at both time points. In summary these results indicate that community shifts due to dysfunctional innate immunity pathways in plant roots grown in natural soil are a poor indicator of aboveground vegetative growth. These findings are not fully consistent with earlier findings (Lebeis et al., 2015), where authors found a significant link between SA pathway and bacterial root community shifts. However, it has to be kept in mind that, first of all, different natural soils were used which can

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harbour strikingly different microbial communities (Durán et al., 2018), and second of all, in my research I have not focused on one innate immunity pathway, which lowers the resolution of observed changes, especially when the described microbial community shifts are relatively subtle (Lebeis et al., 2015).

Most of the microbiome research done in recent years was based on 16S rRNA and ITS1 sequencing (Lundberg et al., 2012; Bodenhausen et al., 2014; Lebeis et al., 2015; Wagner et al., 2016). However, amplicon sequencing may not be the best method to provide sufficient resolution needed to identify strain-level changes present in complex, natural soil microbial communities. Many aspects and questions of microbiota research and the importance of single strain-level changes still remain unknown and unanswered (Berg et al., 2020) and therefore it remains possible that a potential interplay between innate immunity and the root microbiota at strain level resolution was overlooked in the research presented here.

With my results of a wide screen of immunocompromised mutants I propose the hypothesis that plants' innate immune system is highly robust and is able to compensate for different perturbations, lowering the impact of mutations on root microbial community structure. In both experimental systems (greenhouse and FlowPot gnotobiotic system) I observed subtle shifts in microbial community composition, even when drastic changes in plant's phenotype were observed. This suggests that the plant is able to compensate for dysfunctional pathways in order to efficiently maintain host-microbial homeostasis in plant roots. This hypothesis is in line with a hypothesis describing high level of robustness, redundancy and additive effects present within plant's innate immunity network (Tsuda et al., 2009; Roux et al., 2014; Li et al., 2016). An example would be a redundancy between CYP79B2 and CYP79B3 or CYP71A12 and CYP71A13 in tryptophan metabolism and camalexin biosynthesis (Buxdorf et al., 2013) or a high functional redundancy between various WRKY transcription factors (Bakshi & Oelmüller, 2014).

The data suggest that perturbations in different immune sectors only mildly alter microbial community composition and absolute microbial abundance in plant roots under greenhouse conditions. This observation does not exclude the possibility that environmental noise resulting from greenhouse experiments with natural soils obscured the subtle interplay between innate immunity and the root microbiota. Therefore, experiments using gnotobiotic systems with

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germ-free plants and synthetic microbial communities are necessary to more precisely dissect the relevance of the host innate immunity in maintaining host-microbial homeostasis and shaping beneficial plant-microbe interactions.

Plant innate immunity has a key role in mediating microbe-mediated plant growth promotion

By screening several immunocompromised plants in the gnotobiotic FlowPot system, I observed that 9 out of 14 mutants showed a significant decrease in microbe-mediated growth promotion effect in comparison to WT (Figure 7B). The relevance of the plant immune system for microbe-mediated beneficial outcome on plant growth was already shown for individual members of the root microbiota in mono-association with the host (Lahrmann et al., 2015; Hiruma et al., 2016). The results obtained here suggest that, in a community context, the immune system of plant is also required for root microbiota-mediated plant growth promotion. Therefore, the interplay between the microbiota and the host immune system is likely a key factor to maintain homeostatic host-microbial interactions (Hacquard et al., 2017; Vannier et al., 2019). So far majority of studies focused on the role of plant innate immunity in conferring beneficial effects of microbiota under (a)biotic stress conditions (Hiruma et al., 2016; Castrillo et al., 2017; Berendsen et al., 2018), but here I show that innate immunity is also essential for plant growth promotion in absence of (a)biotic stressors. Out of all tested mutants *cyp79b2/b3*, *pad4*, *bak1/bkk1*, *bak1/bkk1/cerk1*, *wrky33/40* and *wrky33* displayed most striking phenotypes, indicating the importance of glucosinolates and/or their hydrolysis products (Rask et al., 2000; Schlaeppi et al., 2010; Sotelo et al., 2015), camalexins (Koprivova et al., 2019), (co-)receptor layer (H. Wang et al., 2019; C. Wang et al., 2019) and WRKY-mediated basal defence (Pandey & Roccaro, 2010; Birkenbihl et al., 2012, 2017) in plants interaction with root microbiota. Out of all the mutants of interest, one mutant (namely *cyp79b2/b3*) displayed a striking phenotype, where an addition of a SynCom not only did not benefit plant's growth, but actually turned detrimental. This phenotype is in line with a well-known overall susceptibility of *cyp79b2/b3* (Schlaeppi et al., 2010; Prince et al., 2017), especially to fungal pathogens (Sanchez-Vallet et al., 2010; Buxdorf et al., 2013), but also to beneficial root endophytes (Lahrmann et al., 2015; Hiruma et al., 2016).

Lack of microbe-mediated growth promotion effect is partly explained by fungal absolute abundance in plants roots

After discovering the striking negative effect of the microbial SynCom on *cyp79b2/b3* (but also other immunocompromised mutants) in FlowPot system, I decided to monitor microbial community composition in root samples. Based on numerous studies, microbial community changes are very likely coupled with changes in plants phenotype and metabolism (Lebeis et al., 2015; Hacquard et al., 2017; Durán et al., 2018; Berens et al., 2019). Overall genotype effect on root microbial community composition varied between 5.89% [p=0.001], 7.62% [p=0.001] and 7.75% [p=0.145] for B-, F- and O-community respectively (Figure 8D-F). However, consistent with data obtained from natural soil experiment, only few genotypes harboured a significantly different microbial community than WT control plants (Table 2). Furthermore, the shifts in microbial community composition did not correlate with lack of microbiota-induced growth promotion phenotype (Supp. Figure 8). I took advantage of the fact that gnotobiotic system was inoculated with clearly defined SynCom and performed additional analyses at OTU-level resolution (Supp. Figure 9, Supp. Table 2) in order to test whether the abundance shifts, although subtle or absent on whole-community level (Figure 8D-F), are present on single OTU level. By inspecting change in relative abundance for different strain variants used in SynCom, I identified genotype-specific difference in the enrichment profiles in respect to WT. Some genotypes harboured several bacterial OTUs whose relative abundance was significantly different from WT (*rar1* [18 OTUs], *pad4* [12 OTUs], or *wrky33/40* [11 OTUs]) and some that harboured only few (*bak1/bkk1/cerk1* [1 OTU], 35SBRI1 [3 OTUs] or *lyk5* [5 OTUs]). Fungal and oomycetes OTUs showed similar variation however, these distinct enrichment signatures measured across mutants are largely inconsistent with aboveground FW phenotypes.

A similar picture was seen in natural soil experiment, where overall genotype effect varied between 4.547% [p=0.001], 3.393% [p=0.314] and 4.39% [p=0.006] for B-, F- and O-community, respectively (Table 2) and, similarly to a FlowPot experiment B-community was more strongly affected by the genotype than F- and O-community and only few genotypes harboured a significantly different microbial community (Supp. Table 1). *cyp79b2/b3*, *apex1*, *apex3* and *bri301* had a consistent significantly different B-community in FlowPot and natural

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soil (in at least one time point) and *wrky33* had a consistently significantly different O-community. F-community did not show any overlap in significantly different genotypes between the two experiments (Figure 4 and 8D-F). ASV-level analysis yielded similar results as OTU-level resolution analysis in vegetative stage FlowPot experiment, with several ASVs/OTUs being affected, but without clear-cut clustering based on phenotypic data (visual comparison) (Supp. Figure 3-5 and 9). Despite lack of substantial changes in root microbiome community composition in vegetative stage FlowPot experiment (Figure 8D-F), I detected a strong variation in total fungal abundance (Figure 9B), correlating with observed lack of microbe-mediated growth promotion effect (Figure 9E). I additionally analysed the correlation between growth promotion effect and bacterial and oomycetes abundance (Figure 9D and F), but I did not detect any significant correlation, strengthening the argument that not the composition, but total abundance of F-community appears to be a key determining factor driving lack of microbe-mediated growth promotion. This result would be in line with an existing hypothesis that even beneficial microbes can become detrimental when they colonized immunocompromised plants, resulting in extensive colonization that negatively affect plant performance (Hiruma et al., 2016). Therefore, maintenance of fungal balance by the host immune system is likely key for promoting plant health in nature. It is important to note that total fungal abundance was poorly influenced by the mutation in different immune sectors in the greenhouse experiment, suggesting that other factors than the immune system can restrict total fungal abundance in natural soils. In case of fungal organisms, *cyp79b2/b3* mutant is exceptionally susceptible (Lahrmann et al., 2015; Hiruma et al., 2016) and additional previous knowledge of *cyp79b2/b3* being overgrown by single inoculated fungi (Bednarek et al., 2009) strengthens the possibility of F-community abundance being the key driver of the phenotype observed in this study.

Increased total fungal abundance in *cyp79b2/b3* is stable during plant's life cycle

Furthermore, when WT and *cyp79b2/b3* were grown until a reproductive stage, an increase in fungal abundance in *cyp79b2/b3* was retained, indicating a life-long dysbiosis of fungi in *A. thaliana* roots (Figure 12G and H). Coupled with an observed negative effect of O- and F-community on both mutants' phenotypic traits, it supports the importance of innate immunity-

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mediated plant interactions with their commensal root microbiota (Hiruma et al., 2016; Vannier et al., 2019), especially in restricting an uncontrolled growth of e.g. filamentous fungi (Bednarek et al., 2009). Glucosinolates and their hydrolysis products (GSHPs) have a strong record of their importance in plant-fungal interactions, e.g. PEN2 and its role in restricting non-adapted fungal pathogen entry in leaves (Hiruma et al., 2010) or the role of GSHPs in plants resistance to *Botrytis cinerea* (Buxdorf et al., 2013).

Additionally, an interesting observation was made that bacterial abundance was consistently increasing with an increase in overall microbial community complexity (Figure 12F). There are two hypotheses that could explain this observation. First, when filamentous eukaryotes are present, the hyphae are able to penetrate the roots more efficiently than bacteria themselves, similarly to ecto- and endomycorrhizal fungi (Luginbuehl & Oldroyd, 2017), facilitating bacterial entry in plant roots through fungus-induced physical damage in epidermal root cells (Jambon et al., 2018) and/or by creating a route for mobile bacteria that can move along their hyphae (Bielčik et al., 2019). Alternatively, presence of fungi and oomycetes (and consequently their exudates or dead hyphae elements) creates additional nutrient source for saprotrophic bacteria (Rudnick et al., 2015), in consequence allowing a higher growth in comparison to single bacterial community.

Fungal abundance in *A. thaliana* roots is controlled by Trp-derived secondary metabolites and bacterial commensals

In order to further test whether fungi are a key factor responsible for the lack of BFO-mediated plant growth promotion in the *cyp79b2/b3* mutant, I tested whether the negative effect was observed in the absence of fungal community in the SynCom. Based on 5-week phenotypic screen in FlowPot system I showed that plants from treatments harboring B- but lacking F-community do have a significantly higher rosette's FW than in treatments where F-community is present, regardless of the presence of B-community (Figure 13). These results validate that the presence of the F-community is driving the lack of microbiota-mediated plant growth promotion phenotype in the *cyp79b2/b3*. The observation that bacterial commensals are needed to control fungal abundance in roots is consistent with previous work (Durán et al., 2018), where the authors observed that fungal alpha and beta diversity was significantly altered in the presence of bacteria in the system. Although I did not detect the same phenotype based on

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alpha diversity indices (small, yet not significant effect of the presence of bacteria on observed fungal OTUs (Supp. Figure 11D)), I did find that total fungal abundance and F-community composition in roots were both significantly altered by the presence of bacteria in the system (Figure 12G). These results corroborate the initial hypothesis according to which bacterial commensals have a key regulatory role in controlling fungal root population, thereby promoting plant survival (Carrión et al., 2019).

Restriction of fungal abundance by bacterial commensals is greater than that mediated by Trp-derived secondary metabolites in *A. thaliana* roots, as in the absence of B-community, a severe negative impact on plant's growth was observed in the WT (Figure 12A and 13), which is in line with previously published research on protective function of B-community against fungal root pathogen (Carrión et al., 2019) and F-community (Durán et al., 2018). These results suggest that fungal colonization of *A. thaliana* roots is tightly controlled by the combined action of bacterial commensals and the Trp-derived secondary metabolites, with the latter being insufficient to fully protect plants from root-associated fungi in the absence of bacterial root commensals. The results further suggest that the maintenance of fungal absolute abundance in *A. thaliana* roots by plant- and bacterial- encoded mechanisms is a key factor needed for plant growth in nature.

Interestingly I observed a trend for oomycetes total abundance to be reduced not only by the presence of B-community, but also by the presence of F-community (Figure 10H). It would be interesting to know whether this observation is related to an important microbe-microbe interaction among filamentous eukaryotes or whether it is due to difference in number of strains between oomycetes and fungi (5 and 25 respectively), as the opposite observation (i.e., decreased fungal abundance due to the presence of oomycetes) was not significant.

Genotype influence on community structure strengthens in reproductive stage

An additional observation was that the influence of host's genotype appeared to increase in importance for community composition determination in comparison to the vegetative stage (Figure 14 and Supp. Figure 13). A somewhat similar trend was found by Edwards et al. (2018) where the authors investigated a field grown rice varieties and the changes in root microbiota

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over the course of plants life cycle. First of all, they found that roots of early and late rice development stages (divided by vegetative and reproductive stage) were dominated by early- and late-colonizers respectively (Edwards et al., 2018). In light of their findings it is not surprising that in reproductive stage FlowPot experiment presented here, at reproductive stage root-associated microbiota is more strongly influenced by host genotype than in vegetative stage. Another argument that is in line with this finding is the fact that in natural soil experiment the time point effect had a clearer effect on endophytic root fraction than bulk soil (especially in case of B-community), indicating that plants development stage and not time per se is the main cause of microbiota shift over time (Figure 3A-C). PERMANOVA analysis of natural soil experiment also support this hypothesis, as the influence of time point factor in dataset with and without soil increased for all three microbial groups (from 5.536%, $p=0.001$ to 7.58%, $p=0.001$ for B-community, from 3.121%, $p=0.001$ to 3.929%, $p=0.001$ for F-community and from 2.341%, $p=0.001$ to 3.655%, $p=0.001$ for O-community) (Table 1). Whether the underlying cause is the same as in rice, related to early- and late-colonizers remains an open question. It also has to be kept in mind that in this report the comparison of genotype effect in vegetative and reproductive stage FlowPot experiment is being done based on two different systems, which lowers the strength of the abovementioned conclusions.

Relationship between microbiota and flowering time is ambiguous

One main discrepancy between the findings of Edwards et al., (2018) and my findings is the fact that I did not observe a simultaneous delay in neither bolting, flowering nor siliques setting time in mutants in comparison to WT, with only one exception of *bak1/bkk1* showing a delayed bolting under O treatment (Supp. Figure 11A-C). Authors use this development-delayed phenotype as the main possible cause (or effect) of changed microbiota giving an example of drought-stricken plants having a delayed flowering time and harbouring microbiota that resembles more the microbial community associated with younger plants. Based on the previously published research and my current results, one hypothesis would be that an increase in host genotype effect on late-colonizers (and so the microbiota from later time points) is linked but not fully intertwined with a possible delay in flowering time. This hypothesis would be more in line with another previously published research of Dombrowski et al., (2017), where a comparison between wild type and flowering mutant of *Arabidopsis alpine* did not yield

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significantly different results in root-associated microbiota. Nevertheless, the subject of microbiota and flowering time is a complex matter, as yet another study showed a clear effect of microbial community structure on flowering time of *A. thaliana* (Panke-Buisse et al., 2015). Additionally, what has to be kept in mind is that the experimental setup that I have used to evaluate fitness proxy of *A. thaliana* is relatively artificial, which may not best reflect the true plant-microbiota interaction during plants reproductive stage in natural soil. The topic of microbiota effect on flowering time is still a very complicated aspect, especially since shift from vegetative to reproductive stage involves major developmental and transcriptional changes within the plant (Poethig, 2013), allowing for complex plant-microbiota interactions that may or may not affect flowering time.

Detrimental effect of microbiota in *cyp79b2/b3* and *bak1/bkk1* is robust at both vegetative and reproductive stage

After a detrimental effect of BFO-community on 5-week old rosette's FW of *cyp79b2/b3* was observed, I performed more detailed experiments on both vegetative and reproductive stage, in order to determine the effect of single-group microbial communities on *cyp79b2/b3* growth and whether the detrimental effect is caused by all microbiota members or only F-community. Results of a vegetative stage experiment with single microbial treatment group showed that *cyp79b2/b3*'s FW is significantly increasing when filamentous eukaryotes are not present in the system (Figure 13), but at the same time a significant difference in FW between BF and BO treatment indicates that fungi and not filamentous eukaryotes overall are the driver behind the observed phenotype. This observation is in line with the known fungal susceptibility of *cyp79b2/b3* (Buxdorf et al., 2013).

On the other hand, *bak1/bkk1*, showed lower susceptibility to fungi (higher survival rate) in comparison to *cyp79b2/b3*, but stronger susceptibility to oomycetes in comparison to WT. *bak1/bkk1* displayed lack of increase in siliques production in O treatment, which was observed in WT, indicating O treatment is posing too high stress on *bak1/bkk1*. Low siliques production was coupled with a strong stem growth retardation (Figure 12D) and a significant rosette's FW reduction in vegetative stage experiment in O treatment in comparison to WT (Figure 13). Additionally, in vegetative stage FlowPot experiment (initial mutant screen), absolute abundance of all three microbial groups was increased (trend) and in reproductive stage in

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BFO-treatment, oomycetes abundance was significantly higher in *bak1/bkk1* than in WT, indicating once more that *bak1/bkk1* mutations cause a disbalance in plant-oomycetes interactions.

Taken together, these results suggest that while *cyp79b2/b3* mutation is a key factor in mediating beneficial/neutral plant-fungal interactions, *bak1/bkk1* is possibly more important in plant-oomycetes interactions. Previous research showed an involvement of BAK1 in oomycetes recognition, strengthening this hypothesis (Raaymakers & Van den Ackerveken, 2016). Additionally, although both *cyp79b2/b3* and *bak1/bkk1* both share lack of microbiota-mediated plant growth promotion effect, there is a strong possibility that the fundamental mechanisms behind both phenotypes are different.

In reproductive stage I observed a consistent detrimental effect of F and O communities in both tested mutants on rosette's water content (Figure 12B) and consequently their FW (Supp. Figure 10D), with lesser, but still present similar effect on stem (Figure 12D and Supp. Figure 10E). Siliques count revealed that *bak1/bkk1* mutant, despite showing an elevated susceptibility to the presence of F- and O-community in the system during vegetative and reproductive stage, was still able to cope with the stress well enough to have a silique production level comparable with WT (with an exception of O treatment) (Figure 12C). In both, WT and *bak1/bkk1* F, and FO treatments induced a higher siliques production (O treatment induced higher siliques production only in WT), in line with a generally accepted theory of plants directing significant amounts of resources into seed production when faced with stresses within their tolerance limits (Zandt et al., 2020). What is interesting is that this phenomenon was not observed for *cyp79b2/b3* where, first of all, all F-, O- and FO-treated plants did not survive (one could call it the most severe fitness penalty score) and despite a clear evidence of higher stress levels in BF and BO treatments in comparison to B treatment alone (based on 5-week old plants FW as well as phenotypic read-outs from 9-week old plants), BF and BO treatments did not yield an increased number of siliques. One hypothesis is that *cyp79b2/b3* plants are already under such high stress levels in these treatments, that it reaches the tipping point and plants are unable to rescue themselves (by ensuring the survival of progeny) by increasing siliques/seed production (Klatt et al., 2018; Sehgal et al., 2018; Zandt et al., 2020). Alternative hypothesis would be that knocking-out *CYP79B2* and *CYP79B3* genes

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has an unexpected effect on the increase in siliques production when faced with high (but tolerable) level of stress. This hypothesis would be partially supported by the fact that AOP2 and AOP3 (ALKENYL HYDROXALKYL PRODUCING 2 and 3), both belonging to methionine-derived aliphatic glucosinolates biosynthesis pathways, were found to alter *A. thaliana*'s flowering time (Jensen et al., 2015). It suggests a possible link between glucosinolates and flowering process. Another phenotypic trait that can be used as fitness proxy was chlorophyll content (CCI). Unfortunately, the experimental setup from this thesis does not allow to draw conclusions from CCI measurements due to too low replicate number.

