Elemental profiling of symbiotic bark and ambrosia beetle fungi in comparison to wood-degrading fungi

von

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Bachelor-Thesis

im Studiengang Biotechnologie
Fakultät Angewandte Naturwissenschaften,
Energie- und Gebäudetechnik
Hochschule Esslingen

vorgelegt am 23. Februar 2023

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gesperrt bis:	……

Abstract

For millions of years, bark and ambrosia beetles utilized weakened or dying trees as their natural habitat. Due to climate change, these beetles pose an increasing challenge to forest ecosystems worldwide. However, bark and ambrosia beetles cannot conquer this habitat alone and hence, live in symbiosis with various beneficial filamentous fungi, yeasts and other organisms. These so-called mutualists have been proposed to help overcome the trees' defenses and to ensure the survival of the beetles through nutritional supplementation. Nonetheless, studies on the actual nutritional benefits of mutualistic filamentous fungi are missing. Here, several mutualistic fungi were compared with wood-degrading fungi and pathogens for their ability to accumulate elements from wood. The ICP-OES and the elemental analyzer were used to determine the elemental accumulation of fourteen biologically relevant elements. The results indicated that the mutualistic fungi cannot be referred to as a generally more nutritious group. The wood-degrading fungi and pathogens showed similar elemental accumulations. Hence, the symbiosis must be driven by other factors e.g., advanced food transportation, possible beneficial secondary metabolites or a far more complex interaction of different microorganisms can be discussed. Further, the ability of fungi to accumulate nutrients could be connected to the sociality of the beetles.

Zusammenfassung

Borken- und Ambrosiakäfer nutzen seit Millionen von Jahren geschwächte oder absterbende Bäume als natürlichen Lebensraum. Aufgrund des Klimawandels stellen diese Käfer die Waldökosysteme weltweit zunehmend vor eine Herausforderung. Borken- und Ambrosiakäfer können diesen Lebensraum jedoch nicht allein bestreiten und leben daher in Symbiose mit verschiedenen nützlichen filamentösen Pilzen, Hefen und anderen Organismen. Diese sogenannten Mutualisten sollen dabei helfen, die Abwehrmechanismen der Bäume zu überwinden und durch Nährstoffbereitstellung das Überleben der Käfer sichern. Studien, zum tatsächlichen Nährstoffgehalt von mutualistischen Pilzen, wurden bisher nicht durchgeführt. In dieser Arbeit wurden mehrere mutualistische Pilze mit holzabbauenden und pathogenen Pilzen auf ihre Fähigkeit, Elemente aus dem Holz anzureichern, verglichen. Die ICP-OES und der Elementaranalysator wurden verwendet, um den Gehalt von vierzehn biologisch relevanten Elementen zu bestimmen. Die Ergebnisse zeigten, dass die mutualistischen Pilze nicht als eine allgemein nährstoffreichere Gruppe bezeichnet werden können. Die holzabbauenden Pilze, sowie die Pathogene zeigten ähnliche Elementgehalte. Als Folge dessen, muss die Symbiose von anderen bzw. zusätzlichen Faktoren abhängig sein, wie verbessertem Nährstofftransport, nützliche sekundär Metabolite oder ein weitaus komplexeres Zusammenspiel verschiedener Mikroorganismen. Darüber hinaus könnte die Fähigkeit von Pilzen, Nährstoffe anzureichern, mit der Sozialität der Käfer in Verbindung gebracht werden.

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Declaration

I certify that I have worked on this thesis independently and without any aids other than those specified. The positions taken directly or indirectly from external sources are marked as such.

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Place, date Signature

1 Introduction

The term symbiosis merely defines the state of two different organisms living together in a relationship (de Bary 1897). The actual interactions between two co-existing organisms are defined through several subcategories. For instance 1: parasitism, one organism harms another by living from its resources (Leung und Poulin 2008), 2: commensalism, when one organism is unaffected by the presence of another organism, while the later gains an advantage (Six und Wingfield 2011), and 3: mutualism, a popular category of a symbiosis, where both organisms are benefitting from the interaction (Six und Wingfield 2011; Leung und Poulin 2008). The subject of this thesis is a rather unknown mutualistic symbiosis, but with a big impact on ecosystems worldwide.

The term Curculionidae describes the family of weevils in the order Coleoptera. Two of the subfamilies of the Curculionidae are the Scolytinae and the Platypodinae, which can also be referred to as bark and ambrosia beetles. Those beetles can be found in many forests worldwide and they spend almost their entire life cycle hidden in dead wood (Kirkendall et al. 2015; Six und Wingfield 2011). The Scolytinae subfamily (bark beetles) includes over 6000 different species, while approximately 1400 species belong to the Platypodinae subfamily (the so-called pine borers) (Kirkendall et al. 2015). The phrase “bark and ambrosia” beetle is jointly used to refer to beetles, which lay their eggs into woody tissue, where the larvae develop to an adult beetle and then spend little time outside of trees (Kirkendall et al. 2015). The term “ambrosia beetle” describes beetles with different characteristics, which evolved independently several times from bark beetles around 21-60 million years ago (Farrell et al. 2001). Hulcr et al. (2015) state that the name ambrosia beetle is not a taxonomic classification because it describes beetle species from the Scolytinae and the Platypodinae subfamily (Batra 1963). This is justified through the requirement of ambrosia beetles to have a fungal symbiont in order to survive, which could be confirmed for species in both of the subfamilies (Hulcr et al. 2015). Beetles and larvae feed on nutritional fungi, actively cultivated by adult beetles within their breeding systems, the so-called galleries (Kirkendall et al. 2015; Francke-Grosmann 1967). Around 3400 species are classified as ambrosia beetles (Farrell et al. 2001).

In contrast, the name “bark beetle” is a taxonomic term because it describes species merely in the Scolytinae, even if they do not colonize the bark (including phloem) (Hulcr et al. 2015). In an ecological term, “bark beetle” describes insects that belong to the Scolytinae subfamily and feed on phloem of trees throughout their entire life (Harrington 2005; Hulcr et al. 2015).

Since recent years some bark and ambrosia beetles became a serious environmental treat to forests, killing an excessive amount of trees worldwide. However, most of the bark and ambrosia beetles mainly attack already weakened or recently dead trees. Only a few species of these beetles are able to infest and kill healthy trees (Six und Elser 2020; Hulcr und Stelinski 2017). In fact, beetles have always been a natural part of the forest ecosystems and exist since more than 100 million years (Cognato und Grimaldi 2009). Though, as a result of climate change, the stress for trees is increasing, which leads to more weakened trees, and thus to more potential breeding substrate for beetles (Hlásny et al. 2021).

Kirkendall et al. (2015) explains, why those beetles chose dead wood or weakened trees as their ecological niche. First, there is the protected environment underneath the bark of the trees. Weather conditions such as temperature changes, wind or rain do not pose a big threat on the beetles. Additionally, the larvae are protected from possible predators or competing insects and can develop in a safe environment. Another advantage are the reduced defense mechanisms of the host tree. A tree with a lack of water supply or with storm damage is only hardly able to produce enough toxic resin, one of the major defense strategies of conifers, to repel the beetles (Kirkendall et al. 2015).

Bark beetles establish their galleries and larval chambers under the bark, more precisely in the phloem of the tree. Here, the female drills a tunnel into the phloem from which the larval galleries expand horizontally (Six 2013; Six und Wingfield 2011). Ambrosia beetles on the other hand create their nests even deeper inside the trees. Those brood chambers can be found in the xylem of the tree (Kirkendall et al. 2015). Nevertheless, bark and ambrosia beetles do not colonize a tree alone, they transfer many microorganisms on their exoskeleton or in their guts from their nest of origin to a new tree (Hofstetter et al. 2015). Mutualistic filamentous fungi

frequently associated with ambrosia beetles are mainly categorized in the ascomycete genera *Ambrosiella* and *Raffaelea* (Farrell et al. 2001), while most fungal bark beetle associates belong to the genera *Grosmannia*, *Ceratocystiopsis*, and *Ophiostoma*. (Six und Elser 2020; Zipfel et al. 2006). However, not only beneficial filamentous fungi can be found in the nests of ambrosia and bark beetles. Pathogenic fungi are additionally present in the nests but in a healthy brood, the growth of pathogens is constrained by adult beetles (Nuotclà et al. 2019). In addition, yeasts can be isolated from various locations around the beetles, such as their gut, the exoskeleton and the walls of the galleries (Six 2013), but for most yeast species, their role remains unknown. Ibarra-Juarez et al. (2020) identified over 450 fungal and bacterial species from the brood and adults of the ambrosia beetle *Xyleborus affinis*. However, Ibarra-Juarez et al. (2020), as well as Six (2013), clearly state, that not all of the organisms found in, on and around the beetles are involved in a symbiosis of any kind with their hosts. Unfortunately, little is known about the function of all those microorganisms because only a few studies in the past years discussed their role in the symbiosis (Douglas 2009; Six 2013; Ibarra-Juarez et al. 2020). One reason for this is that studies on symbiotic partners of bark and ambrosia beetles mainly focused on filamentous fungi.

In 1966, Helene Francke-Grosmann assumed that the symbiosis of filamentous fungi and ambrosia beetles is obligate. According to her findings, the fungi cannot be found in nature without their beetle host, and the brood of the beetle is unsuccessful without the fungal partner (Francke-Grosmann 1966). Concerning the preservation of this symbiosis, the fungi must be transported to the new infestation site by the beetle. Francke-Grosmann (1956) found tube- or sack-shaped reservoirs containing oils/secretions and fungal spores, which made a more efficient the fungal transport possible. These so called mycetangia (Vega und Biedermann 2020; Mayers et al. 2022) can be found in pairs mainly on female beetles and are emptied by muscle movement of the beetle in the new developing nest (Francke-Grosmann 1966, 1956; Batra 1963). Since they developed numerous times separately within the Scolytinae, mycetangia are found in almost all documented ambrosia beetles as well as few bark beetles (e.g. *Dendroctonus* species (Six 2012)). It is even proposed that the mycetangia may be primarily responsible for selecting particular helpful

fungal partners (Bracewell und Six 2015). These morphological adaptations show the long history of this mutualistic relationship (Kirkendall et al. 2015).

The phloem and xylem tissues, inhabited by bark and ambrosia beetles, perform different tasks and are important transportation systems within the tree. Figure 1 shows the different layers of a tree with the bark as the outer, protective layer which is responsible for minimizing the loss of water and damage caused by insects (Matyssek et al. 2012). The next layer is the phloem, which transports nutritional photosynthesis products down to the roots, mainly consisting of amino acids, sugars, peptides, proteins, but also phytohormones. The xylem layer, also termed sapwood, is responsible for water and mineral transportation from the roots to the leaves. The xylem cells are furthermore utilized as a storage compartment for starch and proteins and it supports the stability of the tree. The water transported in the xylem can diffuse horizontally from the xylem to the phloem. Even though the phloem also contains water, the concentration of nutrients is much greater in phloem compared to xylem (relation of 3:1, see Matyssek et al. 2012). The cambium is located between the phloem and xylem (Figure 1). It contains proliferating cells, which develop into new phloem cells outwards and new xylem cells inwards. The heartwood stabilizes the tree and consists of dead cells (Matyssek et al. 2012).

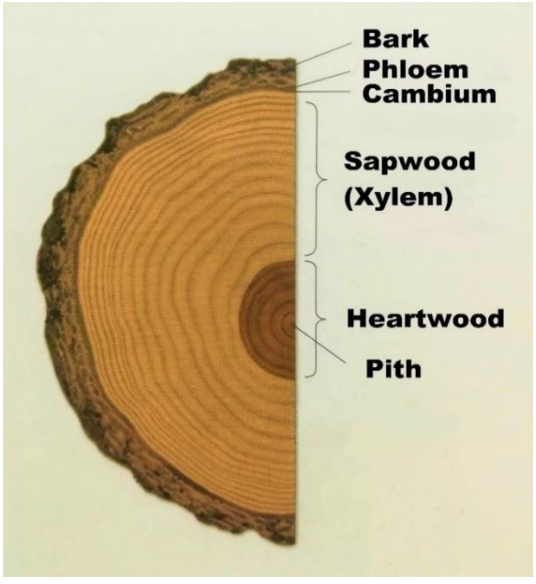

Figure 1: Layers of a conifer trunk (edited from Matyssek et al. (2012)).

Although, living in wood represents a protected habitat, the nutrient supply remains challenging. Filipiak und Weiner (2014) mentioned, that the beetles meet a stoichiometric mismatch between the wood and their nutritional needs. Moreover, wood is difficult to digest and it contains only little amounts of nitrogen and phosphorous (Filipiak et al. 2016; Ayres et al. 2000). Among others, the macro elements nitrogen, potassium and phosphorous pose the most limiting elements during the development of the beetles in the tree (Filipiak und Weiner 2017; Filipiak et al. 2016). Other vital macro elements are carbon, sulfur, calcium and magnesium but also microelements, which are needed in small amounts, such as iron, manganese or zinc are essential for organisms (Lüttge 2017). The options for beetles to obtain nutrients are highly limited. Some species consume large amounts of phloem to meet their nutritional requirements (Ayres et al. 2000), while others seem to utilize their associated microorganisms with different metabolic abilities, such as fungi or yeasts (Douglas 2009). However, bark and ambrosia beetles (as well as their fungi) need to fulfill their nutritional requirements in order to reproduce and survive within a tree. As mentioned above, nitrogen (N) is one of the most important but also most limiting elements (Ayres et al. 2000; Filipiak und Weiner 2014). It can be found in many biologically important molecules such as in the nucleobases of nucleic acids, in amino acids as the amino group ($-NH_2$), and it is consequently also needed for proteins (Matyssek et al. 2012). Phosphorous (P) plays an essential role in the availability of chemical energy in an organism. Here, adenosine triphosphate (ATP) serves as an energy storage and participates in metabolic processes (Matyssek et al. 2012). The elements sodium (Na) and potassium (K) are playing crucial roles in regulating the cell cycle and support the receptor function of neurons as the main participants in the Na^+/K^+-pump (Ling 1987; Pivovarov et al. 2018). For the transmission of stimulus in neurons or muscle cells, calcium (Ca), potassium (K) and sodium (Na) are of essential importance (Kraus et al. 2020). Micro elements like zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) function as cofactors and help enzymes to develop their catalytically active conformation (Kraus et al. 2020). Another vital element for the function of enzymes is sulfur (S), as part of the amino acids methionine and cysteine. Disulfide bridges form between two cysteine residues and help the enzymes to obtain their tertiary or

quarterly structure (Matyssek et al. 2012). Finally, carbon (C) is an abundant part of the main scaffold in biological molecules like carbohydrates, amino acids, vitamins and proteins (Kraus et al. 2020).

Filipiak proclaims in several publications that fungal activity is required to deal with the severe stoichiometric mismatch the beetles encounter while living in wood (Filipiak und Weiner 2014; Filipiak et al. 2016; Filipiak 2018). Filipiak und Weiner (2014) therefore suggest the hypothesis that fungi absorb the nutrients through their mycelium, translocate and accumulate them and serve as food for the beetles in addition to the wood. For this reason, the fungi should help to cover the nutritional requirements of the larvae and beetles. Further, beetles that additionally feed on filamentous fungi must consume less food to obtain their required nitrogen compared to strictly phloem feeding beetles (Ayres et al. 2000). Incidentally, this was reflected in shorter feeding galleries of larvae in the presence of a mutualistic fungi (Ayres et al. 2000). Hence, the beetles efficiently obtain more nutrients through feeding on mycelia, which poses a nutritional advantage in contrary to strictly phloem feeding species (Harrington 2005).

Six und Elser (2019) uncovered in their work that a mutualistic fungus had a more beneficial nitrogen and phosphorous transport for the western pine beetle (*Dendroctonus brevicomis*) than an antagonistic fungus. The antagonist failed to grow from the sapwood all the way into the bark, where the beetle galleries were located, causing the larvae to die. The mutualistic fungus on the other hand was able to grow into the bark, preventing larval starvation by P and N supplementation. Six und Elser (2019) explain that the development of beetles in the bark of trees would not be possible without mutualistic fungi. Moreover, the absence of a mutualistic fungus can lead to brood loss (Bracewell und Six 2015).

The claim of improved nutrient accumulation through the translocation of elements performed by mutualistic fungi could also be supported in the studies of Lehenberger et al. (2021). This study showed that the gallery walls, on which the mutualistic fungi are cultivated by the beetles, were highly enriched with several essential nutrients.

2 Aim of this bachelor thesis

These studies lead to the question whether mutualistic bark and ambrosia beetle fungi stand out with an advanced ability to accumulate nutrients compared to other fungi grown on the same substrate. It is widely assumed that mutualistic fungi help the beetles to meet their nutritional requirements, and thus contribute to the beetle's survival in the tree. If so, this would verify the long-lasting statement of the mutualism being driven by fungal nutrient supplementation. However, detailed studies on the actual elemental composition of symbiotic bark and ambrosia beetle fungi are missing. For this reason, this thesis aims to uncover for the first time the content of biologically relevant elements in several fungal species associated with bark and ambrosia beetles. In aim to compare the ability to accumulate nutrients, several other fungi, such as wood-degrading fungi, pathogenic fungi, and a phylogenetically closely related but non-mutualistic fungus are examined as well. Since there is little known about the yeasts, additionally six yeast strains are analyzed. Although, the focus of this thesis remains on the filamentous fungi. An elemental analyzer, as well an ICP-OES system are utilized to determine the elemental composition of tested fungal biomass. It has to be found out whether the examined fungi contain an enhanced elemental concentration of biologically important elements in comparison to non-mutualistic fungi. Moreover, these results could provide novel insights to the question why bark and ambrosia beetles live in symbiosis with precisely these fungal species.