Glucosinolates biosynthesis pathway is not the main driver behind *cyp79b2/b3* phenotype

Based on my first screening results and known roles of glucosinolates (especially camalexins and their hydrolysis products) in plant-pathogen (Rask et al., 2000; Schlaeppi et al., 2010; Buxdorf et al., 2013; Velasco et al., 2013; Sotelo et al., 2015; Prince et al., 2017) and plant-microbe interactions (Siebers et al., 2018), I hypothesised that glucosinolates and their hydrolysis products are responsible for a striking phenotype of *cyp79b2/b3*. In order to narrow down the list of responsible Trp-derived indole glucosinolates I performed an additional screen with a wide range of glucosinolates knockout mutants. However, neither their decrease in microbe-mediated growth promotion effect (Figure 11A) nor total microbial abundance (Figure 11B-D) provided clear evidence that lack of indole glucosinolates can recapitulate the *cyp79b2/b3*-phenotype. This result is however not completely surprising as it was already suggested in other studies that glucosinolates pathway does not always fully explain *cyp79b2/b3* phenotype, indicating an involvement of other, yet unknown pathways downstream of *CYP79B2* and *CYP79B3* genes (Sanchez-Vallet et al., 2010; Hiruma et al., 2013). However, an interesting observation is the possible involvement of Trp-derived glucosinolates pathway in controlling fungal proliferation in plant roots, while to some extent maintaining microbiota-mediated growth promotion effect. This conclusion comes from the results obtained for a *quadruple* mutant (*myb34/myb51/myb122 /cyp71a13*, previously described as a *quintuple* mutant, see Materials & Methods), where total fungal abundance and effect on plant performance appeared to uncoupled since microbiota-induced growth promotion is retained, despite significantly higher total fungal abundance. The main difference

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between *cyp79b2/b3* and *quadruple* mutant is the functionality of *CYP71A12* gene, which has two main known roles in glucosinolates biosynthesis pathway. First of all, it is partially redundant with *CYP71A13* in converting IAOx (indole-3-acetaldoxime) to IAN (indole-3-acetonitrile) in the camalexin biosynthesis pathway (Koprivova et al., 2019) and secondly it converts IAOx into indole cyanohydrin that is further converted by FOX1 (FAD-LINKED OXIDOREDUCTASE 1) and *CYP82C2* into 4-OH-ICN (4-hydroxy indole-3-carbonyl nitrile) and its hydrolysis product 4-OH-ICA (4-hydroxy indole-3-carboxylic acid) (Figure 1B), (Rajniak et al., 2015). Its second function does sound more promising as a candidate pathway for fungal abundance regulation in roots, especially because in a recent study authors found that *CYP71A12* has an important role in restricting fungal hyphae growth in *A. thaliana* leaves (Pastorczyk et al., 2019).

Future perspectives

Ways of key pathway identification - RNAseq

As the glucosinolates mutant screen indicated that the indole glucosinolate pathway is largely dispensable for microbiota-mediated growth promotion phenotype, I decided to take a step back and focus once again on *cyp79b2/b3* itself. I decided to perform a RNAseq experiment on WT and *cyp79b2/b3* with a goal of identification of plant innate immunity pathways, differentially affected in a mutant by the presence of fungi. In order to do that, I am planning to grow *cyp79b2/b3* and WT plants in 5-week FlowPot settings, focusing on sterile, B and BF treatments. Although ideal comparison would be sterile plants vs F-inoculated ones, due to high WT mortality and 100% mortality of *cyp79b2/b3* in F treatment, I compromised and decided to focus on B and BF treatment comparison. Unfortunately, due to unforeseen delays (failed first two attempts at this experiment, Covid-19 pandemic) this key experiment was delayed and was not completed before the submission of this thesis. Nevertheless, I would like to shortly discuss the current working hypothesis behind this experiment. So far, all my results led to conclusion, that total fungal abundance inside and in the direct surrounding of the roots is a crucial factor linked to beneficial microbe-mediated growth promotion phenotype. Results obtained with *cyp79b2/b3* mutant convinced me that Trp-derived secondary metabolites are an important player in this relationship. With that in mind I performed another FlowPot screen, focusing on various Trp-derived glucosinolates mutants, in an attempt to narrow down the list of possible pathways/chemical compounds responsible for the observed phenotype. Nevertheless, I did not manage to identify any mutant that could explain observed *cyp79b2/b3* phenotype and help narrow down the possible list of chemical compounds. With that in mind the only remaining hypothesis is that there are either further elements within glucosinolates biosynthesis pathway that are yet unknown, or both *CYP79B2* and *CYP79B3* genes have an unknown influence on other, glucosinolates-unrelated pathways. I aim to identify one or more candidate pathways based on transcriptome comparison between B and BF treatments for WT and *cyp79b2/b3* mutants, with the idea that so far presence of F-community seemed to be of crucial importance for the observed phenotype. Additionally, a comparison between sterile and B-inoculated plants would also be done, in order to see if there are any key pathways responding to the presence of B-community in WT that are not activated in *cyp79b2/b3*.

Future perspectives

Identification of such pathways would help create a list of candidate genes and allow a further disentanglement of microbiota-mediated plant growth promotion effect in *A. thaliana* under control conditions.

Does testing one SynCom allows for generalization?

Second important aspect that have not yet been investigated in this thesis, is whether the observed phenotype, especially detrimental effect of F-community, is a common phenotype for all available commensal fungal strains, or whether it is a phenomenon limited to the above-described community of 25 fungi. At the moment my current hypothesis is that the observed phenotype can be extrapolated on other, yet-untested fungal commensal strains. I am planning to perform additional experiments in which I will test one or more different combinations of F-community and determine whether they also do have a detrimental effect on WT's growth and additional, stronger detrimental effect on *cyp79b2/b3* mutant. Based on the results obtained by [Durán et al., \(2018\)](#) with their experiments on single fungal strains and their effect on plant's FW, it stands to reason to assume that as long as one of the individual fungal strains shows a detrimental phenotype, there is a high chance the full SynCom will as well. With that in mind, one strategy would be to compose a SynCom using only beneficial and neutral fungal strains and see whether they remain beneficial/neutral or whether they turn detrimental as a community. Unfortunately, up to date there is not enough data on individual fungi's effect on *A. thaliana* performance in FlowPot system for all strains in the fungal collection that would allow me to devise a 25-member fungal community. With that in mind I believe there are currently two main possibilities to tackle this issue. First, is to semi-randomly design one or more 25-member fungal SynCom(s), using root-associated strains not present in the SynCom tested in this thesis. This approach has an advantage of a (semi-)random design, but a limitation of a high chance of choosing several fungal strains that are detrimental to plants health in the absence of bacterial commensals. In order to diminish the scope of this limitation I could also use available growth data generated in our group, originating from agar-based system. Phenotype observed on agar-plate system may not be perfectly reproducible in a soil-based system, but it increases the chances of choosing a non-pathogenic fungal community. This experiment will hopefully answer the question whether the conclusions drawn from my PhD thesis can be generalized for all root-associated F-communities or not.

Reproductive stage experiments in gnotobiotic system

Albeit not decreasing the value of the findings from reproductive stage FlowPot experiment described in this thesis, it has to be kept in mind that there are still some constraints related to the system used in this study. Although I was able to accommodate *A. thaliana* plants until the flowering and siliques production stage, accumulation of humidity and still-present space-limitations in the system forced me to stop the experiment after 9 weeks of plant growth, as during testing phase I found that inflorescence of 10-week old plants grew high and wide enough to reach Microbox borders and started wilting before setting seeds due to physical contact with borders of the box and consequently physical interaction with water condensation droplets. Taking these constraints into account is crucial, as there is a possibility that fitness score (especially siliques production) could still change if plants were able to grow till the full maturation stage. Unfortunately, without a great change to the FlowPot system that would accommodate a humidity-regulation device, it will be impossible to keep the humidity levels low enough to allow maturation (drying) of the siliques and stem while maintaining axenic and/or gnotobiotic conditions. Secondly, even using two 5l microboxes it was impossible to accommodate a fully mature flowering stem bringing up the second main system constraint that is space-limitation, while maintaining sterility of the system. Nevertheless, as the bolting/flowering and first siliques setting time was not significantly different between WT and the mutants (Supp. Figure 10A-C), and so the conclusions based on 9-week old plants do still carry a biological relevance as WT and mutant plants were harvested not only at the same time but also at the same developmental stage. Additionally, although there are already some studies available that have analysed the microbiota's effect on plants fitness by comparing plants grown in the greenhouse in sterile or re-inoculated soil (Lu et al., 2018; Fitzpatrick et al., 2019), up to my knowledge the study presented in this thesis is the first one where plants fitness was scored for fully-sterile (or inoculated with a known, precise SynCom composition) plants inside a gnotobiotic system.

Materials & Methods

Arabidopsis thaliana mutant lines

In this study a total of 26 *A. thaliana* lines were used. Columbia-0 as a wild type (Col-0, referred to as WT) and 25 mutants in WT background, that are listed in Methods Table 1.

Method Table 1: List of mutants used in this study and their respective mutations in alphabetical order. This figure will be a part of the manuscript in (un)changed form.

mutant name	genes/SALK line	innate immunity pathway	reference
355BR1	overexpression line of BRI1 fused with mCitrine	brassinosteroids	
<i>apex1</i>	Salk_116735C/ATS51560 (NASc info), no name	LRR-receptor kinases	Smakowska-Luzan et al. 2018, Nature
<i>apex2</i>	Salk_055240C/ATS663710 (NASc info), no name	LRR-receptor kinases	Smakowska-Luzan et al. 2018, Nature
<i>apex3</i>	Salk_049669C/ATS645780 (NASc info) CLAVATA3 INSENSITIVE RECEPTOR KINASE 4 (CIK4)	LRR-receptor kinases, clv3-mediated stem cell homeostasis	Smakowska-Luzan et al. 2018, Nature
<i>bak1/bkk1</i>		(co-)receptor	
<i>bak1/bkk1/cerk1</i>		(co-)receptor	
<i>bri1</i>		brassinosteroids	
<i>cyp71a12/a13</i>	<i>cyp71a12/cyp71a13</i>	glucosinolates	
<i>cyp71a27</i>		camalexins	Koprivova et al. 2019, PNAS
<i>cyp79b2/b3</i>	<i>cyp79b2/cyp79b3</i>	glucosinolates/camalexin	
<i>deps</i>	<i>dde2/ein2/pad4/sid2</i>	phytohormones	Tsuda et al. 2009 PLOS Genetics
<i>efr/fhs2/cerk1</i>		(co-)receptor	
<i>lyk5</i>	SALK_131911C	(co-)receptor	
<i>myb34/51/122</i>	<i>myb34/myb51/myb122</i>	glucosinolates	
<i>pad3</i>		camalexins	
<i>pad4</i>		phytohormones	
<i>pen2</i>		glucosinolates hydrolysis products	
<i>pen2/cyp71a12/a13</i>	<i>pen2/cyp71a12/cyp71a13</i>	glucosinolates	
<i>pen2/pad3</i>		glucosinolates	
<i>pyk10/bgglu21</i>		glucosinolates	Nakano et al. 2016 the Plant Journal
<i>quadruple</i>	<i>myb34/myb52/myb122/cyp71a13</i>	glucosinolates	
<i>rar1</i>		regulator of R-gene triggered defences	
<i>wrky33</i>		transcription factor	
<i>wrky33/40</i>	<i>wrky33/wrky40</i>	transcription factor	
<i>wrky40</i>		transcription factor	

quadruple mutant was initially used with the assumption, that it was a *quintuple* mutant (*myb34/myb51/myb122/cyp71a12/cyp71a13*), however after further investigation I have found that *cyp71a12* mutation was not present and so it was renamed to a *quadruple* mutant.

Natural soil experiment

Growth conditions

Natural soil experiment was performed in the greenhouse in short day conditions (8h light) in big square pots (9cm x 9cm) filled with natural Cologne Agricultural Soil (CAS) soil. Plants were grown for either 5 or 8 weeks and the pots were randomized weekly within biological replicates. Approximately two weeks after sowing the extra seedlings were removed and only 5 plants were left per pot. Pots were watered from the bottom whenever necessary and from the top only when the soil was visibly drying out. An experiment was repeated a total of three times, accounting for three independent biological replicates.

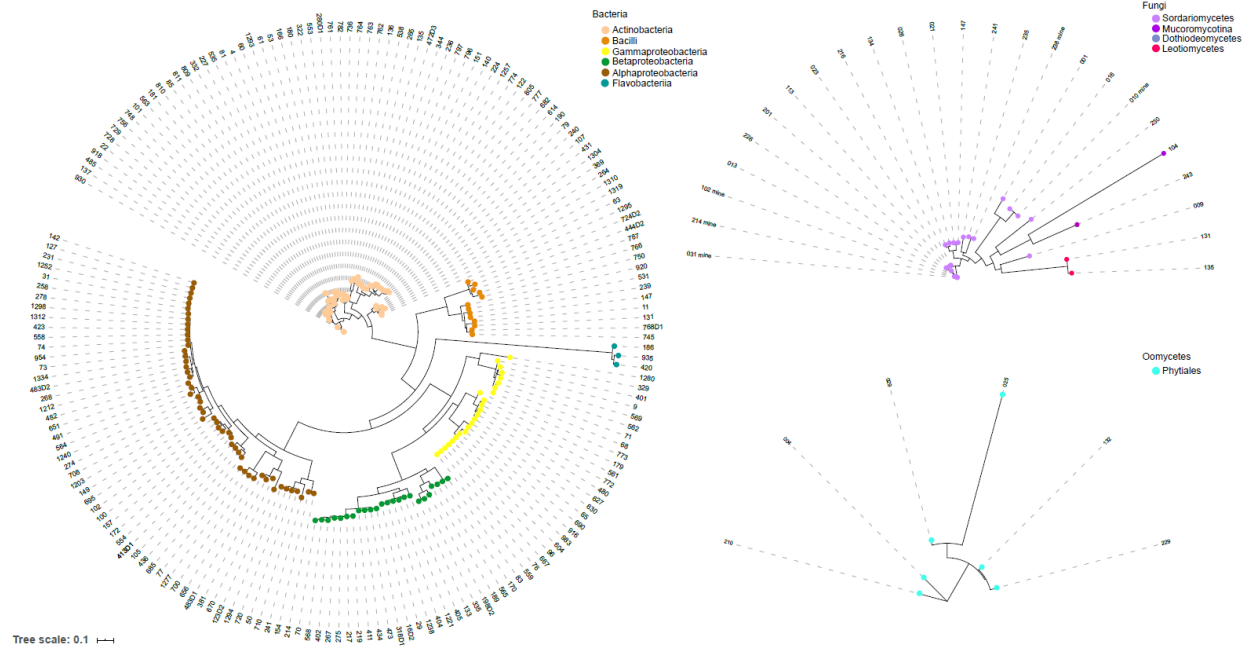
Harvesting

Rosettes of all plants (maximum of 5) were cut and their fresh weight measured. Roots were washed in sterile MQ water three times, then once in detergent (1%Tris-EDTA [TE] + 0.1% Triton X-100), once in 70% ethanol, once in 3% bleach and again three times in sterile MQ water, following the fractionation protocol described before (Durán et al., 2018). Afterwards the roots were dried shortly on the paper filter and frozen in Lysing E matrix tubes (MP Biomedicals) in liquid nitrogen. Soil samples were taken from unplanted pots, first a top 2cm layer of soil was removed and approximately 1g of soil was taken from the middle of the pot into Lysing E matrix tube and immediately frozen in liquid nitrogen. Samples were stored in -80°C until further processing.

Synthetic community preparation

183 bacterial strains (B) isolated from healthy *A. thaliana* roots (Bai et al., 2015) were grown for 7 days in 600µl of 50%TSB (tryptic soy broth) liquid media from the starting glycerol stock. 100µl of each strain was taken, combined together, centrifuged and the pellet was re-suspended in 10mM MgCl₂. 25 fungal (F) and 6 oomycetes (O) strains were grown individually on PGA (potato glucose agar) media for two weeks and harvested one day before the experiments. Harvested F and O mycelium (average of 50mg per strain) was suspended in 1ml of 10mM MgCl₂ inside a sterile 2ml-screw-cap tube containing one stainless steel bead (3.2 mm diameter) and left at 4°C overnight. On the day of the experiment, the mycelium was crushed for 10min in the paint shaker (SK450, Fast & Fluid Management, Sassenheim, Netherlands). B, F and O strains used in this study can be found in Methods Figure 1. Note that over the course of experiments two oomycetes strains (namely 210 and 29) did not survive and so they were only used in the initial screen of innate immunity mutants.

Materials & Methods



Method Figure 1: Phylogenetic tree of microbial synthetic community used in this study.

This figure will be a part of the manuscript in (un)changed form.

FlowPot preparation and growth conditions

Vegetative stage experiment

FlowPots were prepared as described before (Kremer et al., 2018; Durán et al., 2018) with six FlowPots per microbox (SacO2, TD3000+TPD3000, 3l volume). Each BFO-treated FlowPot was inoculated with 200 μ l B-, 200 μ l F- and 80 μ l O-community and each sterile FlowPot was inoculated with 480 μ l of 10mM MgCl₂ to account for the solution used to suspend BFO-culture and placed in light cabinets (Versatile Environmental Test Chamber MLR-352, Panasonic) with 10h light (LUX [luminous flux per unit area] inside the growth chamber average 9627.929, inside Microboxes average 6992.714, data kindly provided by Dr. Marina Cota). Temperature was set at 21°C during the light period and 19°C during the dark period. Seeds were sterilized by rotating at 40rpm for 15 min in 70% ethanol, centrifuged for 1min at 1000rpm in order to remove 70% ethanol, quickly washed with 100% ethanol immediately followed by another centrifugation step (1min, 1000rpm). Afterwards the seeds were dried under the sterile bench, suspended in sterile MQ water and left in the dark at 4°C for 2-3 days. One week after sowing six seeds per FlowPot, extra seedlings were removed under sterile

Materials & Methods

conditions, leaving a maximum of four plants per FlowPot. Plants were harvested after 5 weeks of growth. The sterility of the system was confirmed at the end of the experiment when a soil sample was taken from each sterile box. All soil samples were plated on 50% TSA (tryptic soy agar) plates to check for possible contaminations. If a true contamination was discovered, the samples originating from the contaminated box were removed from further analysis. All treatments lacking bacteria (F, O and FO) were also checked to confirm lack of bacterial contamination. An extreme example was *bri301* mutant in which none of the sterile controls were sterile, indicating an endophytic bacterium/bacterium residing in the seeds and for that reason no sterile FW data is available for *bri301* mutant.

Reproductive stage experiment

The general procedure of system preparation is the same as for vegetative stage FlowPots, with few main differences described below. FlowPots were prepared from the same 50/60ml syringes, but cut at the 45ml mark instead of 25ml. FlowPots were placed in a custom-made metal rack, instead of a plastic one (kindly provided by Dr. Kathrin Wippel and Elke Logemann) inside a big microbox (SacO2 cat. No. TP5000+TPD5000, 5l volume) that was covered with a lid for the first 5 weeks of growth. Afterwards the lid was exchanged with another 5l microbox placed upside down to allow accommodation of the flowering stem in the last 4 weeks of growth. Two boxes were held together with 5cm-wide micropore tape (3M, cat. No. 1530-2). Plants were grown in the greenhouse, first in short-day conditions (8h light) for 3 weeks and then on an open table supplemented with light (16h light) for another 6 weeks, giving in total 9 weeks of growth. Two weeks after sowing, germination/early survival rate was scored and extra seedlings were removed under sterile conditions, leaving one plant per FlowPot. During that time FlowPots were watered with approximately 4ml of 1/2MS (Murashige and Skoog) media each. After 5 weeks of growth (during an exchange of the lid) plants were watered again with the same amount of sterile 1/2MS media. Boxes were randomized on weekly basis within their respective biological replicates and dates of bolting, first flower and silique formation were scored on daily basis for each plant separately. Similarly to vegetative stage experiment, soil samples from sterile treatments were taken to confirm the sterility. All treatments lacking bacteria (F, O and FO) were also checked to confirm lack of bacterial contamination.

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Heat-kill FlowPot control experiment

Methodology of this experiment was the same as for vegetative stage experiment, with use of only WT and an addition of “heat-kill” treatment. “Heat-kill” treatment was done by taking the full BFO-community (prepared as described above) and subjecting it to two subsequent rounds of autoclaving (20min at 121°C for each round). Each FlowPot from “heat-kill” treatment was inoculated with 200µl of heat-killed B-community, 200µl of heat-killed F-community and 80µl of heat-killed O-community.

Harvesting

Vegetative stage experiment

Rosettes of all plants were cut and their FW measured. Four representative FlowPots were chosen from each box and their roots were harvested for microbiome analysis in the following way. Roots were washed four times in sterile MQ water, dried shortly on a paper filter and flash-frozen in Lysing E matrix tubes (MP Biomedicals) in liquid nitrogen. Samples were stored in -80°C until further processing. Experiment was repeated at least three times independently, giving a total of up to 12 replicates per treatment.

Reproductive stage experiment

First, the Chlorophyll Content Index (CCI) (Opti-Sciences Chlorophyll Content Meter CCM-200) was measured. For each plant, three randomly-selected leaves from the middle of the rosette were measured and each of these leaves was measured twice to account for possible measurement variation. Two technical measurements per leaf were averaged and later three averaged values from each plant were averaged again in order to obtain a single representative CCI value per plant. Next, stem was cut, taped to a white sheet of A4 paper, had a picture taken and then placed for 7 days in a bag in the 80°C oven for DW measurements. Separately rosette FW was measured and, similarly to stem, placed in the oven for DW measurements. Stem pictures were later used to count total number and length of the stems (main and side stems), number of branching points and number of siliques. FW and DW measurements were used to calculate water content of both rosettes and stems. Experiment was repeated twice, with up to 5 technical replicates per experiment (1 replicate being an individual plant), giving a total of up to 10 replicates per genotype x treatment combination.