3 Materials and Equipment

3.1 Fungi and Yeasts

Table 1: Organisms from the personal culture collection of Dr. M. Lehenberger at the Max-Planck-Institute for Chemical Ecology, Jena. (A) = Ambrosia beetle. (B) = Bark beetle.

Phylum (order)	Strain	Name	Classification
Ascomycota (Microascales)	P331	*Endoconidiophora polonica*	Mutualist of *Ips typographus* (B)
Ascomycota (Ophiostomatales)	P188	*Grosmannia penicillata*	Mutualist of *Ips typographus* (B)
Ascomycota (Saccharomycetales)	P10	*Alloascoidea hylecoeti*	Mutualist to *Elateroides dermestoides* (A)
Ascomycota (Microascales)	P339	*Ambrosiella grosmanniae*	Mutualist to *Xylosandrus germanus* (A)
Ascomycota (Ophiostomatales)	P159	*Raffaelea sulphurea*	Mutualist to *Xyleborinus saxesenii* (A)
Ascomycota (Ophiostomatales)	P167	*Esteya vermicola*	Phylogenetic control to *R. sulphurea*
Basidiomycota (Polyporales)	P207	*Grifola frondosa*	Wood-degrading fungus
Basidiomycota (Agaricales)	P209	*Pleurotus ulmarius*	Wood-degrading fungus
Basidiomycota (Agaricales)	P211	*Lentinula edodes*	Wood-degrading fungus
Basidiomycota (Polyporales)	P213	*Laetiporus sulphureus*	Wood-degrading fungus
Ascomycota (Sordariales)	P7	*Chaetomium globosum*	Pathogen
Ascomycota (Eurotiales)	P21	*Penicillium commune*	Pathogen
Ascomycota (Hypocreales)	P338	*Trichoderma* sp.	Pathogen
Ascomycota (Saccharomycetales)	It12	*Yamadazyma mexicana*	Associated with *Ips typographus* (B)
Ascomycota (Saccharomycetales)	It13	*Yamadazyma mexicana*	Associated with *Ips typographus* (B)
Ascomycota (Saccharomycetales)	It23	*Cyberlindnera mississippiensis*	Associated with *Ips typographus* (B)
Ascomycota (Saccharomycetales)	Xs1	*Yamadazyma mexicana*	Associated with *Xyleborinus saxesenii* (A)
Ascomycota (Saccharomycetales)	Xs21	*Candida* sp.	Associated with *Xyleborinus saxesenii* (A)
Ascomycota (Saccharomycetales)	Xs22	*Cyberlindnera japonica*	Associated with *Xyleborinus saxesenii* (A)

3.2 Materials

Table 2: Materials

Material	Manufacturer
Beech wood sawdust	MycoGenetics Pilz-Shop, Everswinkel
Spruce phloem	Forest, Jena
Plant agar	Duchefa Biochemie, Haarlem
Cellophane foil	Pure Nature Products Versand GmbH, Idar-Oberstein
Petri dishes (diameter 145 mm)	Greiner Bio-One GmbH, Frickenhausen
Petri dishes (diameter 90 mm)	Carl Roth GmbH & CO., Karlsruhe
Penicillin G sodium salt	Alfa Aesar by Thermo Fisher Scientific, Kandel
Streptomycin sulfate	Duchefa Biochemie, Haarlem
Potato Dextrose Agar	Carl Roth GmbH & CO., Karlsruhe
Toothpicks	Franz Mensch GmbH, Buchloe
Bamboo skewers	Franz Mensch GmbH, Buchloe
Ethanol (<= 100%)	Merck KGaA, Darmstadt
Reaction Tubes (1,5 ml)	Eppendorf SE, Hamburg
Liquid nitrogen	Linde plc, Dublin
Laboratory spatula	Karl Hammacher GmbH, Solingen
Tweezers	Handelsvertretung Schwarz, Steinheim
Tin boats assortment	Elementar Analysensysteme GmbH, Langenselbold
Pelleting press	Elementar Analysensysteme GmbH, Langenselbold
Tungsten-(VI)-oxide	Elementar Analysensysteme GmbH, Langenselbold
Acetanilide (99 %)	Merck KGaA, Darmstadt
24-well Multiwell Plate	Greiner Bio-One GmbH, Frickenhausen
RF220 KP_E29 plant material	Thüringer Landesamt für Landwirtschaft und Ländlichen Raum, Jena
Nitric acid 65% for analysis EMSURE®	Merck KGaA, Darmstadt
Hydrogen peroxide 30% for analysis EMSURE®	Merck KGaA, Darmstadt

Control Sample IPE_208 (*Medicago sativa*)	Wageningen University & Research, Wageningen
Screw cap tube (15 ml)	Sarstedt AG & Co. KG, Nümbrecht
Screw cap tube (50 ml)	Sarstedt AG & Co. KG, Nümbrecht
Single element standard solution for Ca, K, Mg, Na (10 g/l)	Carl Roth GmbH & CO., Karlsruhe
Single element standard solution for Cu, Mn, Sr, Zn (1 g/l)	Merck KGaA, Darmstadt
Single element standard solution for P (10 g/l)	VWR, Radnor (PA)
Single element standard solution for Al, S (10 g/l)	Bernd Kraft GmbH, Duisburg
Single element standard solution for Fe (1 g/l)	Bernd Kraft GmbH, Duisburg
Multi-element stock solution (“Pflanzenaufschlüsse”)	Bernd Kraft GmbH, Duisburg

3.3 Equipment

Table 3: Equipment

Equipment	Manufacturer
VARIOKLAV® steam sterilizer	H+P Labortechnik GmbH, Oberschleissheim
Gilson Pipetman	Gilson Incorporated, Middleton (WI)
Milli-Q® Synthesis A10 Water Purification System	Merck Millipore, Burlington
HERAsafe HS12 saftey cabinet with UV	Heraeus/Kendro Laboratory Products, Hanau
Schott Duran Bottles (500 ml)	DWK Life Sciences GmbH, Mainz
Incubator B 6120	Heraeus/Kendro Laboratory Products, Hanau
Parafilm	Bemis®, Amcor, Zurich
Shaking water bath	Gesellschaft für Labortechnik mbH, Burgwedel

Mettler Toledo PB602-S scale	Mettler-Toledo GmbH, Greifensee
Sartorius BP 211 D scale	Sartorius AG, Göttingen
Function line T6 heating and drying oven	Heraeus/Kendro Laboratory Products, Hanau
Climate chamber MC 1000	Snijders Labs, Tilburg
Freezer	Liebherr-International Deutschland GmbH, Biberach an der Riß
Freeze dryer ALPHA 1-4 LDplus	Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz
XP6 Excellence Plus XP Micro Balance	Mettler-Toledo GmbH, Gießen
Element analyzer vario EL III	Elementar Analysensysteme GmbH, Langenselbold
Universal oven UN55plus	Memmert GmbH + Co. KG, Schwabach
Sample pressure digestion containers	Loftfield Analytische Lösungen, Neueichenberg
Sartorius *Basic* BA 210 S scale	Sartorius AG, Göttingen
iCAP PRO X ICP-OES Duo	Thermo Fisher Scientific, Waltham (MA)
ASX-560 Autosampler	Teledyne CETAC Technologies, Omaha (NE)

3.4 Software

Table 4: Software

Software	Provider
Microsoft 365	Microsoft Corporation, Redmond (WA)
Thermo Scientific Qtegra ISDS Software	Thermo Fisher Scientific, Waltham (MA)
Adobe Illustrator CS5	Adobe, San José (CA)
RStudio	Posit, Boston (MA)
R (Version 4.2.2)	R Core Team

4 Methods

4.1 Fungal samples

The yeasts and fungi examined in this thesis (Table 1) were isolated from field nests of bark and ambrosia beetles by Dr. M. Lehenberger (MPI CE, Jena, Germany) and are stored in the Max-Planck culture collection at – 80 °C. The filamentous fungi include two bark beetle associates and three ambrosia beetle associates. Based on their natural habitat, the bark beetle associates were cultivated on phloem media. However, for the comparability of their nutrient accumulation, these fungi were additionally grown on sawdust (xylem, hereafter referred to as sawdust) media. Ambrosia beetle fungi, pathogens, wood-degrading fungi and the phylogenetic control were cultivated merely on sawdust media. This procedure was also applied to the yeasts. Associates of bark beetles were cultivated on both experimental media types and the ambrosia beetle symbionts solely on sawdust media. The yeast *Yamadazyma mexicana* was isolated from separate nests, thus the terms It12, It13 and Xs1 mark different strains (Table 1). Here, the “It” stands for *Ips typographus* and the “Xs” indicates the isolation from a nest of *Xyleborinus saxesenii*.

4.2 Fungal cultivation

The sawdust was purchased from a supplier offering substrate for growing edible mushrooms. For this reason, there are no oil residues from chainsaws in the media, which would have otherwise affected this project negatively. The phloem was gathered by Max-Plank-Institute staff by manually scarping it off spruces which originate from the forest in Jena. To minimize the risk of bacterial contaminations, penicillin and streptomycin (final concentration: 50 µg/ml) were added to the autoclaved media prior to pouring the plates. To obtain enough biomass, the filamentous fungi on beech wood sawdust were cultivated on petri dishes with a diameter of 145 mm. For the yeast and filamentous fungi cultures on phloem media, petri dishes with a diameter of 90 mm were used, as phloem is richer in nutrients and thus, less substrate is needed for the fungi to produce enough biomass. A sterile cellophane foil was placed on each culture medium prior to the inoculation. This foil prevented the organisms to grow into the media while still allowing access to the

nutrients. This step was necessary to be able to harvest only fungal biomass without residues of culture media.

The fungal cultures were revived on potato dextrose agar (PDA) with an incubation for six to ten days at 25 °C and then stored at 4°C before the experiment. A corkborer with a diameter of 5 mm was used to cut out equal amounts of biomass (plugs) from the precultures, which were then placed onto the experimental medium with a sterilized toothpick. A total of fourteen replicates were inoculated for each organism in order to obtain enough biomass for the following analysis and statistical evaluability. For details about the media, see Table 5 in the Supplements. All fungi and yeast cultures were sealed with Parafilm and incubated in a climate chamber for four weeks at 25 °C with 65 % humidity until the petri dishes were completely covered with biomass. An exception were the two filamentous fungi grown on phloem media because of their faster growth compared to the yeasts, their total growth time was 23 days.

Fourteen plates per experimental medium without fungal material were incubated under the same conditions as described above. These controls were used to determine the content of the elements in the culture media alone.

4.3 General sample preparation

The filamentous fungi and yeasts were scratched off the cellophane foil and individually transferred into reaction tubes. For the control samples, the cellophane foil was removed, and a piece of the media was cut out, using a corkborer with a diameter of 10 mm. The tubes were stored at – 20 °C until all the samples were harvested after exactly four weeks of incubation.

In the next step, all the tubes were opened and frozen with liquid nitrogen before placing them in a freeze dryer. The samples were freeze dried in a vacuum of 0.22 mbar for four days.

4.4 Elemental Analysis

The analysis of carbon (C) and nitrogen (N) was accomplished by using the elemental analyzer vario EL III from the manufacturer Elementar Analysensysteme GmbH. This analysis is the standard procedure to determine the content of carbon

and nitrogen in organic compounds. Here, seven of the total fourteen replicates for each organism were used.

4.4.1 Sample preparation

The freeze-dried samples were individually weighted into tin foil boats and the weight was documented in the software of the elemental analyzer. To achieve a complete combustion, Tungsten-(VI)-oxide was added to each sample, before the tin foil was folded and pressed to a pellet. For every single analysis run, a blank (containing only 10 mg Tungsten-(VI)-oxide), a daily calibration sample, and a control sample were also analyzed. The daily calibration sample, consisting of 4 mg acetanilide with 5 mg Tungsten-(VI)-oxide was later used as a factor to correct the measured values of the samples. The control sample “KP_E29” was a mixture of 4 mg plant material with 5 mg Tungsten-(VI)-oxide to evaluate the overall performance of the elemental analyzer and the user.

4.4.2 Principals of the elemental analyzer

The pellets of the blanks, controls, samples, and daily calibration samples were placed into the sampler of the elemental analyzer. Each pellet was individually combusted with an oxygen flow at over 950 °C. The nitrogen is converted to oxides of nitrogen (NO_x) or nitrogen gas (N_2), while carbon turns into carbon dioxide (CO_2). With the help of the carrier gas helium, the analytes get transported out of the combustion column into a column containing copper. Here, all the excess oxygen is being trapped and all the oxides of nitrogen get converted into nitrogen gas. The H_2O in the gas mixture is bound by phosphorus pentoxide and hence removed. In the next column the N_2 and CO_2 are separated through adsorption. The N_2 gets directly transported into the thermal conductivity detector which consists of two chambers. The gas mixture flows through a measuring cell, while pure helium flows through a reference cell. Because of the different thermal conductivity of the N_2 and helium gas mixture compared to pure helium, a change in the voltage can be measured.

Next, the column that trapped the CO_2 is heated up, causing the CO_2 to desorb and leading to its detection in the same manner as N_2. The measured voltage change is then documented as a function of time and integrated by the computer.

4.4.3 Data evaluation

The analyzer software automatically calculated the percentages of N and C depending on the individual weight of each sample. After the analysis, the correction factor was determined internally using the data of three acetanilide samples and applied to the fungal data. Finally, the contents of carbon and nitrogen in the control sample “KP_E29” were verified with a control chart. The target values can be seen in Table 6 in the Supplements.

4.5 ICP-OES Analysis

The inductively coupled plasma optical emission spectrometry (ICP-OES) was utilized to measure the concentrations of Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Sr and Zn in the fungal samples. This analysis was carried out by the ICP-OES system iCAP pro x duo from ThermoScientific. The remaining seven replicates for each organism were used here.

4.5.1 Sample digestion

In aim to have a homogenous solution, the samples were treated with a nitric acid pressure digestion in an oven. To achieve that, the samples were weighted into pressure digestion containers and 3 ml of 65 % nitric acid and 1 ml of 30 % hydrogen peroxide were added. For each digestion run a blank and a control sample (“KP208”), consisting of plant material (*Medicago sativa*), were also included. Next, the containers were closed tightly and placed into an oven, where they were digested for seven hours at 170 °C. After cooling down, the digested samples were poured into reaction tubes and the vessels were rinsed out with deionized water. The tubes were then filled to 15 ml with the deionized water.

The pressure digestion containers were boiled out with 1 ml 65 % nitric acid and 1 ml deionized water in between digestion runs to prevent cross contamination of the samples.

4.5.2 Preparation of calibration and control samples

The multi-element stock solution ("Pflanzenaufschlüsse") (Table 7) was diluted 1:10 with 20 % nitric acid (HNO_3) to reach the estimated concentrations of the samples. This diluted stock solution was used to prepare the 50 ml calibration standards with the dilutions 1:100, 1:50, 1:10, 1:5, 1:2 and a blank which contained only 20 % HNO_3. The daily control sample called "QC-Standard" was prepared using 5 ml of the 1:10 diluted multi-element stock solution and 45 ml of 20 % HNO_3. To test the calibration, a sample called "QC-Kraft (Pflanze) 1/10" was also analyzed. This sample was pre-made by mixing 5 ml of the multi-element stock solution (not diluted) with 45 ml of 20 % HNO_3. For the use in this analysis, the "QC-Kraft (Pflanze) 1/10" sample was diluted 1:10 with 20 % HNO_3 to get it to a final 1:100 dilution. The calibration standards and the "QC-Kraft (Pflanze)" (1:100) were analyzed prior to the samples. The concentration of the elements in each calibration standard can be seen in Table 8 in the Supplements. The daily control sample "QC-Standard" was measured after every 10th fungal sample to monitor the performance of the analysis run.

The weight of the control sample "KP208" was 0.5 g by default, which was a hundred times higher than the average weight of the fungal samples. Hence, the "KP208" samples were diluted 1:100 using 20 % HNO_3, so that the expected concentrations of the analytes laid within the calibrations. This "KP208" control sample and the blank, which were digested with the fungal samples, were analyzed after every 34th fungal sample.

4.5.3 Principals of the ICP-OES analysis

According to the specifications in the software, the autosampler selects the samples and a pump transports them to the nebulizer, where they are introduced to the carrier gas argon. Larger droplets are removed from the sample aerosol in the spray chamber, while the rest of the aerosol flows into the plasma torch. Here, the atoms get excited by absorbing energy from the argon plasma. This additional energy forces some electrons to shift from their current energy state to a higher state. Since this is an unstable condition, the electrons release this excess energy to get back to their original ground status. Depending on the element, the energy gets emitted as

light of a specific wavelength and can be measured for all the analytes simultaneously. The intensity of the light is used to determine the concentration of each element with the aid of the calibration graph.