Heat-kill FlowPot control experiment

Rosettes of all plants were cut and their FW measured. No further processing was done in this experiment.

DNA extraction and library preparation

DNA was isolated with FastDNA Spin Kit for Soil (MP Biomedicals) following the manufacturer's instructions. Library for sequencing followed the protocol described in [Durán et al., \(2018\)](#). In short, after DNA isolation DNA samples were diluted to 5ng/μl based on Picogreen measurements (Quant-iT™ PicoGreen™ dsDNA Assay-Kit, Invitrogen) and amplified in a two-step PCR with B, F and O specific primers (Primers sequences were published before in [Durán et al., \(2018\)](#), Supplementary Table S2), amplified bacterial products were purified on 1.5% agarose gel with QIAquick Gel Extraction Kit (Qiagen, cat. No. 28704) (B) and fungal and oomycetes products with Agencourt AMPure XP beads (Beckman Coulter, cat. No. A63882). After purification single bacterial, fungal and oomycetes samples were pooled together within their respective microbial groups in equimolar concentrations, cleaned again with Agencourt AMPure XP beads and finally pooled together into one final microbial library sample. Final pooling of bacterial, fungal and oomycetes samples varied between 300 and 850 ng per microbial group, depending on the availability of the samples.

Sequencing data analysis

Prepared libraries were sequenced on a MiSeq machine with pair-end Illumina sequencing (MiSeq reagent Kit v3, 600 cycle, cat. No. MS-102-3003). Primers used for sequencing are as described previously in [Durán et al., \(2018\)](#), Supplementary Table S2. Sequencing reads were mapped at 98% identity to the reference sequence database for bacteria, fungi and oomycete and all statistical analysis were performed in R by adapting previously published scripts ([Zgadzaj et al., 2016](#); [Durán et al., 2018](#)). All sequences with total abundance lower than 0.01% were removed and the samples used for the analysis were filtered with the threshold of minimum 1,000 reads per sample for all microbiota analysis.

Total microbial abundance experiment

Primers tested for specificity are listed in Methods Table 2. Tests for specificity were done with PCR protocol from library preparation protocol, PCR I (Durán et al., 2018). UBQ10 had the highest primer efficiency and showed no signs of non-specificity, and was chosen as plant reference, while 16S (V5-V7), ITS1 and oITS1 were chosen as a bacterial, fungal and oomycetes reference with the main advantage of being the same primer pair used in determining the microbiome composition. Subsequent PCR-tests revealed that fungal and oomycetes primers are fully specific to their respective synthetic communities, and bacteria primers, although to some extent amplifying plant DNA, still show a strong preference for bacterial DNA and Cq readout is highly correlated with increase of bacterial load, regardless of the varying presence of plant DNA (Methods Figure 2). An additional observation was made (and later confirmed with PCR amplification of single oomycetes strains), that each of the tested oomycetes is either harbouring an endophytic bacterium or its DNA is causing an unspecific cross-amplification with 16S primers. Nevertheless, the observed amplification in oomycetes samples is so low in comparison to other samples, that it is highly unlikely to affect the final sample read-out within the true experiment. The main experiment utilized RT-qPCR protocol as follows: 95°C for 3min, 40 cycles (95°C for 15s, 60°C for 30s, 72°C for 30s), 95°C for 10s and melting curve measurement from 55°C to 95°C with increment of 0.5°C. The total microbial load (relative to UBQ10) was calculated with the use of the following formula. Analysis include one reference sample present on each plate in technical triplicates, serving as an inter-plate normalisation (named *ref* in below formula).

$$x = \frac{\left(\frac{2^{-C_{qITS1}}}{2^{-C_{qUBQ10}}} \right)}{2^{-C_{qref}}}$$

Method Table 2: Primers used in the trials for estimation of total microbial abundance.

This table will be a part of the manuscript in (un)changed form.

Materials & Methods

Gene name	Forward primer	Reverse primer
EFR	TTCGGAATTCTACTTTTGGGA	TCA TCCGTTCTCATCCTATC
ACT2	ATCTTCTTCCGCTCTTTCTT	TACCTCTCTTGGATTGTGCT
UBQ10	TGTTTCCGTTCCCTGTTATCT	ATGTTCAAAGCCATCCTTAGA
RNA helix	GGTGGTTGCATATGAAAAAT	TGTCA GAAAAACCAAAATCA
16S (V5-V7)	AACMGGATTAGATACCCCKG	ACGTCATCCCCACCTTCC
16S (V2-V4)	CCTACGGGAGGCAGCAG	GGACTACHVGGGTWTCTAAT
ITS1	CTTGGTCATTTAGAGGAAAGTAA	GCTGCGTTCTTCATCGATGC
oITS	CGGAAGGATCATTACCAC	AGCCTAGACATCCA CTGCTG

Statistical analysis

All statistical analyses were performed in R. For Kruskal-Wallis and Dunn test, FSA package was used and for Dunn control test PMCMR package was used. Other statistical analyses include GLM model and linear model with ANOVA. Example of GLM model with gamma distribution and linear model with ANOVA is provided below:

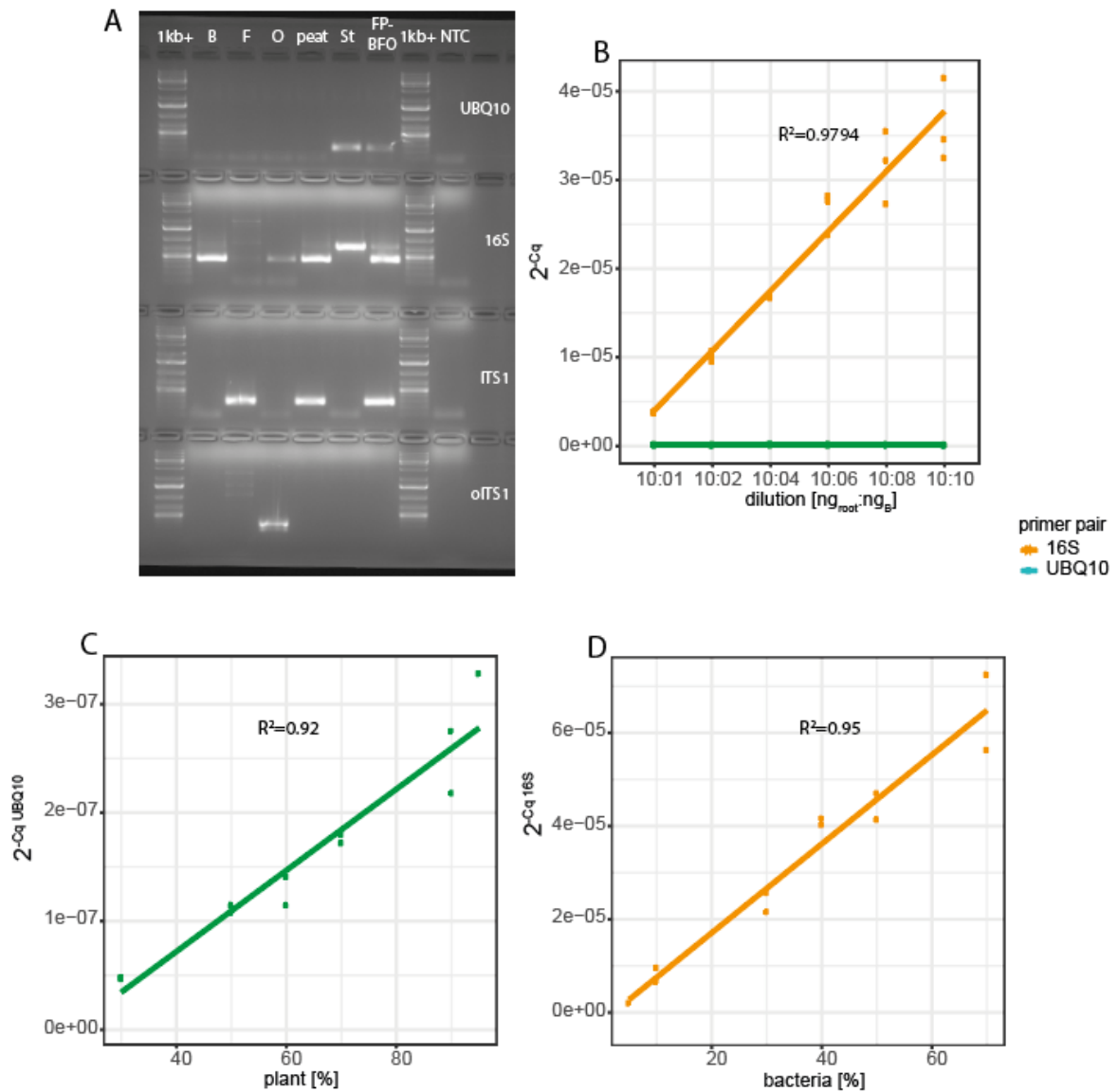
```
mod=glm(data$days_to_bolting~data$treatment*data$genotype,family=Gamma(link="log"),na.action=na.omit)
```

```
mod=lm(log(data$bolt_to_flower_days)~data$genotype*data$treatment, na.action=na.omit)
```

Whenever necessary the response variable was root square- or log-transformed to ensure a normal distribution of the model's residuals.

Statistical analysis for strain enrichment (ASVs in natural soil experiment and OTUs in vegetative stage FlowPot experiment) were calculated in a following way. First, raw sequencing read counts were normalized (TMM normalization, "calcNormFactors" from R package "EdgeR"). Then a GLM including batch effect was used ("glmFit") and finally the significantly enriched ASVs and OTUs were determined with a likelihood ratio test ("glmRT", $p < 0.05$).

Materials & Methods



Method Figure 2: Primer specificity test on 1% agarose gel after PCR amplification (A) and 16S primer efficiency in qRT-PCR (B-D).

Sample code on the gel (A) is as follows: B/F/O – B-/F-/O-initial input; peat – peat used in FlowPots inoculated with BFO-community; St – 3-week old WT seedlings grown on sterile 1% 1/2MS agar plates; FP-BFO – roots from BFO-inoculated FlowPot plant; 1kb+ – 1kb+ ladder; NTC – negative control. Samples in each row were amplified with use of the following primer pairs: UBQ10, 16S, ITS1 and oITS1, respectively (Methods Table 2). For qRT-PCR results (B-D), 16S primer efficiency with UBQ10 as a control in a dilution series of bacterial DNA with stable sterile root DNA input (B). UBQ10 (C) and 16S (D) primer efficiency in a dilution series with varying root and bacteria DNA inputs. In panel C and D numbers on x axis indicate percentage value of plant (C) and bacterial (D) input. UBQ10 and 16S are presented on a separate graph for clarity reasons. This figure will be a part of the manuscript in (un)changed form.

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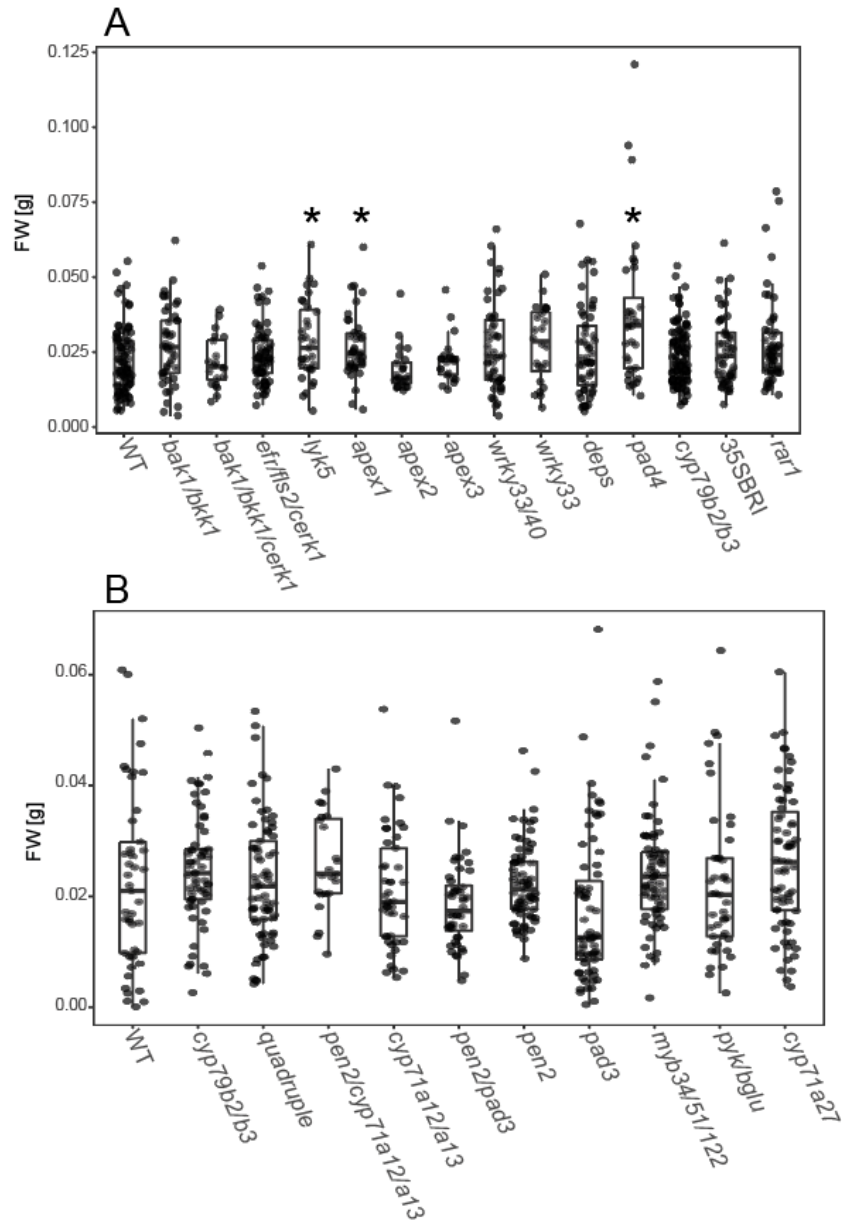
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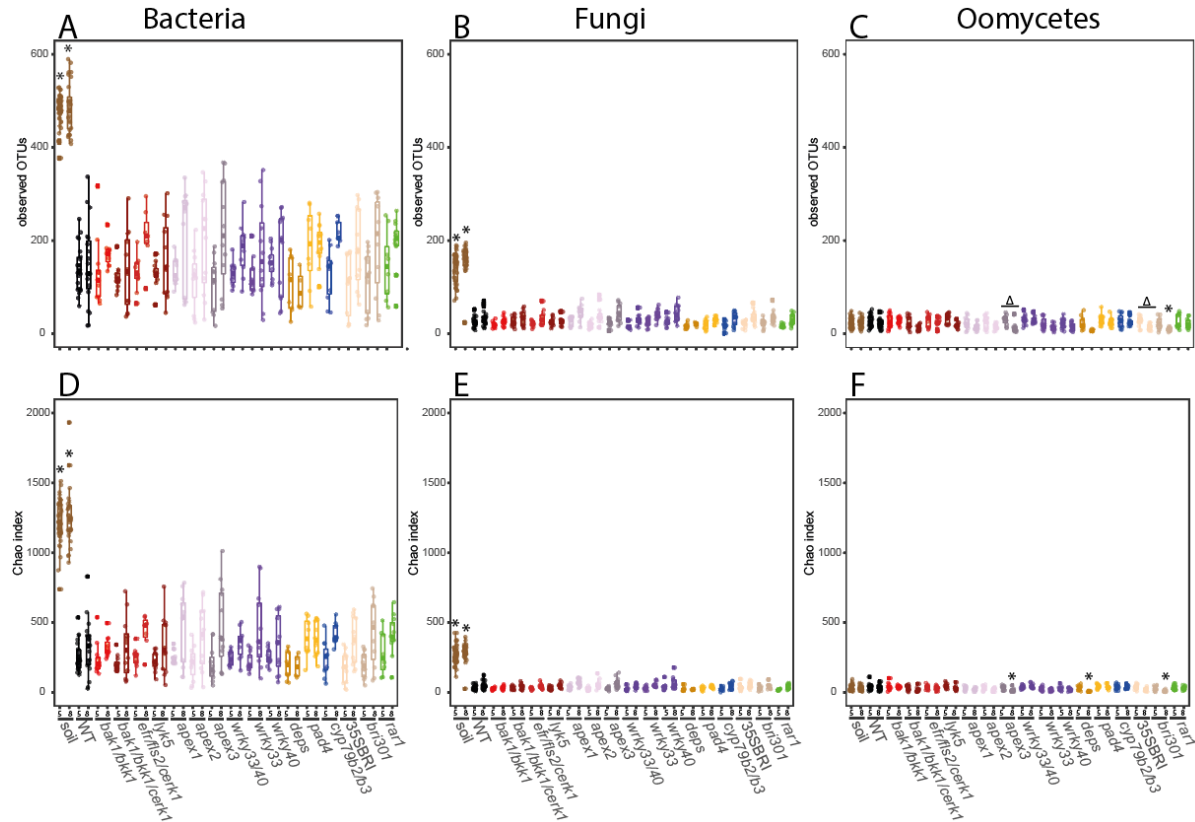
Supplementary Figures



Supplementary Figure 1: FW measurements of 5-week old sterile *A. thaliana* plants grown in gnotobiotic system FlowPot.

Genotypes grown in the initial 5-week FlowPot mutant screen (A) and in 5-week dissection of glucosinolate mutants (B). Asterisks indicate genotypes with FW significantly different from WT within each panel. Statistical analyses were done using Kruskal-Wallis and Dunn control test ($p=0.05$) with WT as a control. This figure will be a part of the manuscript in (un)changed form. I could not confirm the effect of mutation under sterile conditions for *bri301* and *wrky40* due to technical limitations.

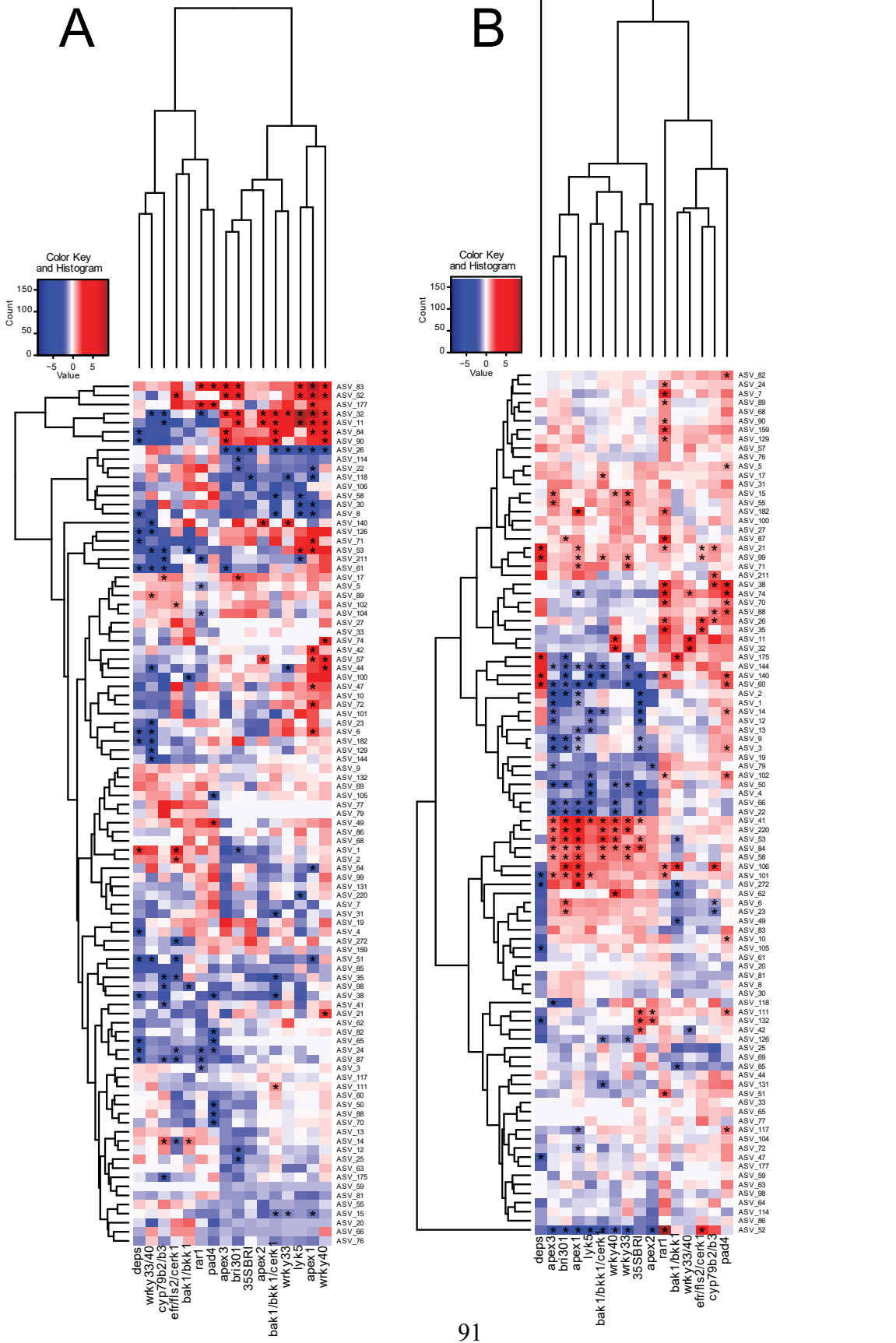
Supplementary Figures



Supplementary Figure 2: Alpha diversity indices of endophytic root microbiome from natural soil.