4.5.4 Data evaluation

Of some elements, two or more wavelengths have been detected (e.g., aluminum at 237.31 nm and 396.15 nm). This is important in case substances in the samples interfere with a wavelength which an analyte emits. For that reason, all the element peaks of one sample had to be checked manually for any unwanted interference. Preferably, high peaks with a clean symmetric shape and without any additional peaks were selected. The chosen wavelength for each element was adopted to all the other samples and the remaining data of other wavelengths was not considered further.

The measured element concentrations were received in mg/l from the ICP-OES system. The following equation (1) was used to calculate the mass fraction of each element depending on the individual weight of the samples.

$$w\left(\frac{mg}{kg}\right) = c\left(\frac{mg}{l}\right) * \frac{0{,}0015\ l\ (sample\ volume)}{sample\ weight\ (kg)} \qquad (1)$$

The accuracy of the analysis was verified using the control sample “KP208”. For this, the mean values in mg/l for the blanks of each element were calculated in Microsoft Excel. Those mean values were then subtracted from the measured data of the control sample “KP208”. With formula (1), the concentrations were converted from mg/l into mg/kg, which was followed by calculating the mean values for each element of the control sample. Finally, the data had to be multiplied by 100 because of the dilution steps before the analysis run.

Those calculated mean values were compared with given mass fractions (see Table 9 in the Supplements) and had to be close to the target value to ensure accurate analysis run. Ultimately, the calculated mean values of the blanks were also

subtracted from the fungal samples. The equation (1) was then utilized to calculate the mass fractions of the elements in mg/kg.

4.5.5 Limit of quantification

The limit of quantification was determined by combining all the blank samples, which were digested along with the fungal samples, and equally distributing them into six test tubes. The calibration standards, the "QC-Standard" and the "QC-Kraft (Pflanze) 1/10" were prepared as described above in chapter 4.5.2. After generating the calibration graph, each of the six test tubes containing the blank mixture was analyzed twice to get twelve replicates.

The mean value ($\bar{x}$) and the standard deviation (s) for each element of the blank replicates were calculated in Excel. The setpoints for the limit of quantification were determined using the equation (2) below.

Limit of quantification (LOQ): $$LOQ = 10 * s \quad (2)$$

The obtained LOQ setpoints for each element were then rounded up and a solution containing those exact LOQ concentrations was prepared. The LOQ-test solution was prepared by uniting different volumes of the single element solutions (see Table 2 for concentrations of the standard solutions) in a concentration hundred times higher than the LOQ. In the next step this solution was filled up to 100 ml using the 20 % HNO_3 and then diluted 1:100 using the 20 % HNO_3, as well. The solution was divided into five test tubes.

The calibration standards, the "QC-Standard", the "QC-Kraft (Pflanze) 1/10" and two blanks were prepared as described above in chapter 4.5.2 and analyzed together with the LOQ-test solution samples.

In Excel, the mean values of the blanks were calculated and subtracted from the measured values of the LOQ-test solution samples. Then the mean values ($\bar{x}$) and the standard deviations (s) of the LOQ-test solution were determined and used to calculate the following parameters with the equations (3), (4) and (5) for each individual element. Those parameters show whether the previously defined LOQ setpoints (x_s) for the limit of quantification could be verified.

Coefficient of variation (CV) (max. 20 %): $$CV = \left(\frac{s}{\bar{x}}\right) * 100 \quad (3)$$

Recovery rate (RR): $$RR = \left(\frac{\bar{x}}{x_s}\right) * 100 \quad (4)$$

Inaccuracy (I) (max. 40 %): $$I = RR - 100 \quad (5)$$

The final limits of quantification can be seen in Table 10 in the Supplements.

4.6 Statistical Analysis

The statistical analysis of the generated data was performed using Microsoft Excel and R (Version 4.2.2). The Tukey´s method (Tukey 1977) was used to identify outliers in Excel. This method utilizes the first (Q1) and third (Q3) quartile of the evaluated data and the inner quartile range (IQR). The IQR is the Q1 subtracted from the Q3. The following formulas (6) and (7) show how the lower threshold, and the top threshold were calculated.

Lower threshold: $$Q1 - 1{,}5 * IQR \quad (6)$$

Top threshold: $$Q3 + 1{,}5 * IQR \quad (7)$$

Every data point that could be found below the lower threshold or above the top threshold was declared an outlier and subsequently removed from the data set.

The boxplots were generated using the package “ggplot2” (Wickham 2009) in R. For the heatmaps, the R package “pheatmap” (Kolde 2018) was applied. The Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test were utilized to calculate the *p*-values between the different fungal samples in R. Due to the vast amount of data, it was not possible to show significant differences within the boxplots. For this reason, the *p*-values can be seen in the Supplements from Table 14 to Table 41.

5 Results

The nutritional value of mutualistic bark and ambrosia beetle fungi in comparison to wood-degrading fungi and pathogens was examined by taking a closer look at the elemental composition of these fungi. The contents of carbon and nitrogen were determined with an elemental analyzer and the other elements were detected using an ICP-OES system. The other elements were aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorous (P), sulfur (S), strontium (Sr) and zinc (Zn). This elemental profiling was performed to compare the ability of nutritional accumulation of mutualistic fungi with other fungi which naturally grow on the same substrate.

It is important to mention, that not all the obtained data can be evaluated in detail. As it has already been stated in the aims of this study, the focus of this thesis is on the filamentous fungi. There will be a closer look on the mutualistic filamentous fungi in contrast to the wood-degrading fungi and pathogens. A comparison between wood-degrading fungi and pathogens is not being pursued. The yeasts will be addressed in a brief chapter below. Detailed information about the mean values (*M*) and standard deviations (*SD*) (Overview in Table 12 and Table 13), as well as the *p*-values (Table 14 to Table 41) can be seen in the Supplements.

Additionally, the elemental composition of the used phloem and xylem is a record of the nutritional content to the moment the healthy tree was harvested. It must be kept in mind that the available nutrients in phloem and xylem could vary depending on the time of day or other factors such as previous precipitation.

5.1 Measured elemental contents in filamentous fungi

The boxplots below (see Figure 2 to Figure 11) show the mass fractions or percentages of each individual element in the controls, the filamentous mutualistic fungi with one phylogenetic control, wood-degrading fungi, and pathogens. The detailed evaluation will be carried out for ten of the fourteen analyzed elements. The additional four elements are displayed in the Supplements in Figure 13 to Figure 16.

5.1.1 Evaluation of macro elements in filamentous fungi

The first boxplot in Figure 2 shows the mass fractions of calcium in the examined filamentous fungi. The two mutualists of *Ips typographus*, *E. polonica* and *G. penicilliata*, contained on phloem nearly the same calcium content (M = 3264 mg/kg, SD = 237 mg/kg and M = 3588 mg/kg, SD = 744 mg/kg). *E. polonica* accumulated on sawdust less calcium with just 2999 mg/kg (SD = 202 mg/kg). Only *G. penicilliata* on sawdust media accumulated 7869 mg/kg (SD = 105 mg/kg) calcium, even though the sawdust control contains less calcium than the phloem control. This mutualist has a significantly higher (p = 0.00399 – 0.00647) calcium content than the wood-degraders and pathogens. Just *G. frondosa*, with 9861 mg/kg (SD = 4418 mg/kg), accumulated more calcium. The mutualist *A. hylecoeti* has the least amount of calcium with 995 mg/kg (SD = 119 mg/kg), this was statistically significantly lower (p = 0.00067 – 0.00233) than all the other samples displayed in this graphic. *A. grosmanniae* shows a significantly higher (p = 0.00193 – 0.00233) calcium content than all three pathogens and two of the wood degrading fungi (*P. ulmarius* and *L. edodes*). Compared to *G. frondosa* and *L. sulphureus*, there was no statistically significant difference detectable (p = 0.0612 and p = 0.861).

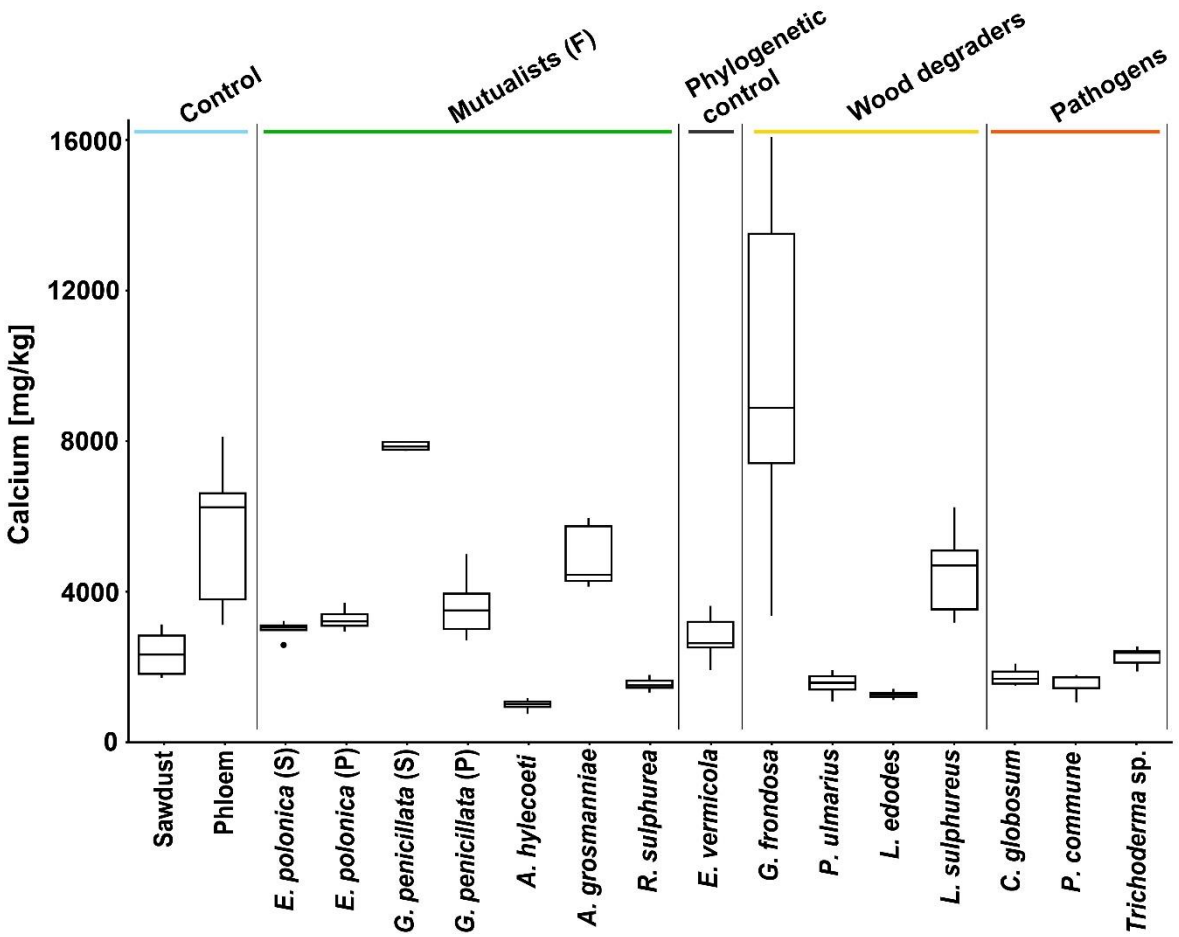

Figure 2: Calcium (Ca) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

In Figure 3, *E. polonica* accumulated with 6664 mg/kg (*SD* = 686 mg/kg) more potassium (K) from sawdust than from phloem media with only 2459 mg/kg (*SD* = 428 mg/kg). In contrast, *G. penicillata* is showing a significantly higher K accumulation from phloem media (*M* = 15256 mg/kg, *SD* = 1676 mg/kg) with $p = 0.00327$ compared to *G. penicillata* cultivated on sawdust media (*M* = 5198 mg/kg, *SD* = 1956 mg/kg). The mutualist *A. hylecoeti* has with 17462 mg/kg (*SD* = 2143 mg/kg) a significantly higher potassium content compared to all the wood-degrading fungi and pathogens, with *p*-values reaching from 0.00067 to 0.00603.

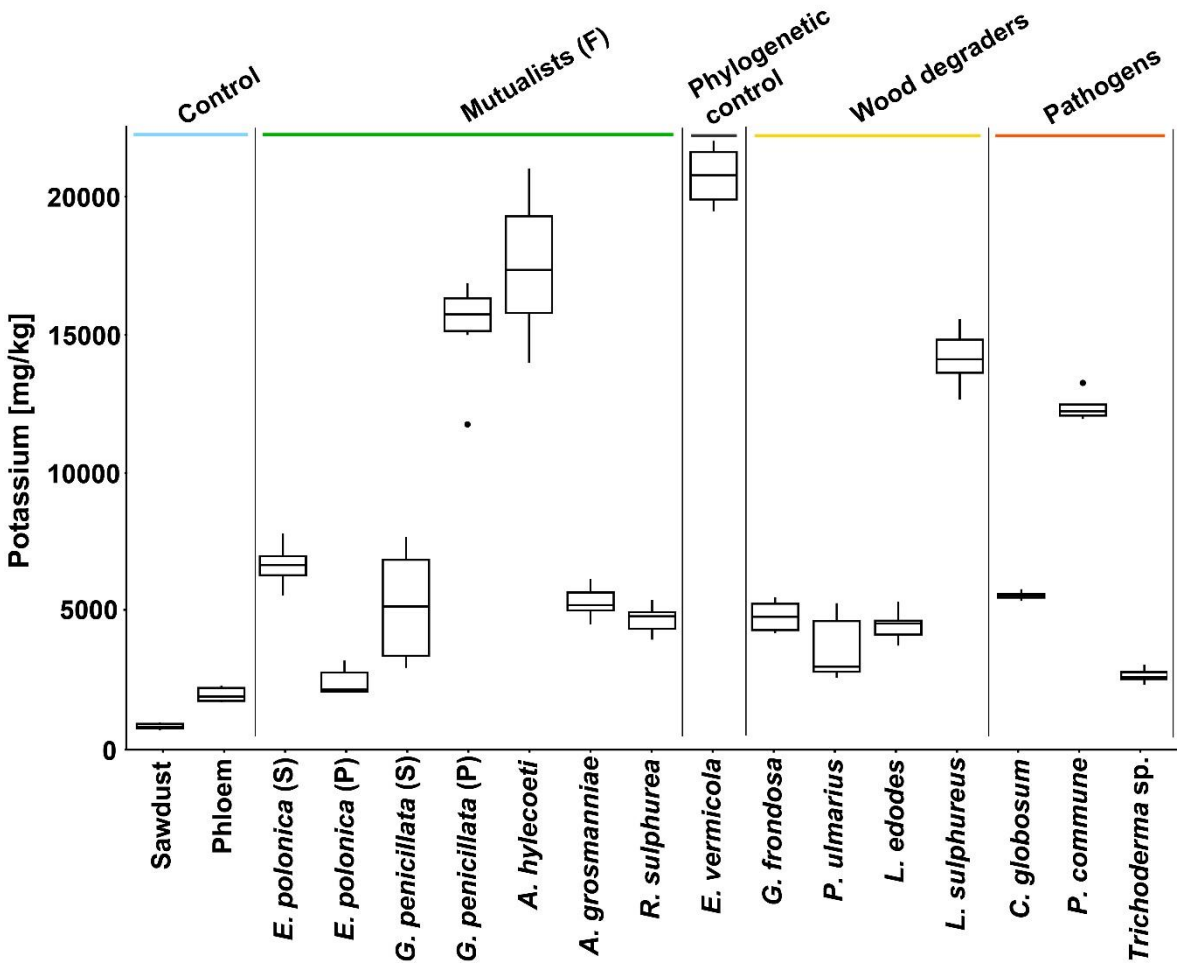

Figure 3: Potassium (K) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

Remarkably is the very high potassium content in the phylogenetic control *E. vermicola* with a mean value of 20747 mg/kg (*SD* = 956 mg/kg). While the closely related *R. sulphurea* contained a mean value of 4696 mg/kg (*SD* = 457 mg/kg) potassium. *A. grosmanniae* and *R. sulphurea* are not significantly different in their potassium content than the wood degraders *G. frondosa*. (*A. grosmanniae* $p = 0.33676$, *R. sulphurea* $p = 0.75866$) or *L. edodes* (*A. grosmanniae* $p = 0.06118$, *R. sulphurea* $p = 0.48419$).