Observed OTUs (A, B and C) and Chao index (D, E and F) for bacteria, fungi and oomycetes, respectively. Statistical analysis for all indices were done using Kruskal-Wallis and Dunn test with Bonferroni-Hochberg correction ($p=0.05$). Asterisk indicate significant difference from WT, while delta indicates significant difference within genotype, between two time points. Colours indicate genotypes, 5 – week 5; 8 – week 8.

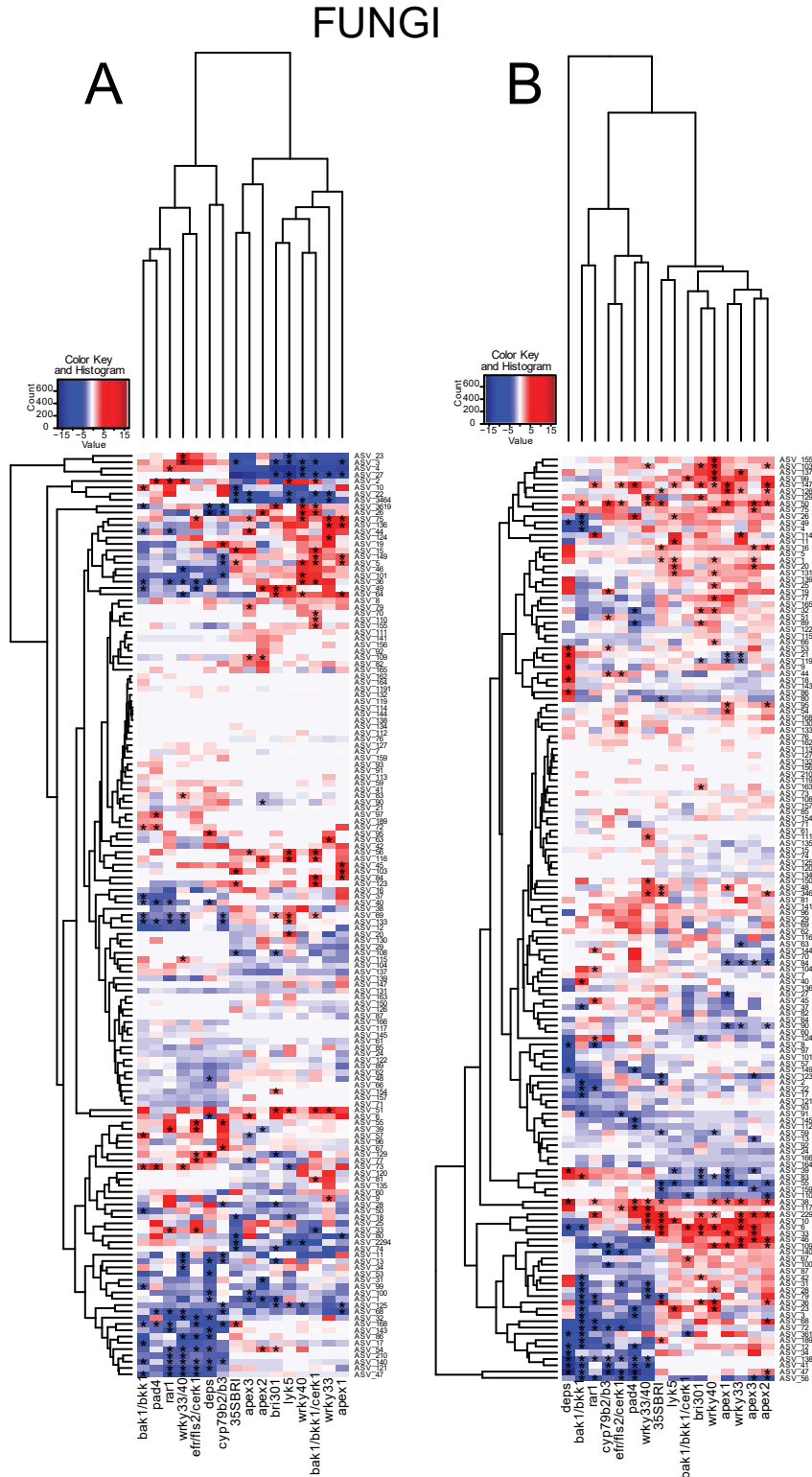
BACTERIA



Supplementary Figures

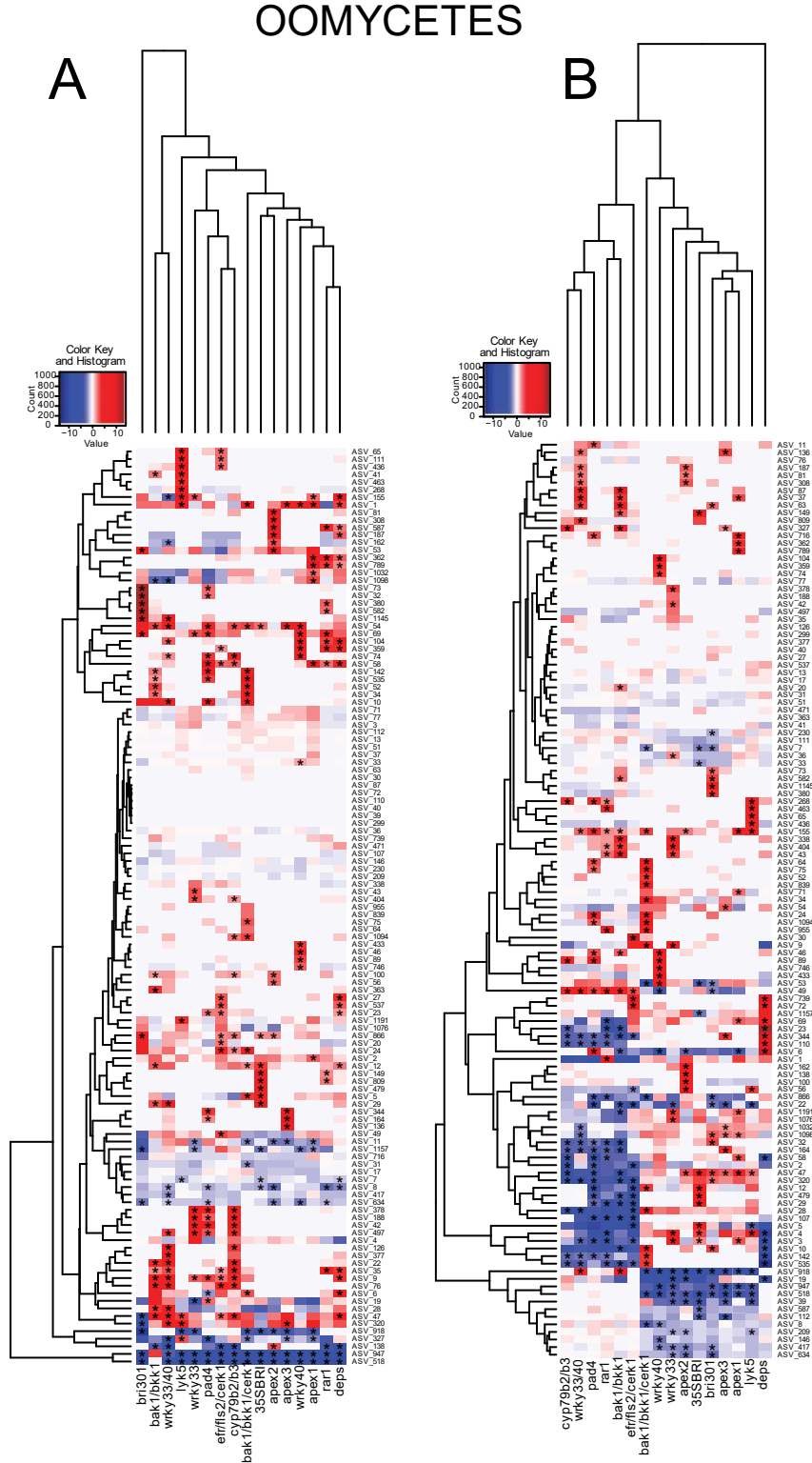
Supplementary Figure 3: Genotype-induced changes in bacterial abundance on strain-level in natural soil are subtle.

Genotype-induced changes in strain abundance in comparison to WT, based on mean abundance values for bacteria in week 5 (A) and week 8 (B). Significantly enriched ASVs were determined based on a likelihood ratio test ($p < 0.05$), for more details see Materials & Methods section “Statistical analysis”.



Supplementary Figure 4: Genotype-induced changes in fungal abundance on strain-level in natural soil are subtle.

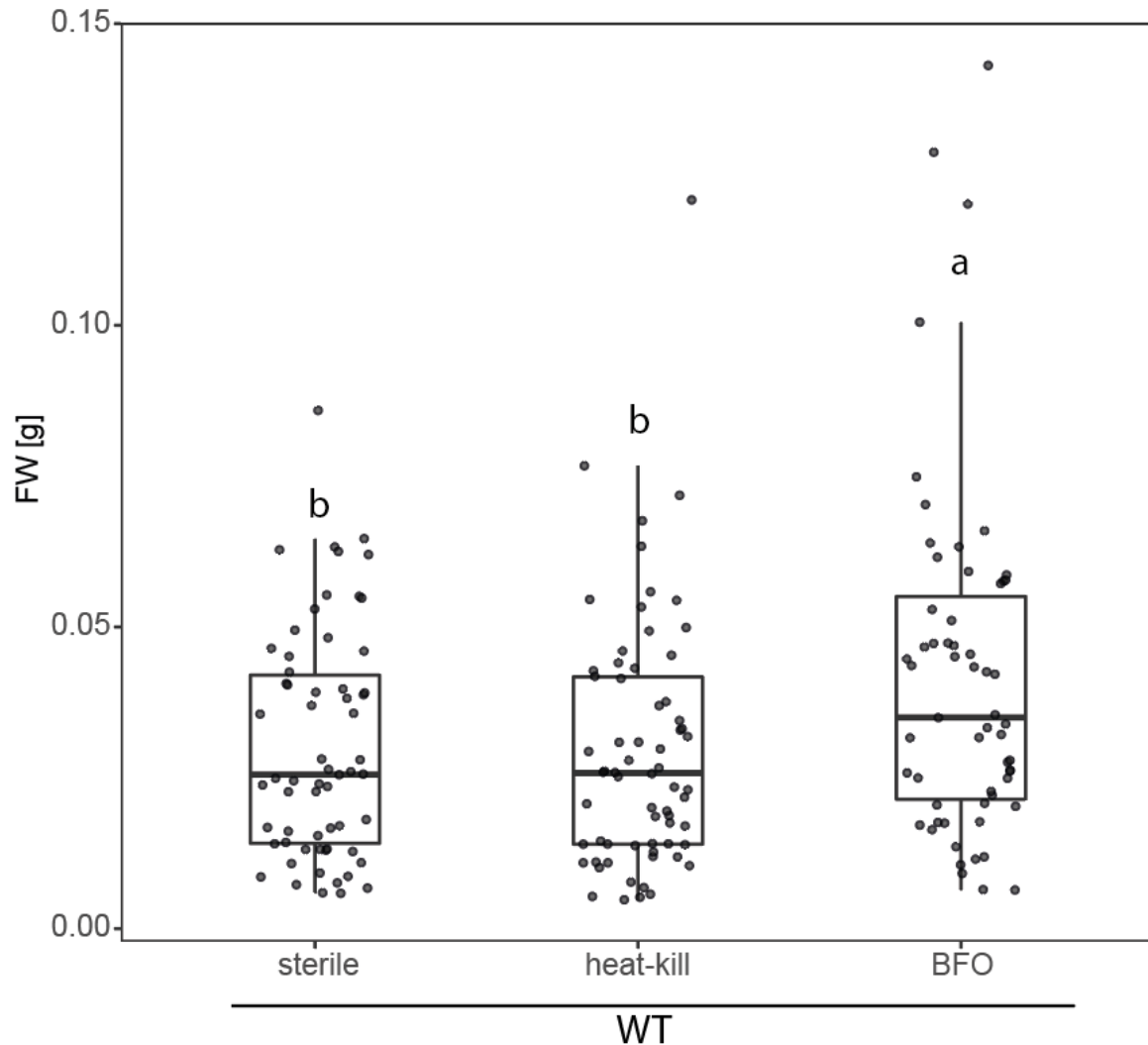
Genotype-induced changes in strain abundance in comparison to WT, based on mean abundance values for fungi in week 5 (A) and week 8 (B). Significantly enriched ASVs were determined based on a likelihood ratio test ($p < 0.05$), for more details see Materials & Methods section “Statistical analysis”.



Supplementary Figures

Supplementary Figure 5: Genotype-induced changes in oomycetes abundance on strain-level in natural soil are subtle.

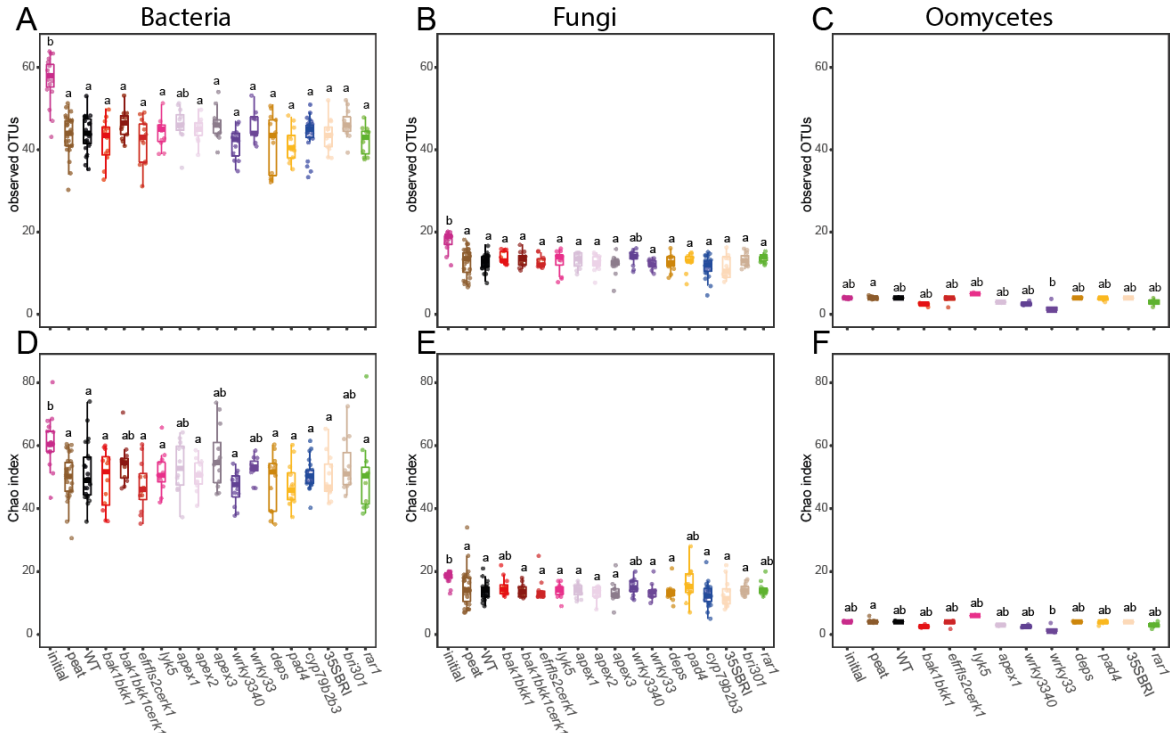
Genotype-induced changes in strain abundance in comparison to WT, based on mean abundance values for oomycetes in week 5 (A) and week 8 (B). Significantly enriched ASVs were determined based on a likelihood ratio test ($p < 0.05$), for more details see Materials & Methods section “Statistical analysis”.



Supplementary Figure 6: Heat-kill control experiment in FlowPot.

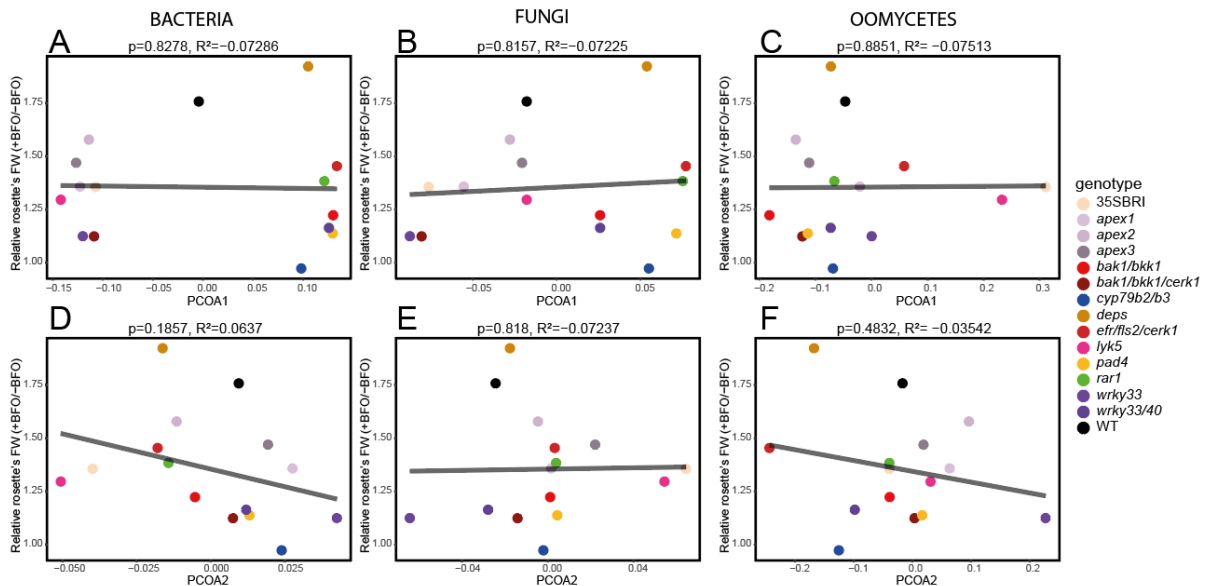
5-week old WT plants were grown in sterile, heat-killed and BFO conditions (for details see Materials & Methods, section “heat-kill FlowPot control experiment”). Statistical analysis was done using Kruskal-Wallis and Dunn test with Bonferroni-Hochberg correction ($p = 0.05$). Plant number: 59-62, median: 0.02760, mean: 0.03370.

Supplementary Figures



Supplementary Figure 7: Alpha diversity indices of root-associated microbiome from vegetative stage FlowPot experiment.

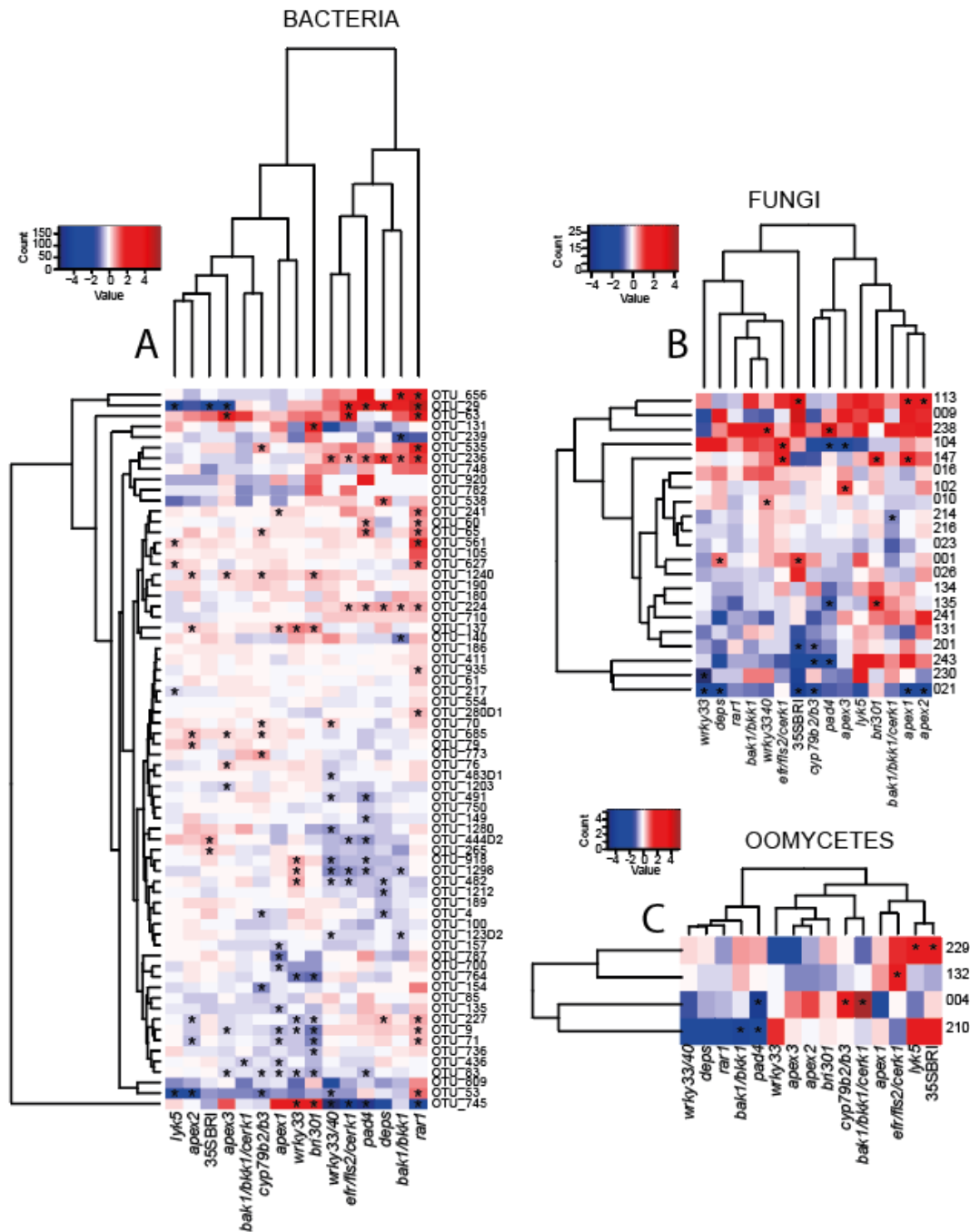
Observed OTUs (A, B and C) and Chao index (D, E and F) for bacteria, fungi and oomycetes, respectively. Statistical analyses for all indices were done using Kruskal-Wallis and Dunn test with Bonferroni-Hochberg correction ($p=0.05$). This figure will be a part of the manuscript in (un)changed form.