Figure 4 presents the magnesium mass fractions in the filamentous fungi. The mutualists of *Ips typographus*, *E. polonica* and *G. penicillata* contained significantly higher mass fractions of magnesium while growing on sawdust compared to phloem (*E. polonica* $p = 0.0034$, *G. penicillata* $p = 0.0021$). *G. penicillata* (S) has with 1609 mg/kg (*SD* = 64 mg/kg) the highest magnesium content, which is significantly higher in comparison to all the wood degraders and pathogens ($p = 0.0021 – 0.0057$). The second highest magnesium accumulation can be observed from the mutualist *A. grosmanniae* with 1098 mg/kg (*SD* = 195 mg/kg), which was closely followed by the wood degrader *L. sulphureus* (*M* = 1088 mg/kg, *SD* = 20 mg/kg) and the pathogen *Trichoderma* sp. (*M* = 1007 mg/kg, *SD* = 94 mg/kg). The lowest magnesium content of the mutualists was found in *R. sulphurea* with a mean value 650 mg/kg (*SD* = 35 mg/kg). This was significantly lower compared to one wood degrader (*L. sulphureus* $p = 0.0035$) and two pathogens (*P. commune* and *Trichoderma* sp. both with $p = 0.0021$). On the other hand, this was also significantly higher compared to two wood degraders (*P. ulmarius* and *L. edodes* both with $p = 0.0021$) and one pathogen (*C. globosum* $p = 0.0175$).

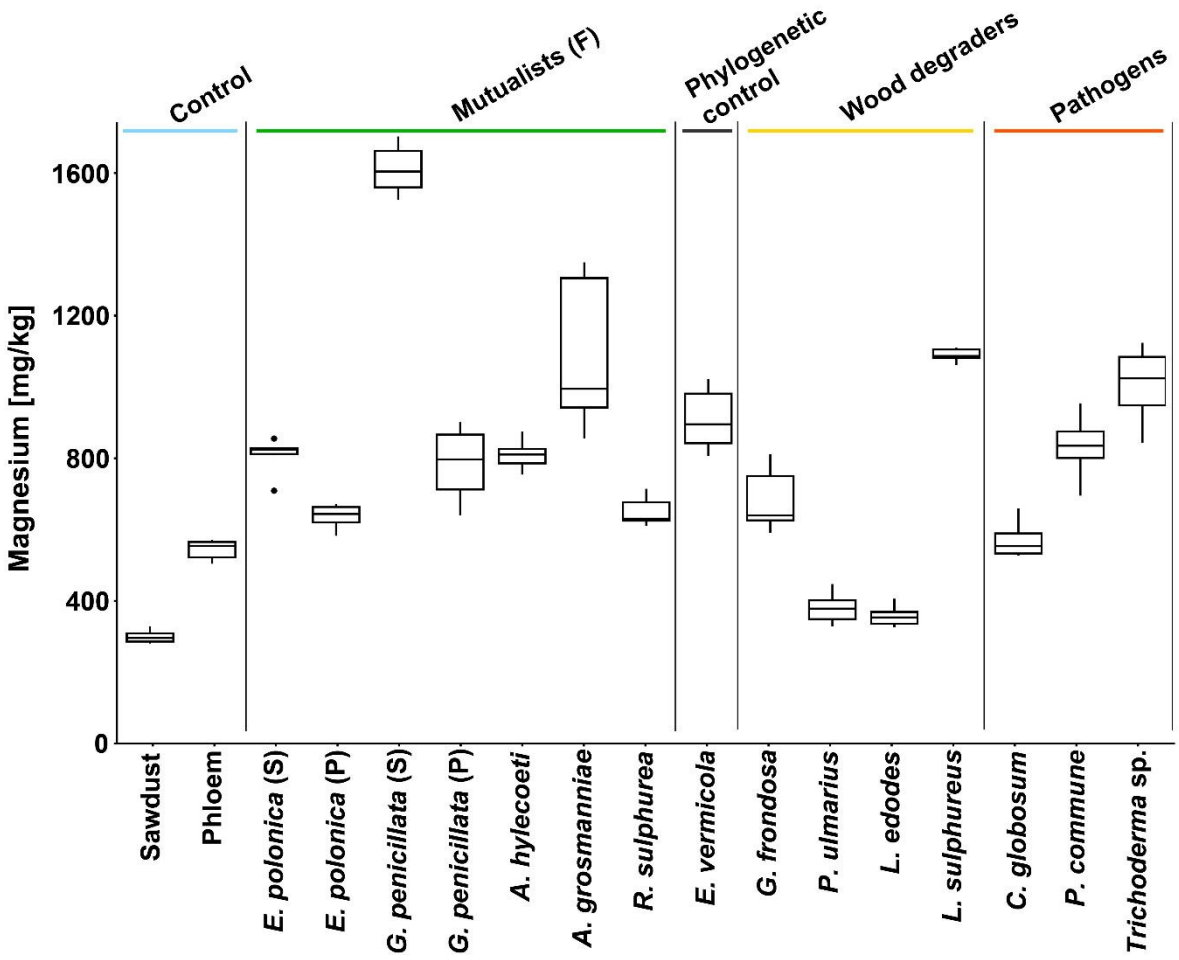

Figure 4: Magnesium (Mg) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

The nitrogen contents in Figure 5 are given as a percentage of the total elemental composition of a fungus. The pathogens and wood degraders appear to have a rather similar nitrogen content. The wood degrader *L. sulphureus* is an exception with a total mean value of 4.95 % (*SD* = 0.12 %) nitrogen. The mutualists, on the other hand, differ greatly from one another. *E. polonica* accumulated three times more of this element while being cultivated on sawdust (*M* = 3.01 %, *SD* = 0.13 %) compared to the cultivation on phloem (*M* = 0.948 %, *SD* = 0.245 %). *G. penicillata* shows a contrary accumulation with 0.923 % (*SD* = 0.574 %) nitrogen from sawdust media and 1.77 % (*SD* = 0.06 %) from phloem media. *E. polonica* (S) and *A. hylecoeti* accumulated significantly more nitrogen than all the pathogens and three out of the four wood degraders (*E. polonica* p = 0.00180 – 0.00320,

A. hylecoeti p = 0.00049 – 0.00113). The mutualist *R. sulphurea* contained a significantly higher nitrogen content than the tree wood degraders *G. frondosa* (p = 0.00991), *P. ulmarius* (p = 0.00539) and *L. edodes* (p = 0.00147) but showed no significant differences to the three pathogens *C. globosum* (p = 0.343), *P. commune* (p = 0.401) and *Trichoderma* sp. (p = 0.726). The two mutualists *G. penicillata* (S) and *A. grosmanniae* accumulated nearly the same amount of nitrogen with 0.923 % (*SD* = 0.575 %) and 0.928 % (*SD* = 0.346 %), respectively. Only the wood degrader *L. edodes* contained with 0.88 % (*SD* = 0.15 %) less nitrogen than the two just mentioned mutualists.