Supplementary Figure 8: Microbial composition is not a likely candidate behind lack of microbiota-mediated growth promotion phenotype.

Supplementary Figures

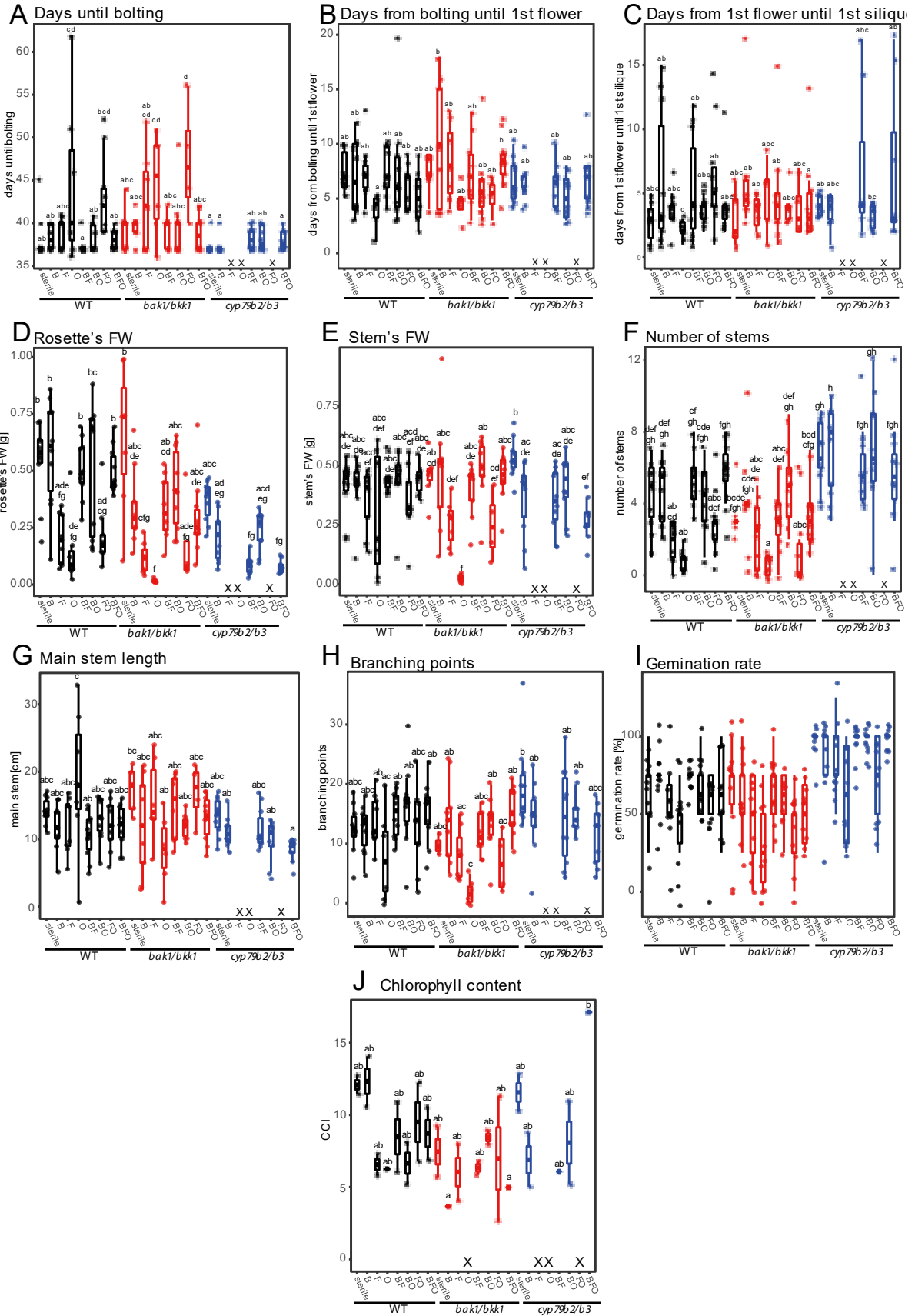
Regression analysis of relative rosette's FW and microbial community composition first and second principal component for bacteria (A and B), fungi (C and D) and oomycetes (E and F). p value and R² were obtained from ANOVA. This figure will be a part of the manuscript in (un)changed form.



Supplementary Figure 9: Genotype-induced changes in abundance on strain-level in vegetative stage FlowPot experiment are subtle.

Genotype-induced changes in strain abundance in comparison to WT, based on mean abundance values for bacteria (A), fungi (B) and oomycetes (C). This figure will be a part of the manuscript in (un)changed form.

Supplementary Figures

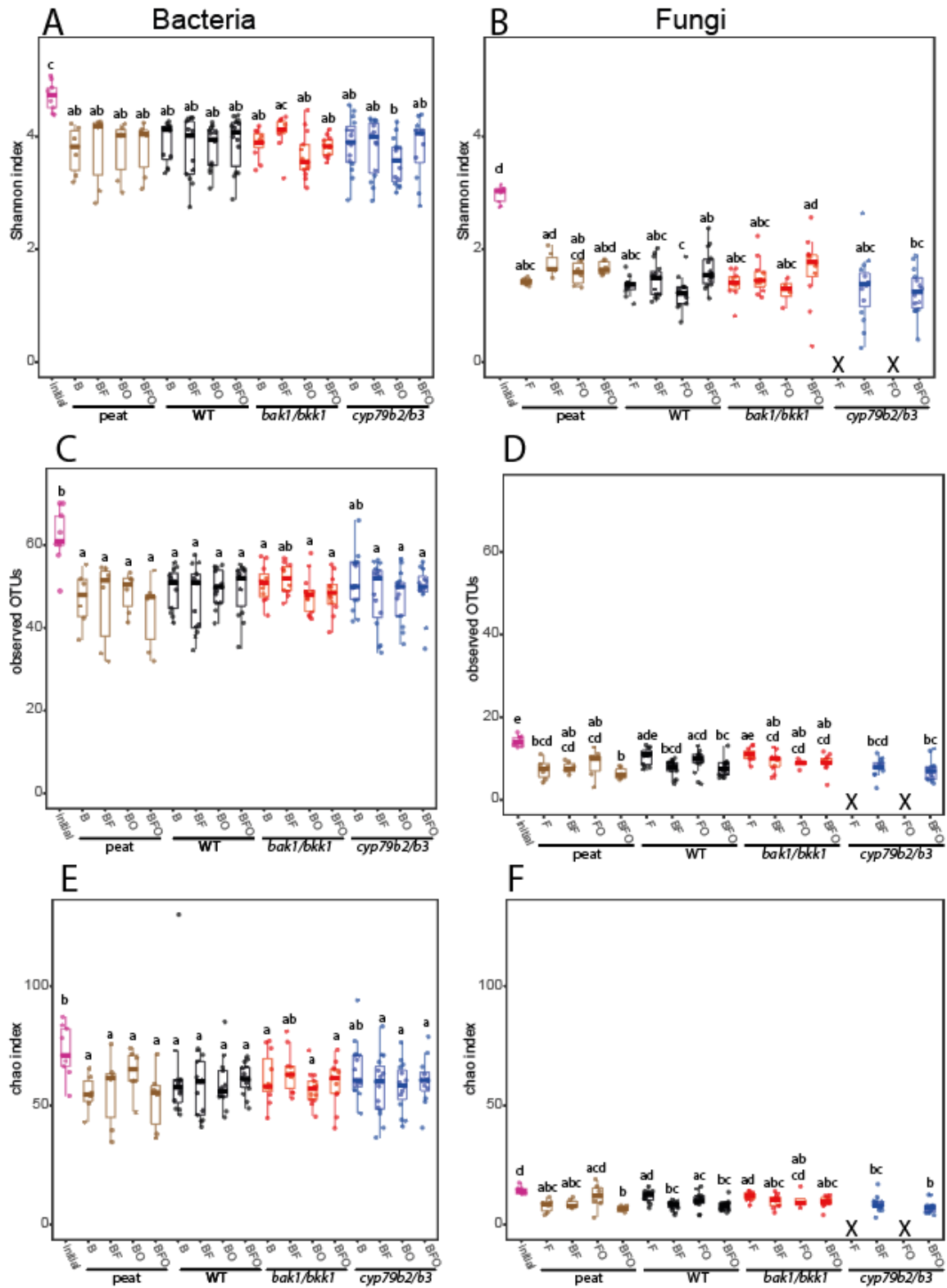


Supplementary Figures

Supplementary Figure 10: Interaction between innate immunity and microbial composition affects several phenotypic traits, which are not directly linked to plant's fitness, but it does not have a strong influence on developmental stages of the plants.

Panels A-C show the number of days needed for the plant to reach different developmental stages, days until bolting (plant number: 0-10, median: 38.50, mean: 39.39) (A), days from bolting until 1st flower (plant number: 0-10, median: 6.0, mean: 6.78) (B), days from 1st flower until 1st silique (plant number: 0-10, median: 4.0, mean: 4.429) (C). Panels D-I present fitness-(un)related traits, rosette's FW (plant number: 0-10, median: 0.2768, mean: 0.3235) (D), stem's FW (plant number: 0-10, median: 0.4329, mean: 0.3899) (E), number of stems (plant number: 0-10, median: 4.0, mean: 4.088) (F), main stem length (plant number: 0-10, median: 12.50, mean: 12.53) (G), branching points (plant number: 0-10, median: 13.00, mean: 13.14) (H), germination rate (plant number: 10, median: 75.00, mean: 66.46) (I, no significant differences) and chlorophyll content (plant number: 0-30, median: 7.650, mean: 8.476) (J). Statistical analysis for days until bolting (A) and number of stems (F) were done using general linearized model (GLM), followed by a Likelihood Ratio Test and post-hoc pairwise comparisons. Statistical analysis for days from bolting until 1st flower (B), main stem length (G) and chlorophyll content (J) were done using ANOVA followed by Tukey HSD. Statistical analysis for the remaining panels (C-E and H-I) were done using Kruskal-Wallis and Dunn test with Bonferroni-Hochberg correction ($p=0.05$). This figure will be a part of the manuscript in (un)changed form.

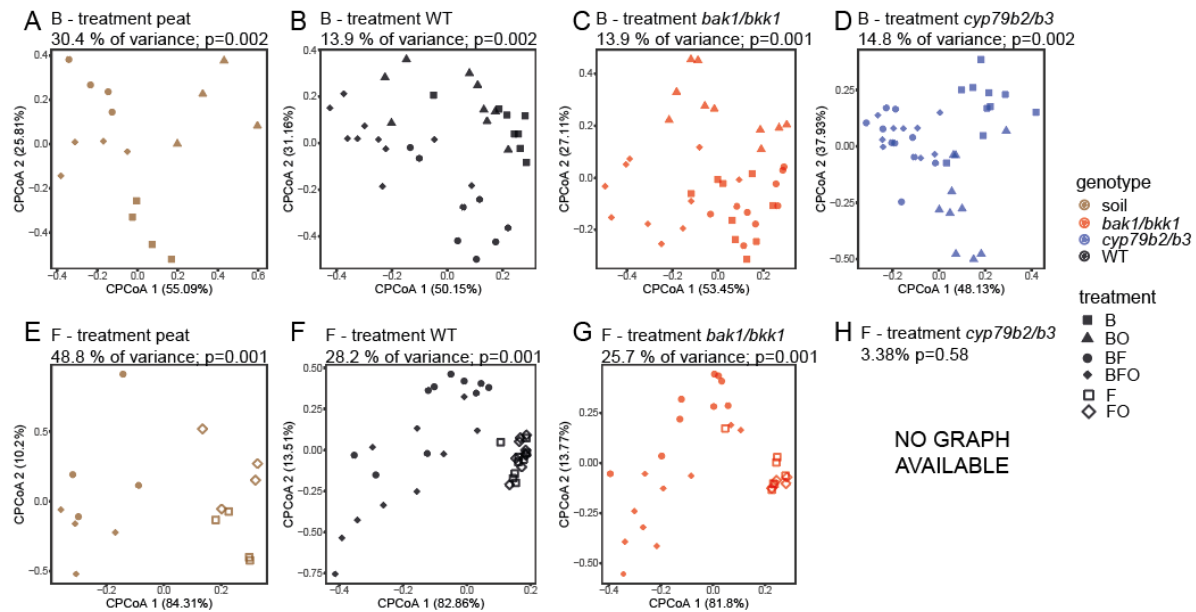
Supplementary Figures



Supplementary Figure 11: Alpha diversity indices of root-associated microbiome from reproductive stage FlowPot experiment.

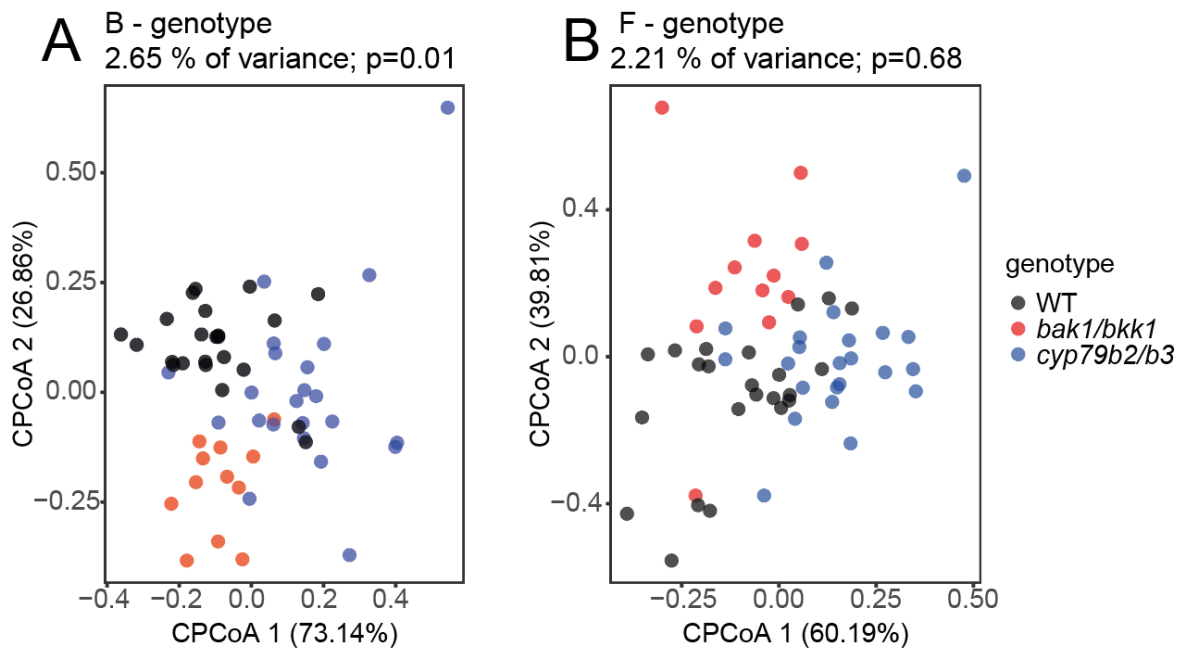
Supplementary Figures

Shannon index (A and B), observed OTUs (C and D) and Chao index (E and F) for bacteria and fungi, respectively. Statistical analyses for all indices were done using Kruskal-Wallis and Dunn test with Bonferroni-Hochberg correction ($p=0,05$). This figure will be a part of the manuscript in (un)changed form.



Supplementary Figure 12: Treatment effect has a strong effect on microbial root communities of 9-week old *A. thaliana* plants.

CPCoA based on Bray-Curtis distances, constrained by treatment for peat, WT, *bak1/bkk1* and *cyp79b2/b3* in bacterial (A-D respectively) and fungal (E-H respectively) community. Panel H does not contain a graph as *cyp79b2/b3* plants did not survive any treatment lacking bacterial community, which does not allow a graphical representation. This figure will be a part of the manuscript in (un)changed form.



Supplementary Figures

Supplementary Figure 13: Subtle genotype effect on bacterial and fungal community in initial 5-week FlowPot screen calculated for a subset of data.

CPCoA plot based on Bray-Curtis distances, constrained by genotype, calculated for a subset of data including WT, *bak1/bkk1* and *cyp79b2/b3* mutants.

Supplementary Tables

Supplementary Table 1: Percentage of variance explained by genotype effect in natural soil experiment presented in Figure 4.

genotype	BACTERIA				FUNGI				OOMYCETES			
	week 5		week 8		week 5		week 8		week 5		week 8	
	% of variance	p value	% of variance	p value	% of variance	p value	% of variance	p value	% of variance	p value	% of variance	p value
<i>bak1/bkk1</i>	3.52	0.061	2.76	0.028	4.68	0.065	3.38	0.191	6.09	0.012	2.59	0.642
<i>bak1/bkk1/cerk1</i>	2.27	0.429	1.63	0.731	2.66	0.832	4.16	0.089	3.83	0.287	2.51	0.684
<i>efr/fls2/cerk1</i>	4.87	0.004	2.72	0.01	3.26	0.381	2.39	0.72	6.73	0.005	2.83	0.371
<i>lyk5</i>	1.88	0.784	1.60	0.718	2.23	0.932	3.23	0.416	3.35	0.454	1.92	0.927
<i>apex1</i>	3.53	0.011	3.94	0.012	3.00	0.698	2.90	0.616	2.98	0.581	2.55	0.697
<i>apex2</i>	2.25	0.532	4.00	0.014	2.46	0.887	3.59	0.225	3.43	0.364	1.33	0.988
<i>apex3</i>	2.26	0.439	5.39	0.002	2.19	0.924	3.78	0.173	2.84	0.655	4.63	0.052
<i>wrky33</i>	2.18	0.509	1.26	0.953	2.46	0.889	3.87	0.115	3.70	0.332	6.09	0.007
<i>wrky40</i>	1.83	0.82	2.18	0.296	2.4	0.892	2.99	0.547	3.29	0.448	2.14	0.86
<i>wrky33/40</i>	4.75	0.003	2.61	0.059	4.42	0.05	3.24	0.186	4.17	0.099	2.29	0.749
<i>deps</i>	4.31	0.008	4.43	0.006	5.76	0.005	5.83	0.013	4.35	0.173	2.77	0.717
<i>pad4</i>	7.73	0.001	2.28	0.155	4.67	0.032	3.60	0.095	9.25	0.001	2.37	0.65
<i>cyp79b2/b3</i>	4.28	0.015	3.24	0.002	3.95	0.176	2.97	0.272	3.17	0.452	2.37	0.616
35SBRI	2.31	0.428	5.17	0.002	2.41	0.892	4.34	0.068	3.35	0.417	1.87	0.915
<i>bri301</i>	2.22	0.476	4.62	0.006	2.49	0.845	3.97	0.146	4.17	0.215	4.90	0.049
<i>rar1</i>	8.50	0.001	3.06	0.003	5.71	0.007	2.66	0.545	9.57	0.001	3.41	0.203

Supplementary Table 2: List of bacterial, fungal and oomycetes strains and their assignment to single OTU clusters, based on sequence similarity.

Supplementary Tables

List includes all strains with read count above 1000. Sequence of the strain number indicated in the OTU name is the one used as a reference OTU sequence in the analysis.

BACTERIA		BACTERIA		BACTERIA		BACTERIA		BACTERIA		FUNGI	
OTU	strains	OTU	strains	OTU	strains	OTU	strains	OTU	strains	OTU	strains
OTU_100	100		140		29		627	OTU_750	750	OTU_235	235
	105		151		404	OTU_627	480		766	OTU_131	131
OTU_105	413D1	OTU_140	796	OTU_29	1221		772	OTU_756	756	OTU_104	104
OTU_107	107		797		1238		63		76	OTU_230	230
	1203	OTU_147	147		16D2		431		96	OTU_16	16
OTU_1203	708	OTU_149	149			OTU_63	1295		559	OTU_9	9
	1212		154	OTU_335	133		1304	OTU_76	604	OTU_13	13
OTU_1212	268	OTU_154	214	OTU_4	4		1319		667	OTU_113	113
	122	OTU_157	157	OTU_405	405		65		916	OTU_23	23
OTU_122	774	OTU_180	180		411	OTU_65	630		983	OTU_135	135
	123D2	OTU_186	186		434	OTU_656	656	OTU_764	764	OTU_243	243
OTU_123D2	123D2	OTU_189	189	OTU_411	473	OTU_685	685	OTU_773	773	OTU_226	226
	1240		190		318D1	OTU_690	690	OTU_777	777	OTU_1	1
OTU_1240	274	OTU_190	614	OTU_420	420	OTU_70	70	OTU_782	805	OTU_201	201
	1280		682	OTU_436	436	OTU_700	700	OTU_787	782	OTU_216	216
	1298	OTU_198D2	198D2	OTU_444D2	444D2		77	OTU_79	787	OTU_241	241
	954		217	OTU_482	482		1277		79	OTU_134	134
	74		219		483D1	OTU_71	71	OTU_809	240	OTU_147	147
	127	OTU_217	267	OTU_483D1	381		68		809	OTU_26	26
	142		275		670		562		332	OTU_21	21
	231		402	OTU_483D2	483D2		329		83	OTU_238	238
	258		568		491		710	OTU_83	170	OTU_102	102
	278		224	OTU_491	564	OTU_710	50		565	OTU_31	31
	558	OTU_224	1257		651		720		85	OTU_214	214
	1252	OTU_227	227	OTU_53	53		1294	OTU_85	181	OTU_10	10
	1312		236	OTU_531	531	OTU_724D2	724D2		810		
	31		344		535	OTU_728	728		811		
	423	OTU_236	472D3	OTU_535	81	OTU_729	729		9		
OTU_131	131		239	OTU_538	538		736	OTU_9	401		
	11	OTU_239	239		554		761		569		
	1334	OTU_241	241		102	OTU_736	762		918		
OTU_1334	73		264	OTU_554	172		763	OTU_918	22		
	135	OTU_264	369		695		745		920		
OTU_135	135		1310		561	OTU_745	768D1	OTU_920	920	OTU_229	229
OTU_136	136	OTU_265	265	OTU_561	179		748	OTU_935	935	OTU_004	4
	137		280D1		60		101			OTU_210	210
OTU_137	485	OTU_280D1	166	OTU_60	1293	OTU_748	563				
	930		553	OTU_61	61						

Supplementary Table 3: Pairwise comparisons of bacterial community composition between genotypes and treatments from the reproductive stage FlowPot experiment.

Supplementary Tables

Values presented in the table show p-values (with FDR correction). Significant comparisons are highlighted in bold with light-green background.

		<i>bak1/bkk1</i>				<i>cyp79b2/b3</i>				WT		
		B	BF	BFO	BO	B	BF	BFO	BO	B	BF	BFO
<i>bak1/bkk1</i>	BF	0.2513	-	-	-	-	-	-	-	-	-	-
	BFO	0.0143	0.0094	-	-	-	-	-	-	-	-	-
	BO	0.2640	0.2359	0.0596	-	-	-	-	-	-	-	-
<i>cyp79b2/b3</i>	B	0.0589	0.1760	0.0073	0.0143	-	-	-	-	-	-	-
	BF	0.0094	0.0914	0.0073	0.0717	0.0094	-	-	-	-	-	-
	BFO	0.0073	0.1151	0.0094	0.1699	0.0143	0.3014	-	-	-	-	-
	BO	0.1131	0.1248	0.0073	0.7341	0.0406	0.0440	0.0717	-	-	-	-
WT	B	0.0592	0.4295	0.0073	0.0598	0.2460	0.0073	0.0143	0.0594	-	-	-
	BF	0.0620	0.4982	0.0143	0.1886	0.0957	0.5427	0.8640	0.1232	0.2513	-	-
	BFO	0.0143	0.0733	0.0124	0.0143	0.0343	0.0073	0.0073	0.0094	0.0073	0.0868	-
	BO	0.2017	0.6579	0.0165	0.1941	0.2774	0.0124	0.0767	0.1014	0.2017	0.1914	0.1200

Supplementary Table 4: Pairwise comparisons of fungal community composition between genotypes and treatments from the reproductive stage FlowPot experiment.

Values presented in the table show p-values (with FDR correction). Significant comparisons are highlighted in bold with light-green background.

		<i>bak1/bkk1</i>		<i>cyp79b2/b3</i>		WT
		BF	BFO	BF	BFO	BF
<i>bak1/bkk1</i>	BFO	0.619	-	-	-	-
<i>cyp79b2/b3</i>	BF	0.075	0.075	-	-	-
	BFO	0.216	0.216	0.661	-	-
WT	BF	0.804	0.235	0.075	0.216	-
	BFO	0.296	0.688	0.235	0.296	0.216

Supplementary Table 5: Pairwise comparisons of bacterial and fungal community composition between genotypes and treatments from the reproductive stage FlowPot experiment.

Supplementary Tables

Values presented in the table show p-values (with FDR correction). Significant comparisons are highlighted in bold with light-green background.

BACTERIA

WT	B	BF	BFO
BF	0.216	-	-
BFO	0.012	0.122	-
BO	0.203	0.203	0.122

<i>bak1/bkk1</i>	B	BF	BFO
BF	0.249	-	-
BFO	0.009	0.009	-
BO	0.249	0.241	0.08

<i>cyp79b2/b3</i>	B	BF	BFO
BF	0.006	-	-
BFO	0.009	0.257	-
BO	0.022	0.022	0.022

FUNGI

WT	BF	BFO	F
BFO	0.098	-	-
F	0.002	0.003	-
FO	0.002	0.002	0.266

<i>bak1/bkk1</i>	BF	BFO	F
BFO	0.558	-	-
F	0.012	0.012	-
FO	0.024	0.022	0.561

<i>cyp79b2/b3</i>	BF	BFO	F
BFO	0.569	-	-
F	-	-	-
FO	-	-	-

Contributions

Brigitte Pickel assisted in harvesting process of natural soil experiment and vegetative stage FlowPot experiment. Brigitte Pickel and Sjoerd Gremmen assisted in harvesting of reproductive stage FlowPot experiment. Sjoerd Gremmen assisted in harvesting of glucosinolates screen experiment.

Genotyping of glucosinolates mutants and fungal synthetic community was done by Lug Trémulot.

Script used for analysis presented on Figure 4 and Figure 8D-F was provided by Dr. Thorsten Thiergart.

Methods Figure 1 and Supp. Figure 3-5 and 9 were provided by Dr. Thorsten Thiergart

Panels D-G from Figure 10 and Supplementary Figure 8 as well as statistical output presented in Table 2 and 4 and Supplementary Table 3-5 were provided by Dr. Nathan Vannier.

Origin of data presented on Figure 11B-D: DNA was isolated by Sjoerd Gremmen, RT-qPCRs were done by Brigitte Pickel and I have performed the analysis based on the generated data.

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First of all, I would like to thank my direct supervisor Dr. Stéphane Hacquard and Prof. Dr. Paul Schulze-Lefert for hosting me during my PhD project at the Max Planck Institute for Plant Breeding Research in Cologne. I would also like to extend my thanks to the Examination Committee for evaluating my dissertation and to my both TAC co-advisors, Prof. Dr. Jane Parker and Prof. Dr. Marcel Bucher for their regular feedback and comments on my project.

Furthermore, I would like to give my thanks to those group and department members that were always supporting and helpful through the duration of my stay. Their friendly and team-spirited attitude was a crucial factor for enjoying my time as a doctoral researcher. I would also like to thank the PhD Coordinator Dr. Stephan Wagner for an amazing support and help with all non-scientific aspects of the PhD.

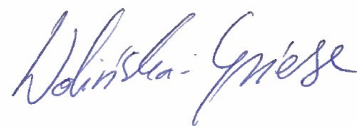
Finally, I would like to thank my family and friends for supporting me during the whole doctoral journey, I could always count on them to lend an ear whenever the work-related stress was reaching its heights. A big thank you also goes to my office-buddy Maria, who always knows how to lighten up the mood.

Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegt worden ist, sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.

Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Paul Schulze-Lefert betreut worden.

Ich versichere, dass ich alle Angaben wahrheitsgemäß nach bestem Wissen und Gewissen gemacht habe und verpflichte mich, jedmögliche, die obigen Angaben betreffenden Veränderungen, dem Dekanat unverzüglich mitzuteilen.



Köln, 18. 02. 2021

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Curriculum Vitae



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October 2014 – January 2015 (3,5 months) Internship at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences. „Characterization of novel wild sources of resistance to Potato Virus Y infection” under supervision of prof. Jacek Hennig

January 2014 – September 2014 (9 months) BSc thesis + Internship in Laboratory of Genetics at WUR “Validating candidate genes expression under drought+*B.cinerea* combinatory stress conditions in *A.thaliana* natural accessions” under supervision of Pingping Huang and dr. Mark Aarts

April-June 2013 (3 months) Internship in a Department of Chemistry at Faculty of Food Sciences at Warsaw University of Life Sciences

Academic year 2012/2013 – Student assistant in a Department of Chemistry at Faculty of Food Sciences at Warsaw University of Life Sciences

Additional experience

1.12.2018 – 31.12.2019 Member of PhDNet Secretary Working Group

May 2019 – November 2019 Member of the organization committee of PhDNet Event Mental Health Awareness Week (MHAW) at MPIPZ

1.11.2018 – 20.07.2020 External PhD representative of MPIPZ

Workshops/courses

- “Social Media in Science” by PLANT 2030 ACADEMY
- Career training webinar series by Sarah Blackford
- “Legal Basics for Plant Scientists” by PLANT 2030 ACADEMY
- Lecture “Technology Patents & Technology Licensing” by Dr. Erik Jongedijk (KWS)
- Workshop “Introduction into Patent Issues” by Prof. Dr. Peter Schreier
- “Understanding the psyche better – accepting individuality, recognizing and acting on warning signals” by Dr. Angela Heinrich
- “Introduction to RNA-seq data analysis and ChIP-seq data analysis” by Dr. Barbara Kracher
- Participation in Summer School Green Genetics 2014 organized by WUR

Languages

Polish – Native speaker

English – C1/C2

German – B2

French – A2

Dutch – A2

Publications

Articles

Katarzyna W. Wolinska, and Matthias L. Berens "Optimal Defense Theory 2.0: tissue-specific stress defense prioritization as an extra layer of complexity." *Communicative & Integrative Biology* 12.1 (2019): 91-95.

Matthias L. Berens, Katarzyna W. Wolinska, Stijn Spaepen, Jörg Ziegler, Tatsuya Nobori, Aswin Nair, Verena Krüler, Thomas M. Winkelmüller, Yiming Wang, Akira Mine, Dieter Becker, Ruben Garrido-Oter, Paul Schulze-Lefert, Kenichi Tsuda “Balancing trade-offs between biotic and abiotic stress responses through leaf age-dependent variation in stress hormone cross-talk” *Proceedings of the National Academy of Sciences* 2019, 201817233; DOI: 10.1073/pnas.1817233116

PhD thesis chapters

“**Molecular determination and genetic modification of flower colour in *Lilium* spp**” PhD thesis of Dr. Nur Fatihah Hasan Nudin

Nur Fatihah Hasan Nudin, Katarzyna Wolinska, Jan G. Schaart, Marian Oortwijn, Richard G.F. Visser, Frans A. Krens “Molecular mechanisms regulating anthocyanin biosynthesis during flower development in *Lilium* spp.”

“Exploring the genetics underlying the responses to consecutive combinations of biotic stresses and drought in *Arabidopsis thaliana*” PhD thesis of Dr. Pingping Huang

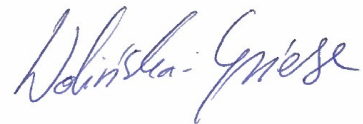
Pingping Huang, Mohamed El-Soda, Katarzyna W. Wolinska, Nelson H. Davila Olivas, Marcel Dike, Kaige Zhao, Jan van Kan, Mark G. M. Aarts „Genetic variation in the response of *Arabidopsis thaliana* to consecutive biotic (*Pieris rapae* and *Botrytis cinerea*) stresses on subsequent drought”

Pingping Huang, Mohamed El-Soda, Katarzyna W. Wolinska, Silvia Coolen, Hans van Pelt, Corné Pieterse, Jan van Kan, Mark G. M. Aarts „Genome-wide association mapping of biomass responses to sequential drought and *Botrytis cinerea* treatments in *Arabidopsis thaliana*”

Posters

Katarzyna W. Wolinska, Brigitte Pickel, Thorsten Thiergart and Stéphane Hacquard “Role of the plant innate immune system in controlled accommodation of beneficial microbes in *Arabidopsis thaliana* roots”. Poster presented on Phytobiomes conference in Montpellier, France on 4-6 December 2018

Pingping Huang, Katarzyna Wolinska, Silvia Coolen, Hans van Pelt, Corné Pieterse and Mark G.M. Aarts „Genome-wide association analysis identifies a gene required for tolerance to the combined stress of drought and *Botrytis* infection”. Poster presented on Gordon Research Conference Salt & Water Stress in Plants in Newry, ME in United States August 2014



Annex II

bacterial community			bacterial community			bacterial community		
phylum/class	genotype	Letter	Family	genotype	Letter	Family	genotype	Letter
Actinobacteria	input	d	Alcaligenaceae	input	abc	Microbacteriaceae	input	abc
Actinobacteria	peat	cd	Alcaligenaceae	peat	cd	Microbacteriaceae	peat	b
Actinobacteria	WT	ab	Alcaligenaceae	WT	bcd	Microbacteriaceae	WT	ab
Actinobacteria	bak1/bkk1	bcd	Alcaligenaceae	bak1/bkk1	abc	Microbacteriaceae	bak1/bkk1	ac
Actinobacteria	bak1/bkk1/ork1	ab	Alcaligenaceae	bak1/bkk1/ork1	ab	Microbacteriaceae	bak1/bkk1/ork1	abc
Actinobacteria	efr/fls2/ork1	abcd	Alcaligenaceae	efr/fls2/ork1	abc	Microbacteriaceae	efr/fls2/ork1	ac
Actinobacteria	lyk5	a	Alcaligenaceae	lyk5	bcd	Microbacteriaceae	lyk5	ab
Actinobacteria	apex1	ab	Alcaligenaceae	apex1	abcd	Microbacteriaceae	apex1	ab
Actinobacteria	apex2	ab	Alcaligenaceae	apex2	a	Microbacteriaceae	apex2	c
Actinobacteria	apex3	ab	Alcaligenaceae	apex3	ab	Microbacteriaceae	apex3	abc
Actinobacteria	wrky33/40	abcd	Alcaligenaceae	wrky33/40	bcd	Microbacteriaceae	wrky33/40	ab
Actinobacteria	wrky33	ab	Alcaligenaceae	wrky33	abc	Microbacteriaceae	wrky33	abc
Actinobacteria	deps	abcd	Alcaligenaceae	deps	ab	Microbacteriaceae	deps	c
Actinobacteria	pad4	abcd	Alcaligenaceae	pad4	bcd	Microbacteriaceae	pad4	abc
Actinobacteria	cyp79b2/b3	abc	Alcaligenaceae	cyp79b2/b3	a	Microbacteriaceae	cyp79b2/b3	ac
Actinobacteria	355BR1	a	Alcaligenaceae	355BR1	ab	Microbacteriaceae	355BR1	abc
Actinobacteria	br1301	abc	Alcaligenaceae	br1301	ab	Microbacteriaceae	br1301	c
Actinobacteria	rar1	abcd	Alcaligenaceae	rar1	d	Microbacteriaceae	rar1	abc
Alphaproteobacteria	input	e	Bradyrhizobiaceae	input	ade	Mycobacteriaceae	input	a
Alphaproteobacteria	peat	ab	Bradyrhizobiaceae	peat	c	Mycobacteriaceae	peat	de
Alphaproteobacteria	WT	bef	Bradyrhizobiaceae	WT	bcd	Mycobacteriaceae	WT	abcde
Alphaproteobacteria	bak1/bkk1	de	Bradyrhizobiaceae	bak1/bkk1	abcd	Mycobacteriaceae	bak1/bkk1	abc
Alphaproteobacteria	bak1/bkk1/ork1	ab	Bradyrhizobiaceae	bak1/bkk1/ork1	abcd	Mycobacteriaceae	bak1/bkk1/ork1	abcde
Alphaproteobacteria	efr/fls2/ork1	de	Bradyrhizobiaceae	efr/fls2/ork1	abcd	Mycobacteriaceae	efr/fls2/ork1	abcde
Alphaproteobacteria	lyk5	ac	Bradyrhizobiaceae	lyk5	abcd	Mycobacteriaceae	lyk5	cde
Alphaproteobacteria	apex1	ac	Bradyrhizobiaceae	apex1	abcd	Mycobacteriaceae	apex1	abc
Alphaproteobacteria	apex2	ab	Bradyrhizobiaceae	apex2	abcd	Mycobacteriaceae	apex2	abc
Alphaproteobacteria	apex3	ab	Bradyrhizobiaceae	apex3	abcd	Mycobacteriaceae	apex3	ab
Alphaproteobacteria	wrky33/40	de	Bradyrhizobiaceae	wrky33/40	abc	Mycobacteriaceae	wrky33/40	abcde
Alphaproteobacteria	wrky33	ac	Bradyrhizobiaceae	wrky33	bc	Mycobacteriaceae	wrky33	abcd
Alphaproteobacteria	deps	def	Bradyrhizobiaceae	deps	e	Mycobacteriaceae	deps	abc
Alphaproteobacteria	pad4	de	Bradyrhizobiaceae	pad4	abcd	Mycobacteriaceae	pad4	bcde
Alphaproteobacteria	cyp79b2/b3	bf	Bradyrhizobiaceae	cyp79b2/b3	ae	Mycobacteriaceae	cyp79b2/b3	a
Alphaproteobacteria	355BR1	ab	Bradyrhizobiaceae	355BR1	abcd	Mycobacteriaceae	355BR1	abcd
Alphaproteobacteria	br1301	ac	Bradyrhizobiaceae	br1301	abcd	Mycobacteriaceae	br1301	abc
Alphaproteobacteria	rar1	d	Bradyrhizobiaceae	rar1	bc	Mycobacteriaceae	rar1	e
Bacteroidetes	input	df	Caulobacteraceae	input	ab	Paenibacillaceae	input	ab
Bacteroidetes	peat	df	Caulobacteraceae	peat	c	Paenibacillaceae	peat	b
Bacteroidetes	WT	bcd	Caulobacteraceae	WT	ab	Paenibacillaceae	WT	ab
Bacteroidetes	bak1/bkk1	ab	Caulobacteraceae	bak1/bkk1	abc	Paenibacillaceae	bak1/bkk1	ab
Bacteroidetes	bak1/bkk1/ork1	abc	Caulobacteraceae	bak1/bkk1/ork1	ab	Paenibacillaceae	bak1/bkk1/ork1	a
Bacteroidetes	efr/fls2/ork1	e	Caulobacteraceae	efr/fls2/ork1	abc	Paenibacillaceae	efr/fls2/ork1	a
Bacteroidetes	lyk5	abc	Caulobacteraceae	lyk5	abc	Paenibacillaceae	lyk5	ab
Bacteroidetes	apex1	abc	Caulobacteraceae	apex1	abc	Paenibacillaceae	apex1	ab
Bacteroidetes	apex2	abc	Caulobacteraceae	apex2	ab	Paenibacillaceae	apex2	ab
Bacteroidetes	apex3	abc	Caulobacteraceae	apex3	a	Paenibacillaceae	apex3	ab
Bacteroidetes	wrky33/40	eg	Caulobacteraceae	wrky33/40	abc	Paenibacillaceae	wrky33/40	ab
Bacteroidetes	wrky33	ac	Caulobacteraceae	wrky33	ab	Paenibacillaceae	wrky33	ab
Bacteroidetes	deps	efg	Caulobacteraceae	deps	ab	Paenibacillaceae	deps	ab
Bacteroidetes	pad4	efg	Caulobacteraceae	pad4	bc	Paenibacillaceae	pad4	ab
Bacteroidetes	cyp79b2/b3	bc	Caulobacteraceae	cyp79b2/b3	a	Paenibacillaceae	cyp79b2/b3	ab
Bacteroidetes	355BR1	abc	Caulobacteraceae	355BR1	ab	Paenibacillaceae	355BR1	ab
Bacteroidetes	br1301	abc	Caulobacteraceae	br1301	ab	Paenibacillaceae	br1301	ab
Bacteroidetes	rar1	e	Caulobacteraceae	rar1	bc	Paenibacillaceae	rar1	ab
Betaproteobacteria	input	fb	Cellulomonadaceae	input	a	Promicromonosporaceae	input	abc
Betaproteobacteria	peat	fb	Cellulomonadaceae	peat	b	Promicromonosporaceae	peat	abc
Betaproteobacteria	WT	ac	Cellulomonadaceae	WT	ab	Promicromonosporaceae	WT	abc
Betaproteobacteria	bak1/bkk1	ac	Cellulomonadaceae	bak1/bkk1	ab	Promicromonosporaceae	bak1/bkk1	abc
Betaproteobacteria	bak1/bkk1/ork1	ac	Cellulomonadaceae	bak1/bkk1/ork1	ab	Promicromonosporaceae	bak1/bkk1/ork1	abc
Betaproteobacteria	efr/fls2/ork1	ac	Cellulomonadaceae	efr/fls2/ork1	ab	Promicromonosporaceae	efr/fls2/ork1	abc
Betaproteobacteria	lyk5	ac	Cellulomonadaceae	lyk5	ab	Promicromonosporaceae	lyk5	abc
Betaproteobacteria	apex1	ac	Cellulomonadaceae	apex1	ab	Promicromonosporaceae	apex1	abc
Betaproteobacteria	apex2	ac	Cellulomonadaceae	apex2	ab	Promicromonosporaceae	apex2	abc
Betaproteobacteria	apex3	ac	Cellulomonadaceae	apex3	ab	Promicromonosporaceae	apex3	abc
Betaproteobacteria	wrky33/40	ac	Cellulomonadaceae	wrky33/40	ab	Promicromonosporaceae	wrky33/40	abc
Betaproteobacteria	wrky33	ac	Cellulomonadaceae	wrky33	ab	Promicromonosporaceae	wrky33	abc
Betaproteobacteria	deps	ac	Cellulomonadaceae	deps	ab	Promicromonosporaceae	deps	ab
Betaproteobacteria	pad4	ac	Cellulomonadaceae	pad4	ab	Promicromonosporaceae	pad4	abc
Betaproteobacteria	cyp79b2/b3	c	Cellulomonadaceae	cyp79b2/b3	ab	Promicromonosporaceae	cyp79b2/b3	abc
Betaproteobacteria	355BR1	ab	Cellulomonadaceae	355BR1	ab	Promicromonosporaceae	355BR1	abc
Betaproteobacteria	br1301	ac	Cellulomonadaceae	br1301	ab	Promicromonosporaceae	br1301	abc
Betaproteobacteria	rar1	ac	Cellulomonadaceae	rar1	ab	Promicromonosporaceae	rar1	c
Firmicutes	input	e	Comamonadaceae	input	ab	Sphingomonadaceae	input	ab
Firmicutes	peat	d	Comamonadaceae	peat	ab	Sphingomonadaceae	peat	e
Firmicutes	WT	abc	Comamonadaceae	WT	ab	Sphingomonadaceae	WT	abcd
Firmicutes	bak1/bkk1	abc	Comamonadaceae	bak1/bkk1	ab	Sphingomonadaceae	bak1/bkk1	ab
Firmicutes	bak1/bkk1/ork1	abcd	Comamonadaceae	bak1/bkk1/ork1	ab	Sphingomonadaceae	bak1/bkk1/ork1	abcde
Firmicutes	efr/fls2/ork1	bc	Comamonadaceae	efr/fls2/ork1	ab	Sphingomonadaceae	efr/fls2/ork1	abcde
Firmicutes	lyk5	abc	Comamonadaceae	lyk5	ab	Sphingomonadaceae	lyk5	bcde
Firmicutes	apex1	ad	Comamonadaceae	apex1	ab	Sphingomonadaceae	apex1	abcde
Firmicutes	apex2	abcd	Comamonadaceae	apex2	ab	Sphingomonadaceae	apex2	abcd
Firmicutes	apex3	abd	Comamonadaceae	apex3	ab	Sphingomonadaceae	apex3	abc
Firmicutes	wrky33/40	bc	Comamonadaceae	wrky33/40	ab	Sphingomonadaceae	wrky33/40	bcde
Firmicutes	wrky33	abcd	Comamonadaceae	wrky33	b	Sphingomonadaceae	wrky33	de
Firmicutes	deps	bc	Comamonadaceae	deps	ab	Sphingomonadaceae	deps	a
Firmicutes	pad4	abc	Comamonadaceae	pad4	ab	Sphingomonadaceae	pad4	bcde
Firmicutes	cyp79b2/b3	abc	Comamonadaceae	cyp79b2/b3	ab	Sphingomonadaceae	cyp79b2/b3	a
Firmicutes	355BR1	abc	Comamonadaceae	355BR1	ab	Sphingomonadaceae	355BR1	abcd
Firmicutes	br1301	abd	Comamonadaceae	br1301	a	Sphingomonadaceae	br1301	abcd
Firmicutes	rar1	ce	Comamonadaceae	rar1	ab	Sphingomonadaceae	rar1	cde
Gammaproteobacteria	input	f	Flavobacteriaceae	input	ab			
Gammaproteobacteria	peat	cdg	Flavobacteriaceae	peat	d			
Gammaproteobacteria	WT	actdg	Flavobacteriaceae	WT	a			
Gammaproteobacteria	bak1/bkk1	ef	Flavobacteriaceae	bak1/bkk1	ab			
Gammaproteobacteria	bak1/bkk1/ork1	ab	Flavobacteriaceae	bak1/bkk1/ork1	abcd			
Gammaproteobacteria	efr/fls2/ork1	eg	Flavobacteriaceae	efr/fls2/ork1	ab			
Gammaproteobacteria	lyk5	b	Flavobacteriaceae	lyk5	abcd			
Gammaproteobacteria	apex1	abc	Flavobacteriaceae	apex1	a			
Gammaproteobacteria	apex2	abcd	Flavobacteriaceae	apex2	abc			
Gammaproteobacteria	apex3	abc	Flavobacteriaceae	apex3	abc			
Gammaproteobacteria	wrky33/40	efg	Flavobacteriaceae	wrky33/40	ab			
Gammaproteobacteria	wrky33	deg	Flavobacteriaceae	wrky33	ab			
Gammaproteobacteria	deps	deg	Flavobacteriaceae	deps	a			
Gammaproteobacteria	pad4	deg	Flavobacteriaceae	pad4	bcd			
Gammaproteobacteria	cyp79b2/b3	cdg	Flavobacteriaceae	cyp79b2/b3	a			
Gammaproteobacteria	355BR1	abc	Flavobacteriaceae	355BR1	ab			
Gammaproteobacteria	br1301	actdg	Flavobacteriaceae	br1301	a			
Gammaproteobacteria	rar1	efg	Flavobacteriaceae	rar1	cd			

Annex II

fungal community		oomycetes community			
Family	Group	Letter	OTU	Group	Letter
Ascomycota	input	b	OTU_229	input	ab
Ascomycota	peat	a	OTU_229	peat	ab
Ascomycota	WT	ab	OTU_229	WT	abc
Ascomycota	bak1/bkk1	ab	OTU_229	bak1/bkk1	abc
Ascomycota	bak1/bkk1/øerk1	ab	OTU_229	bak1/bkk1/øerk1	c
Ascomycota	efr/fls2/øerk1	ab	OTU_229	efr/fls2/øerk1	b
Ascomycota	lyk5	ab	OTU_229	lyk5	abc
Ascomycota	apex1	ab	OTU_229	apex1	abc
Ascomycota	apex2	ab	OTU_229	apex2	ac
Ascomycota	apex3	ab	OTU_229	apex3	abc
Ascomycota	wrky33/40	ab	OTU_229	wrky33/40	ab
Ascomycota	wrky33	ab	OTU_229	wrky33	ac
Ascomycota	deps	ab	OTU_229	deps	abc
Ascomycota	pad4	ab	OTU_229	pad4	abc
Ascomycota	cyp79b2/b3	a	OTU_229	355BRI	ab
Ascomycota	355BRI	ab	OTU_229	bri301	abc
Ascomycota	bri301	ab	OTU_229	rar1	abc
Ascomycota	rar1	ab	OTU_229	cyp79b2b3	abc
Hypocreales	input	b	OTU_29	input	ace
Hypocreales	peat	a	OTU_29	peat	abcdef
Hypocreales	WT	a	OTU_29	WT	abcdef
Hypocreales	bak1/bkk1	a	OTU_29	bak1/bkk1	cd
Hypocreales	bak1/bkk1/øerk1	a	OTU_29	bak1/bkk1/øerk1	abef
Hypocreales	efr/fls2/øerk1	a	OTU_29	efr/fls2/øerk1	cd
Hypocreales	lyk5	a	OTU_29	lyk5	bdf
Hypocreales	apex1	a	OTU_29	apex1	abcdef
Hypocreales	apex2	a	OTU_29	apex2	abcdef
Hypocreales	apex3	a	OTU_29	apex3	abcdef
Hypocreales	wrky33/40	a	OTU_29	wrky33/40	cdef
Hypocreales	wrky33	a	OTU_29	wrky33	abcdef
Hypocreales	deps	a	OTU_29	deps	cd
Hypocreales	pad4	a	OTU_29	pad4	cdef
Hypocreales	cyp79b2/b3	a	OTU_29	355BRI	ab
Hypocreales	355BRI	a	OTU_29	bri301	abcdef
Hypocreales	bri301	a	OTU_29	rar1	cd
Hypocreales	rar1	a	OTU_29	cyp79b2b3	abcdef
Hyponectriaceae	input	b			
Hyponectriaceae	peat	ab			
Hyponectriaceae	WT	a			
Hyponectriaceae	bak1/bkk1	ab			
Hyponectriaceae	bak1/bkk1/øerk1	ab			
Hyponectriaceae	efr/fls2/øerk1	a			
Hyponectriaceae	lyk5	ab			
Hyponectriaceae	apex1	ab			
Hyponectriaceae	apex2	a			
Hyponectriaceae	apex3	ab			
Hyponectriaceae	wrky33/40	ab			
Hyponectriaceae	wrky33	a			
Hyponectriaceae	deps	ab			
Hyponectriaceae	pad4	a			
Hyponectriaceae	cyp79b2/b3	a			
Hyponectriaceae	355BRI	a			
Hyponectriaceae	bri301	a			
Hyponectriaceae	rar1	ab			
Lulworthiaceae	input	b			
Lulworthiaceae	peat	b			
Lulworthiaceae	WT	ab			
Lulworthiaceae	bak1/bkk1	ab			
Lulworthiaceae	bak1/bkk1/øerk1	a			
Lulworthiaceae	efr/fls2/øerk1	ab			
Lulworthiaceae	lyk5	ab			
Lulworthiaceae	apex1	ab			
Lulworthiaceae	apex2	ab			
Lulworthiaceae	apex3	ab			
Lulworthiaceae	wrky33/40	ab			
Lulworthiaceae	wrky33	ab			
Lulworthiaceae	deps	ab			
Lulworthiaceae	pad4	ab			
Lulworthiaceae	cyp79b2/b3	ab			
Lulworthiaceae	355BRI	ab			
Lulworthiaceae	bri301	ab			
Lulworthiaceae	rar1	ab			
Nectriaceae	input	abcd			
Nectriaceae	peat	e			
Nectriaceae	WT	abcde			
Nectriaceae	bak1/bkk1	abcde			
Nectriaceae	bak1/bkk1/øerk1	a			
Nectriaceae	efr/fls2/øerk1	abcde			
Nectriaceae	lyk5	abc			
Nectriaceae	apex1	abc			
Nectriaceae	apex2	abc			
Nectriaceae	apex3	abcd			
Nectriaceae	wrky33/40	bcde			
Nectriaceae	wrky33	ac			
Nectriaceae	deps	abcde			
Nectriaceae	pad4	de			
Nectriaceae	cyp79b2/b3	bde			
Nectriaceae	355BRI	abcd			
Nectriaceae	bri301	a			
Nectriaceae	rar1	bde			
Pleosporaceae	input	c			
Pleosporaceae	peat	bc			
Pleosporaceae	WT	ab			
Pleosporaceae	bak1/bkk1	abc			
Pleosporaceae	bak1/bkk1/øerk1	ab			
Pleosporaceae	efr/fls2/øerk1	abc			
Pleosporaceae	lyk5	abc			
Pleosporaceae	apex1	abc			
Pleosporaceae	apex2	ab			
Pleosporaceae	apex3	a			
Pleosporaceae	wrky33/40	abc			
Pleosporaceae	wrky33	a			
Pleosporaceae	deps	ab			
Pleosporaceae	pad4	ab			
Pleosporaceae	cyp79b2/b3	a			
Pleosporaceae	355BRI	a			
Pleosporaceae	bri301	ab			
Pleosporaceae	rar1	abc			

Annex III

bacterial community				bacterial community				bacterial community			
phylum/class	genotype	treatment	letter	Family	genotype	treatment	letter	Family	genotype	treatment	letter
Actinobacteria	input		b	Alcaligenaceae	input		e	Hyphomicrobiaceae	input		b
Actinobacteria	peat	B	ab	Alcaligenaceae	peat	B	de	Hyphomicrobiaceae	peat	B	ab
Actinobacteria	peat	BF	ab	Alcaligenaceae	peat	BF	bde	Hyphomicrobiaceae	peat	BF	ab
Actinobacteria	peat	BO	b	Alcaligenaceae	peat	BO	bde	Hyphomicrobiaceae	peat	BO	ab
Actinobacteria	peat	BFO	ab	Alcaligenaceae	peat	BFO	de	Hyphomicrobiaceae	peat	BFO	ab
Actinobacteria	WT	B	a	Alcaligenaceae	WT	B	ac	Hyphomicrobiaceae	WT	B	ab
Actinobacteria	WT	BF	a	Alcaligenaceae	WT	BF	ac	Hyphomicrobiaceae	WT	BF	ab
Actinobacteria	WT	BO	a	Alcaligenaceae	WT	BO	ac	Hyphomicrobiaceae	WT	BO	ab
Actinobacteria	WT	BFO	a	Alcaligenaceae	WT	BFO	abc	Hyphomicrobiaceae	WT	BFO	ab
Actinobacteria	bak1/bk1	B	a	Alcaligenaceae	bak1/bk1	B	abc	Hyphomicrobiaceae	bak1/bk1	B	ab
Actinobacteria	bak1/bk1	BF	ab	Alcaligenaceae	bak1/bk1	BF	abd	Hyphomicrobiaceae	bak1/bk1	BF	ab
Actinobacteria	bak1/bk1	BO	a	Alcaligenaceae	bak1/bk1	BO	ac	Hyphomicrobiaceae	bak1/bk1	BO	ab
Actinobacteria	bak1/bk1	BFO	a	Alcaligenaceae	bak1/bk1	BFO	ac	Hyphomicrobiaceae	bak1/bk1	BFO	ab
Actinobacteria	cy7/2/b3	B	a	Alcaligenaceae	cy7/2/b3	B	abc	Hyphomicrobiaceae	cy7/2/b3	B	ab
Actinobacteria	cy7/2/b3	BF	a	Alcaligenaceae	cy7/2/b3	BF	ac	Hyphomicrobiaceae	cy7/2/b3	BF	a
Actinobacteria	cy7/2/b3	BO	a	Alcaligenaceae	cy7/2/b3	BO	c	Hyphomicrobiaceae	cy7/2/b3	BO	ab
Actinobacteria	cy7/2/b3	BFO	ab	Alcaligenaceae	cy7/2/b3	BFO	abc	Hyphomicrobiaceae	cy7/2/b3	BFO	ab
Alphaproteobacteria	input		b	Bacillaceae	input		b	Intrasporangiaceae	input		b
Alphaproteobacteria	peat	B	a	Bacillaceae	peat	B	a	Intrasporangiaceae	peat	B	ab
Alphaproteobacteria	peat	BF	a	Bacillaceae	peat	BF	a	Intrasporangiaceae	peat	BF	ab
Alphaproteobacteria	peat	BO	a	Bacillaceae	peat	BO	a	Intrasporangiaceae	peat	BO	ab
Alphaproteobacteria	peat	BFO	a	Bacillaceae	peat	BFO	a	Intrasporangiaceae	peat	BFO	ab
Alphaproteobacteria	WT	B	a	Bacillaceae	WT	B	a	Intrasporangiaceae	WT	B	a
Alphaproteobacteria	WT	BF	a	Bacillaceae	WT	BF	a	Intrasporangiaceae	WT	BF	a
Alphaproteobacteria	WT	BO	a	Bacillaceae	WT	BO	a	Intrasporangiaceae	WT	BO	a
Alphaproteobacteria	WT	BFO	ab	Bacillaceae	WT	BFO	a	Intrasporangiaceae	WT	BFO	a
Alphaproteobacteria	bak1/bk1	B	a	Bacillaceae	bak1/bk1	B	a	Intrasporangiaceae	bak1/bk1	B	a
Alphaproteobacteria	bak1/bk1	BF	a	Bacillaceae	bak1/bk1	BF	a	Intrasporangiaceae	bak1/bk1	BF	a
Alphaproteobacteria	bak1/bk1	BO	a	Bacillaceae	bak1/bk1	BO	a	Intrasporangiaceae	bak1/bk1	BO	a
Alphaproteobacteria	bak1/bk1	BFO	a	Bacillaceae	bak1/bk1	BFO	a	Intrasporangiaceae	bak1/bk1	BFO	a
Alphaproteobacteria	cy7/2/b3	B	a	Bacillaceae	cy7/2/b3	B	a	Intrasporangiaceae	cy7/2/b3	B	a
Alphaproteobacteria	cy7/2/b3	BF	a	Bacillaceae	cy7/2/b3	BF	a	Intrasporangiaceae	cy7/2/b3	BF	a
Alphaproteobacteria	cy7/2/b3	BO	a	Bacillaceae	cy7/2/b3	BO	a	Intrasporangiaceae	cy7/2/b3	BO	a
Alphaproteobacteria	cy7/2/b3	BFO	a	Bacillaceae	cy7/2/b3	BFO	a	Intrasporangiaceae	cy7/2/b3	BFO	a
Bacteroidetes	input		c	Bradyrhizobiaceae	input		c	Methylobacteriaceae	input		e
Bacteroidetes	peat	B	bc	Bradyrhizobiaceae	peat	B	ab	Methylobacteriaceae	peat	B	abcde
Bacteroidetes	peat	BF	abc	Bradyrhizobiaceae	peat	BF	ab	Methylobacteriaceae	peat	BF	abc
Bacteroidetes	peat	BO	abc	Bradyrhizobiaceae	peat	BO	b	Methylobacteriaceae	peat	BO	abcde
Bacteroidetes	peat	BFO	bc	Bradyrhizobiaceae	peat	BFO	ab	Methylobacteriaceae	peat	BFO	cde
Bacteroidetes	WT	B	ab	Bradyrhizobiaceae	WT	B	ab	Methylobacteriaceae	WT	B	abd
Bacteroidetes	WT	BF	ab	Bradyrhizobiaceae	WT	BF	ab	Methylobacteriaceae	WT	BF	abcde
Bacteroidetes	WT	BO	ab	Bradyrhizobiaceae	WT	BO	ab	Methylobacteriaceae	WT	BO	ab
Bacteroidetes	WT	BFO	b	Bradyrhizobiaceae	WT	BFO	a	Methylobacteriaceae	WT	BFO	ab
Bacteroidetes	bak1/bk1	B	ab	Bradyrhizobiaceae	bak1/bk1	B	ab	Methylobacteriaceae	bak1/bk1	B	abcde
Bacteroidetes	bak1/bk1	BF	ab	Bradyrhizobiaceae	bak1/bk1	BF	ab	Methylobacteriaceae	bak1/bk1	BF	abcd
Bacteroidetes	bak1/bk1	BO	a	Bradyrhizobiaceae	bak1/bk1	BO	ab	Methylobacteriaceae	bak1/bk1	BO	ab
Bacteroidetes	bak1/bk1	BFO	a	Bradyrhizobiaceae	bak1/bk1	BFO	ab	Methylobacteriaceae	bak1/bk1	BFO	a
Bacteroidetes	cy7/2/b3	B	ab	Bradyrhizobiaceae	cy7/2/b3	B	ab	Methylobacteriaceae	cy7/2/b3	B	abcde
Bacteroidetes	cy7/2/b3	BF	ab	Bradyrhizobiaceae	cy7/2/b3	BF	ab	Methylobacteriaceae	cy7/2/b3	BF	abcd
Bacteroidetes	cy7/2/b3	BO	a	Bradyrhizobiaceae	cy7/2/b3	BO	a	Methylobacteriaceae	cy7/2/b3	BO	abcde
Bacteroidetes	cy7/2/b3	BFO	ab	Bradyrhizobiaceae	cy7/2/b3	BFO	ab	Methylobacteriaceae	cy7/2/b3	BFO	bcde
Betaproteobacteria	input		bc	Caulobacteraceae	input		a	Microbacteriaceae	input		b
Betaproteobacteria	peat	B	def	Caulobacteraceae	peat	B	c	Microbacteriaceae	peat	B	ab
Betaproteobacteria	peat	BF	f	Caulobacteraceae	peat	BF	c	Microbacteriaceae	peat	BF	ab
Betaproteobacteria	peat	BO	f	Caulobacteraceae	peat	BO	bc	Microbacteriaceae	peat	BO	ab
Betaproteobacteria	peat	BFO	f	Caulobacteraceae	peat	BFO	c	Microbacteriaceae	peat	BFO	ab
Betaproteobacteria	WT	B	abc	Caulobacteraceae	WT	B	abc	Microbacteriaceae	WT	B	a
Betaproteobacteria	WT	BF	abc	Caulobacteraceae	WT	BF	abc	Microbacteriaceae	WT	BF	a
Betaproteobacteria	WT	BO	abc	Caulobacteraceae	WT	BO	abc	Microbacteriaceae	WT	BO	a
Betaproteobacteria	WT	BFO	abc	Caulobacteraceae	WT	BFO	abc	Microbacteriaceae	WT	BFO	a
Betaproteobacteria	bak1/bk1	B	abcd	Caulobacteraceae	bak1/bk1	B	abc	Microbacteriaceae	bak1/bk1	B	a
Betaproteobacteria	bak1/bk1	BF	abc	Caulobacteraceae	bak1/bk1	BF	abc	Microbacteriaceae	bak1/bk1	BF	a
Betaproteobacteria	bak1/bk1	BO	abcd	Caulobacteraceae	bak1/bk1	BO	abc	Microbacteriaceae	bak1/bk1	BO	a
Betaproteobacteria	bak1/bk1	BFO	abcd	Caulobacteraceae	bak1/bk1	BFO	abc	Microbacteriaceae	bak1/bk1	BFO	a
Betaproteobacteria	cy7/2/b3	B	c	Caulobacteraceae	cy7/2/b3	B	abc	Microbacteriaceae	cy7/2/b3	B	a
Betaproteobacteria	cy7/2/b3	BF	abc	Caulobacteraceae	cy7/2/b3	BF	abc	Microbacteriaceae	cy7/2/b3	BF	ab
Betaproteobacteria	cy7/2/b3	BO	abc	Caulobacteraceae	cy7/2/b3	BO	ab	Microbacteriaceae	cy7/2/b3	BO	a
Betaproteobacteria	cy7/2/b3	BFO	abcd	Caulobacteraceae	cy7/2/b3	BFO	abc	Microbacteriaceae	cy7/2/b3	BFO	a
Firmicutes	input		d	CeLulomonadaceae	input		a	Mycobacteriaceae	input		d
Firmicutes	peat	B	abc	CeLulomonadaceae	peat	B	abc	Mycobacteriaceae	peat	B	c
Firmicutes	peat	BF	abc	CeLulomonadaceae	peat	BF	d	Mycobacteriaceae	peat	BF	bc
Firmicutes	peat	BO	abc	CeLulomonadaceae	peat	BO	abc	Mycobacteriaceae	peat	BO	bc
Firmicutes	peat	BFO	abc	CeLulomonadaceae	peat	BFO	bc	Mycobacteriaceae	peat	BFO	c
Firmicutes	WT	B	cd	CeLulomonadaceae	WT	B	a	Mycobacteriaceae	WT	B	abc
Firmicutes	WT	BF	abc	CeLulomonadaceae	WT	BF	abc	Mycobacteriaceae	WT	BF	abd
Firmicutes	WT	BO	abc	CeLulomonadaceae	WT	BO	a	Mycobacteriaceae	WT	BO	abc
Firmicutes	WT	BFO	abc	CeLulomonadaceae	WT	BFO	abc	Mycobacteriaceae	WT	BFO	ab
Firmicutes	bak1/bk1	B	abc	CeLulomonadaceae	bak1/bk1	B	abc	Mycobacteriaceae	bak1/bk1	B	ab
Firmicutes	bak1/bk1	BF	ab	CeLulomonadaceae	bak1/bk1	BF	abc	Mycobacteriaceae	bak1/bk1	BF	abc
Firmicutes	bak1/bk1	BO	abc	CeLulomonadaceae	bak1/bk1	BO	abc	Mycobacteriaceae	bak1/bk1	BO	abd
Firmicutes	bak1/bk1	BFO	a	CeLulomonadaceae	bak1/bk1	BFO	abc	Mycobacteriaceae	bak1/bk1	BFO	ad
Firmicutes	cy7/2/b3	B	c	CeLulomonadaceae	cy7/2/b3	B	a	Mycobacteriaceae	cy7/2/b3	B	bc
Firmicutes	cy7/2/b3	BF	abc	CeLulomonadaceae	cy7/2/b3	BF	abc	Mycobacteriaceae	cy7/2/b3	BF	abcd
Firmicutes	cy7/2/b3	BO	abc	CeLulomonadaceae	cy7/2/b3	BO	a	Mycobacteriaceae	cy7/2/b3	BO	abd
Firmicutes	cy7/2/b3	BFO	bc	CeLulomonadaceae	cy7/2/b3	BFO	abc	Mycobacteriaceae	cy7/2/b3	BFO	bc
Gammaproteobacteria	input		d	Comamonadaceae	input		bcd	Nocardioideae	input		a
Gammaproteobacteria	peat	B	ce	Comamonadaceae	peat	B	d	Nocardioideae	peat	B	ab
Gammaproteobacteria	peat	BF	bce	Comamonadaceae	peat	BF	d	Nocardioideae	peat	BF	ab
Gammaproteobacteria	peat	BO	abce	Comamonadaceae	peat	BO	cd	Nocardioideae	peat	BO	b
Gammaproteobacteria	peat	BFO	a	Comamonadaceae	peat	BFO	d	Nocardioideae	peat	BFO	ab
Gammaproteobacteria	WT	B	abce	Comamonadaceae	WT	B	ab	Nocardioideae	WT	B	ab
Gammaproteobacteria	WT	BF	abce	Comamonadaceae	WT	BF	ab	Nocardioideae	WT	BF	ab
Gammaproteobacteria	WT	BO	abce	Comamonadaceae	WT	BO	a	Nocardioideae	WT	BO	ab
Gammaproteobacteria	WT	BFO	ab	Comamonadaceae	WT	BFO	ab	Nocardioideae	WT	BFO	ab
Gammaproteobacteria	bak1/bk1	B	abc	Comamonadaceae	bak1/bk1	B	ab	Nocardioideae	bak1/bk1	B	ab
Gammaproteobacteria	bak1/bk1	BF	abc	Comamonadaceae	bak1/bk1	BF	ab	Nocardioideae	bak1/bk1	BF	ab
Gammaproteobacteria	bak1/bk1	BO	abd	Comamonadaceae	bak1/bk1	BO	ab	Nocardioideae	bak1/bk1	BO	ab
Gammaproteobacteria	bak1/bk1	BFO	ad	Comamonadaceae	bak1/bk1	BFO	abc	Nocardioideae	bak1/bk1	BFO	ab
Gammaproteobacteria	cy7/2/b3	B	ab	Comamonadaceae	cy7/2/b3	B	a	Nocardioideae	cy7/2/b3	B	ab
Gammaproteobacteria	cy7/2/b3	BF	abc	Comamonadaceae	cy7/2/b3	BF	ab	Nocardioideae	cy7/2/b3	BF	ab
Gammaproteobacteria	cy7/2/b3	BO	abd	Comamonadaceae	cy7/2/b3	BO	ab	Nocardioideae	cy7/2/b3	BO	ab
Gammaproteobacteria	cy7/2/b3	BFO	abce	Comamonadaceae	cy7/2/b3	BFO	bcd	Nocardioideae	cy7/2/b3	BFO	ab
Flavobacteriaceae	input		c	Flavobacteriaceae	input		c	Paenibacillaceae	input		e
Flavobacteriaceae	peat	B	bc	Flavobacteriaceae	peat	B	bc	Paenibacillaceae	peat	B	abcde
Flavobacteriaceae	peat	BF	abc	Flavobacteriaceae	peat	BF	abc	Paenibacillaceae	peat	BF	abcd
Flavobacteriaceae	peat	BO	abc	Flavobacteriaceae	peat	BO	bc	Paenibacillaceae	peat	BO	abcd
Flavobacteriaceae	peat	BFO	bc	Flavobacteriaceae	peat	BFO	bc	Paenibacillaceae	peat	BFO	abcd
Flavobacteriaceae	WT	B	ab	Flavobacteriaceae	WT	B	ab	Paenibacillaceae	WT	B	de
Flavobacteriaceae	WT	BF	ab	Flavobacteriaceae	WT	BF	ab	Paenibacillaceae	WT	BF	abcd
Flavobacteriaceae	WT	BO	ab	Flavobacteriaceae	WT	BO	ab	Paenibacillaceae	WT	BO	abcd
Flavobacteriaceae	WT	BFO	b	Flavobacteriaceae	WT	BFO	b	Paenibacillaceae	WT	BFO	abcd
Flavobacteriaceae	bak1/bk1	B	ab	Flavobacteriaceae	bak1/bk1	B	ab	Paenibacillaceae	bak1/bk1	B	abcd
Flavobacteriaceae	bak1/bk1	BF	ab	Flavobacteriaceae	bak1/bk1	BF	ab	Paenibacillaceae	bak1/bk1	BF	ab
Flavobacteriaceae	bak1/bk1	BO	a	Flavobacteriaceae	bak1/bk1	BO	a	Paenibacillaceae	bak1/bk1	BO	abcd
Flavobacteriaceae	bak1/bk1	BFO	a	Flavobacteriaceae	bak1/bk1	BFO	a	Paenibacillaceae	bak1/bk1	BFO	a
Flavobacteriaceae	cy7/2/b3	B	ab	Flavobacteriaceae	cy7/2/b3	B	ab	Paenibacillaceae	cy7/2/b3	B	cde
Flavobacteriaceae	cy7/2/b3	BF	ab	Flavobacteriaceae	cy7/2/b3	BF	ab	Paenibacillaceae	cy7/2/b3	BF	abcd
Flavobacteriaceae	cy7/2/b3	BO	a	Flavobacteriaceae	cy7/2/b3	BO	a	Paenibacillaceae	cy7/2/b3	BO	abcd
Flavobacteriaceae	cy7/2/b3	BFO	ab	Flavobacteriaceae	cy7/2/b3	BFO	ab	Paenibacillaceae	cy7/2/b3	BFO	abcd

Annex III

bacterial community				fungal community			
Family	genotype	treatment	Letter	Family	genotype	treatment	Letter
Phyllobacteriaceae	input		b	Ascomycota	input		c
Phyllobacteriaceae	peat	B	ab	Ascomycota	peat	F	abcd
Phyllobacteriaceae	peat	BF	ab	Ascomycota	peat	BF	abcd
Phyllobacteriaceae	peat	BO	abc	Ascomycota	peat	FO	abcd
Phyllobacteriaceae	peat	BFO	ab	Ascomycota	peat	BFO	abd
Phyllobacteriaceae	WT	B	c	Ascomycota	WT	F	c
Phyllobacteriaceae	WT	BF	ac	Ascomycota	WT	BF	b
Phyllobacteriaceae	WT	BO	ac	Ascomycota	WT	FO	cd
Phyllobacteriaceae	WT	BFO	ac	Ascomycota	WT	BFO	b
Phyllobacteriaceae	bak1/bk1	B	ab	Ascomycota	bak1/bk1	F	c
Phyllobacteriaceae	bak1/bk1	BF	ab	Ascomycota	bak1/bk1	BF	ab
Phyllobacteriaceae	bak1/bk1	BO	ab	Ascomycota	bak1/bk1	FO	acd
Phyllobacteriaceae	bak1/bk1	BFO	ac	Ascomycota	bak1/bk1	BFO	ab
Phyllobacteriaceae	cy p7/3/2/3	B	ab	Ascomycota	cy p7/3/2/3	BF	b
Phyllobacteriaceae	cy p7/3/2/3	BF	ab	Ascomycota	cy p7/3/2/3	BFO	ab
Phyllobacteriaceae	cy p7/3/2/3	BO	ab	Hypocreales	input		b
Phyllobacteriaceae	cy p7/3/2/3	BFO	ab	Hypocreales	peat	F	a
Promicromonosporaceae	input		e	Hypocreales	peat	BF	a
Promicromonosporaceae	peat	B	de	Hypocreales	peat	FO	a
Promicromonosporaceae	peat	BF	cde	Hypocreales	peat	BFO	a
Promicromonosporaceae	peat	BO	abcde	Hypocreales	WT	F	a
Promicromonosporaceae	peat	BFO	abcde	Hypocreales	WT	BF	a
Promicromonosporaceae	WT	B	abcd	Hypocreales	WT	FO	a
Promicromonosporaceae	WT	BF	abc	Hypocreales	WT	BFO	a
Promicromonosporaceae	WT	BO	b	Hypocreales	bak1/bk1	F	a
Promicromonosporaceae	WT	BFO	abc	Hypocreales	bak1/bk1	BF	a
Promicromonosporaceae	bak1/bk1	B	abcd	Hypocreales	bak1/bk1	FO	a
Promicromonosporaceae	bak1/bk1	BF	abcd	Hypocreales	bak1/bk1	BFO	a
Promicromonosporaceae	bak1/bk1	BO	abcd	Hypocreales	cy p7/3/2/3	BF	a
Promicromonosporaceae	bak1/bk1	BFO	abcd	Hypocreales	cy p7/3/2/3	BFO	a
Promicromonosporaceae	cy p7/3/2/3	B	abc	Hyponectriaceae	input		e
Promicromonosporaceae	cy p7/3/2/3	BF	ab	Hyponectriaceae	peat	F	de
Promicromonosporaceae	cy p7/3/2/3	BO	b	Hyponectriaceae	peat	BF	abc
Promicromonosporaceae	cy p7/3/2/3	BFO	acd	Hyponectriaceae	peat	FO	abcd
Pseudomonadaceae	input		abcdeg	Hyponectriaceae	peat	BFO	abc
Pseudomonadaceae	peat	B	g	Hyponectriaceae	WT	F	abcd
Pseudomonadaceae	peat	BF	beg	Hyponectriaceae	WT	BF	abc
Pseudomonadaceae	peat	BO	bcg	Hyponectriaceae	WT	FO	abcd
Pseudomonadaceae	peat	BFO	eg	Hyponectriaceae	WT	BFO	abc
Pseudomonadaceae	WT	B	bcdeg	Hyponectriaceae	bak1/bk1	F	acd
Pseudomonadaceae	WT	BF	abcdf	Hyponectriaceae	bak1/bk1	BF	ab
Pseudomonadaceae	WT	BO	abcde	Hyponectriaceae	bak1/bk1	FO	cde
Pseudomonadaceae	WT	BFO	abcdf	Hyponectriaceae	bak1/bk1	BFO	abcd
Pseudomonadaceae	bak1/bk1	B	abcde	Hyponectriaceae	cy p7/3/2/3	BF	b
Pseudomonadaceae	bak1/bk1	BF	abcdf	Hyponectriaceae	cy p7/3/2/3	BFO	abc
Pseudomonadaceae	bak1/bk1	BO	adf	Lulworthiaceae	input		e
Pseudomonadaceae	bak1/bk1	BFO	f	Lulworthiaceae	peat	F	c
Pseudomonadaceae	cy p7/3/2/3	B	abcd	Lulworthiaceae	peat	BF	ac
Pseudomonadaceae	cy p7/3/2/3	BF	af	Lulworthiaceae	peat	FO	ac
Pseudomonadaceae	cy p7/3/2/3	BO	af	Lulworthiaceae	peat	BFO	ac
Pseudomonadaceae	cy p7/3/2/3	BFO	acdf	Lulworthiaceae	WT	F	abc
Rhizobiaceae	input		e	Lulworthiaceae	WT	BF	ac
Rhizobiaceae	peat	B	d	Lulworthiaceae	WT	FO	abc
Rhizobiaceae	peat	BF	cd	Lulworthiaceae	WT	BFO	ac
Rhizobiaceae	peat	BO	d	Lulworthiaceae	bak1/bk1	F	abc
Rhizobiaceae	peat	BFO	acd	Lulworthiaceae	bak1/bk1	BF	abc
Rhizobiaceae	WT	B	abc	Lulworthiaceae	bak1/bk1	FO	abc
Rhizobiaceae	WT	BF	ab	Lulworthiaceae	bak1/bk1	BFO	abc
Rhizobiaceae	WT	BO	ab	Lulworthiaceae	cy p7/3/2/3	BF	ab
Rhizobiaceae	WT	BFO	b	Lulworthiaceae	cy p7/3/2/3	BFO	ac
Rhizobiaceae	bak1/bk1	B	abc	Nectriaceae	input		ce
Rhizobiaceae	bak1/bk1	BF	abc	Nectriaceae	peat	F	abce
Rhizobiaceae	bak1/bk1	BO	abc	Nectriaceae	peat	BF	ce
Rhizobiaceae	bak1/bk1	BFO	ab	Nectriaceae	peat	FO	abde
Rhizobiaceae	cy p7/3/2/3	B	ab	Nectriaceae	peat	BFO	e
Rhizobiaceae	cy p7/3/2/3	BF	abcd	Nectriaceae	WT	F	abd
Rhizobiaceae	cy p7/3/2/3	BO	abc	Nectriaceae	WT	BF	ce
Rhizobiaceae	cy p7/3/2/3	BFO	abc	Nectriaceae	WT	FO	d
Streptomycetaceae	input		b	Nectriaceae	WT	BFO	ce
Streptomycetaceae	peat	B	a	Nectriaceae	bak1/bk1	F	ad
Streptomycetaceae	peat	BF	a	Nectriaceae	bak1/bk1	BF	abcd
Streptomycetaceae	peat	BO	a	Nectriaceae	bak1/bk1	FO	abd
Streptomycetaceae	peat	BFO	a	Nectriaceae	bak1/bk1	BFO	abc
Streptomycetaceae	WT	B	a	Nectriaceae	cy p7/3/2/3	BF	bc
Streptomycetaceae	WT	BF	a	Nectriaceae	cy p7/3/2/3	BFO	ce
Streptomycetaceae	WT	BO	a	Plectosphaerellaceae	input		e
Streptomycetaceae	WT	BFO	a	Plectosphaerellaceae	peat	F	abcde
Streptomycetaceae	bak1/bk1	B	a	Plectosphaerellaceae	peat	BF	de
Streptomycetaceae	bak1/bk1	BF	a	Plectosphaerellaceae	peat	FO	abcde
Streptomycetaceae	bak1/bk1	BO	a	Plectosphaerellaceae	peat	BFO	de
Streptomycetaceae	bak1/bk1	BFO	a	Plectosphaerellaceae	WT	BF	ab
Streptomycetaceae	cy p7/3/2/3	B	a	Plectosphaerellaceae	WT	BF	acd
Streptomycetaceae	cy p7/3/2/3	BF	a	Plectosphaerellaceae	WT	FO	b
Streptomycetaceae	cy p7/3/2/3	BO	a	Plectosphaerellaceae	WT	BFO	cde
Streptomycetaceae	cy p7/3/2/3	BFO	a	Plectosphaerellaceae	bak1/bk1	F	ab
Xanthomonadaceae	input		c	Plectosphaerellaceae	bak1/bk1	BF	abc
Xanthomonadaceae	peat	B	ac	Plectosphaerellaceae	bak1/bk1	FO	abc
Xanthomonadaceae	peat	BF	ab	Plectosphaerellaceae	bak1/bk1	BFO	abcd
Xanthomonadaceae	peat	BO	abc	Plectosphaerellaceae	cy p7/3/2/3	BF	acde
Xanthomonadaceae	peat	BFO	ac	Plectosphaerellaceae	cy p7/3/2/3	BFO	cde
Xanthomonadaceae	WT	B	ab	Pleosporaceae	input		c
Xanthomonadaceae	WT	BF	b	Pleosporaceae	peat	F	abd
Xanthomonadaceae	WT	BO	ab	Pleosporaceae	peat	BF	ab
Xanthomonadaceae	WT	BFO	a	Pleosporaceae	peat	FO	abd
Xanthomonadaceae	bak1/bk1	B	a	Pleosporaceae	peat	BFO	ab
Xanthomonadaceae	bak1/bk1	BF	ab	Pleosporaceae	WT	F	cd
Xanthomonadaceae	bak1/bk1	BO	ab	Pleosporaceae	WT	BF	a
Xanthomonadaceae	bak1/bk1	BFO	ab	Pleosporaceae	WT	FO	bd
Xanthomonadaceae	cy p7/3/2/3	B	a	Pleosporaceae	WT	BFO	a
Xanthomonadaceae	cy p7/3/2/3	BF	ab	Pleosporaceae	bak1/bk1	F	cd
Xanthomonadaceae	cy p7/3/2/3	BO	ab	Pleosporaceae	bak1/bk1	BF	ab
Xanthomonadaceae	cy p7/3/2/3	BFO	ab	Pleosporaceae	bak1/bk1	FO	abcd
				Pleosporaceae	bak1/bk1	BFO	ab
				Pleosporaceae	cy p7/3/2/3	BF	ab
				Pleosporaceae	cy p7/3/2/3	BFO	ab