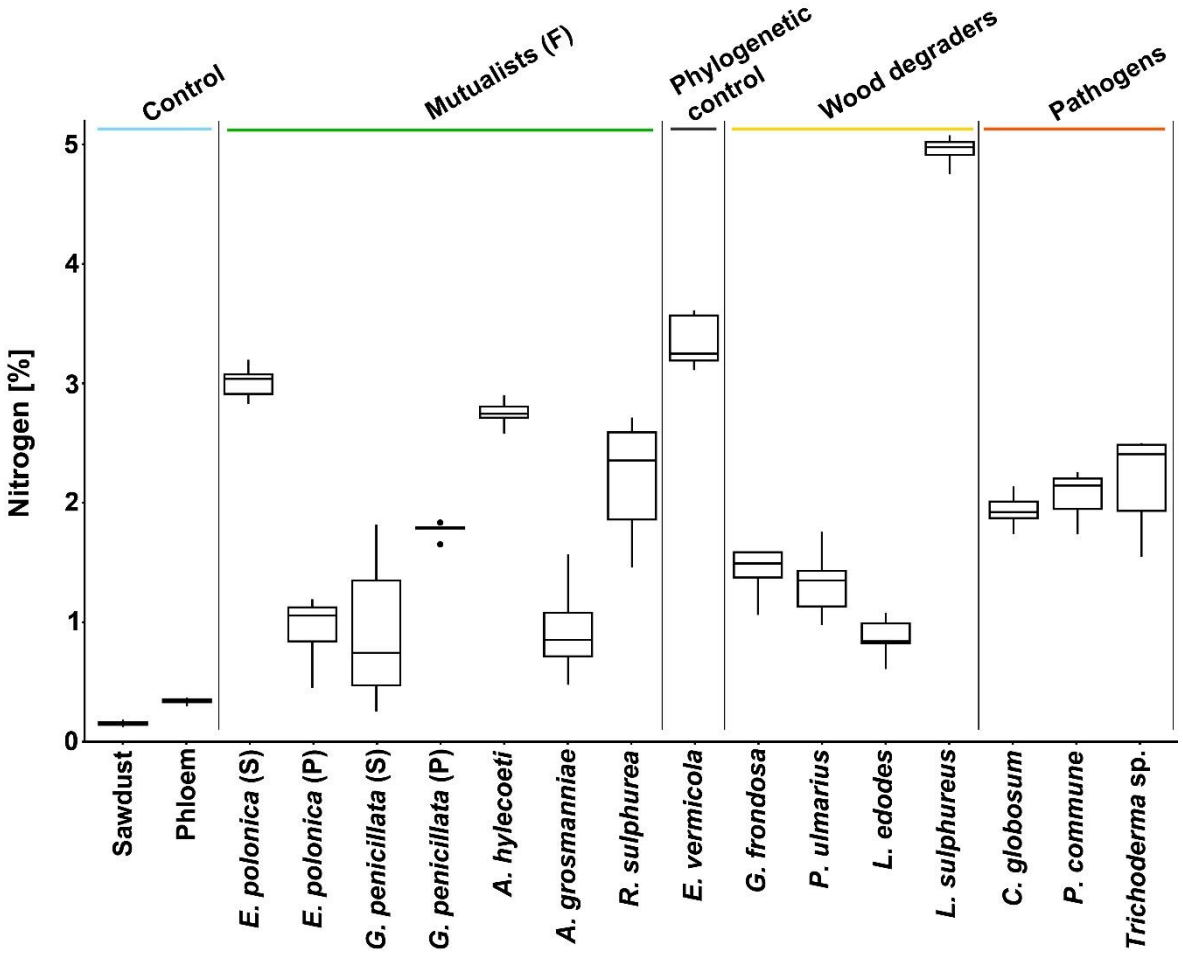

Figure 5: Nitrogen (N) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in a percentage (%). The analysis was carried out with an elemental analyzer. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

E. polonica shows in Figure 6 once again the pattern of absorbing more of phosphorous from the sawdust media than from the phloem media. On sawdust *E. polonica* accumulated 3978 mg/kg (*SD* = 152 mg/kg) phosphorous and grown on phloem media it was 2025 mg/kg (*SD* = 331 mg/kg). For *G. penicillata*, the opposite case can be seen in Figure 6. This fungus contained a mean phosphorous content grown on sawdust media of 1840 mg/kg (*SD* = 157 mg/kg) and 2478 mg/kg (*SD* = 104 mg/kg) from phloem media.

E. polonica (S) contained a significantly higher mass fraction compared to the three pathogens (*C. globosum* p = 0.00350, *P. commune* and *Trichoderma* sp. p = 0.00223) and the three wood degraders *G. frondosa*, *P. ulmarius* and *L. edodes* (p = 0.00223). *E. polonica* (S) had a significantly lower phosphorous content than the wood degrader *L. sulphureus* (p = 0.00350).

The mutualist, *A. hylecoeti* had 7530 mg/kg (*SD* = 1048 mg/kg) phosphorous, which was the highest out of the tested mutualists. However, the wood degrader *L. sulphureus* accumulated with 8424 mg/kg (*SD* = 144 mg/kg) even more phosphorous. Nonetheless, *A. hylecoeti* showed a significantly higher phosphorous accumulation compared to the other pathogens and wood degraders (*G. frondosa* p = 0.00105, *P. ulmarius* p = 0.00077, *L. edodes* p = 0.00105, *C. globosum* p = 0.00223, *P. commune* p = 0.00077, *Trichoderma* sp. p = 0.00105).

R. sulphurea showed with 3837 mg/kg (*SD* = 179 mg/kg) phosphorous a significantly higher mass fraction (p = 0.00223 – 0.00834) than all the pathogens and the three wood degraders *G. frondosa*, *P. ulmarius* and *L. edodes*. But this mutualist has a significantly lower phosphorous content than the wood degrader *L. sulphureus* (p = 0.00350). *A. grosmanniae* showed no significant differences from *G. frondosa* (p = 0.05764), *P. commune* (p = 0.27340) and *Trichoderma* sp. (p = 0.30613).

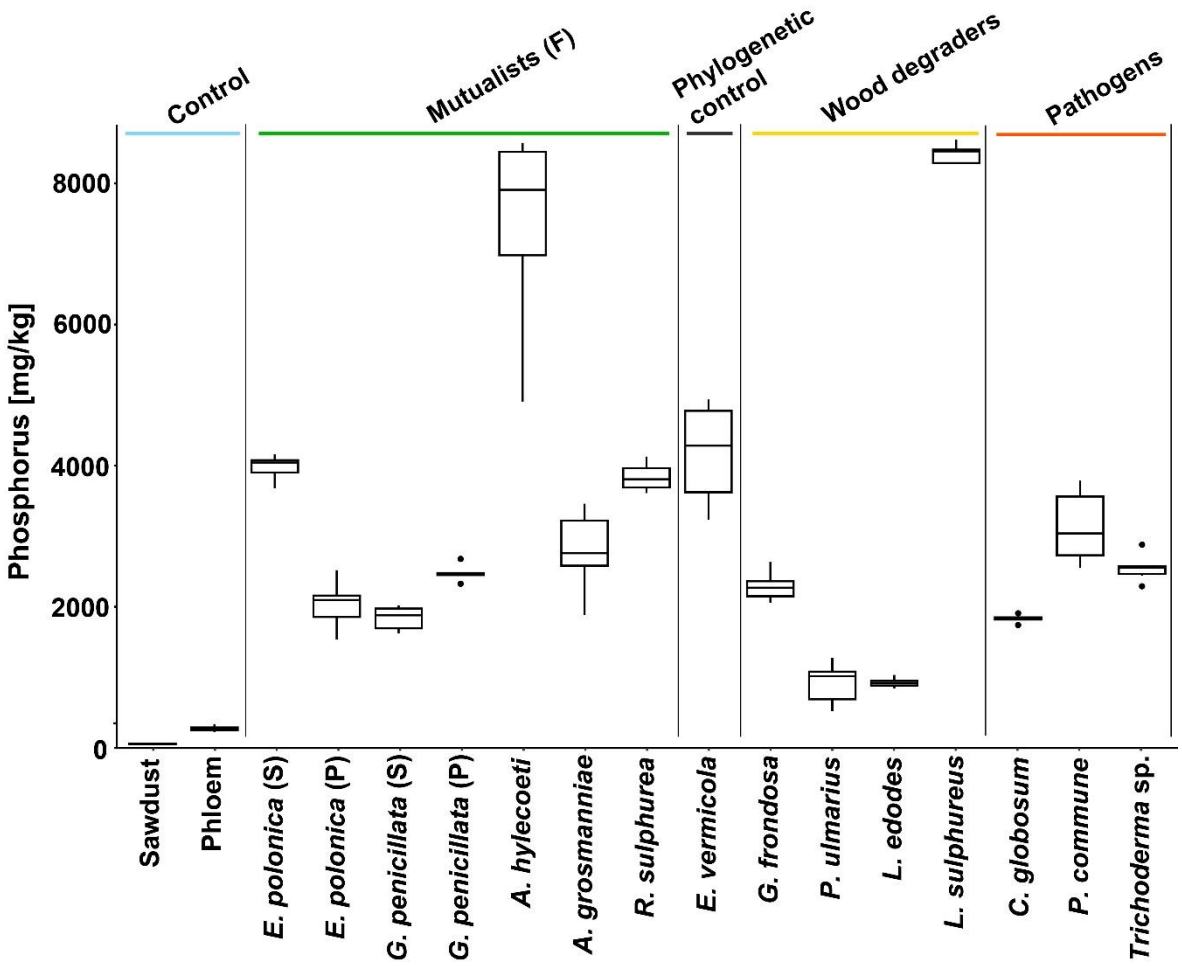

Figure 6: Phosphorus (P) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

The tree mutualists *A. hylecoeti* (*M* = 1943 mg/kg, *SD* = 201 mg/kg), *A. grosmanniae* (*M* = 1862.01 mg/kg, *SD* = 84.2 mg/kg) and *R. sulphurea* (*M* = 1803.0 mg/kg, *SD* = 67.2 mg/kg) have an analogous sulfur accumulation, as visualized in Figure 7. *G. penicillata* has a distinctly higher sulfur content from both culture media compared to the other mutualists. From sawdust media, *G. penicillata* accumulated 2847 mg/kg (*SD* = 298 mg/kg) sulfur and from phloem media 3655 mg/kg (*SD* = 469 mg/kg). The sulfur content from *G. penicillata* (S) is significantly higher than compared to three wood degraders (*G. frondosa* $p = 0.0033$, *P. ulmarius* $p = 0.0019$, *L. edodes* $p = 0.0019$) and two of the pathogens (*C. globosum* $p = 0.0182$, *Trichoderma* sp. $p = 0.0058$).

The differences between *G. penicillata* (P) compared to all the pathogens and wood degraders were overall significant (p = 0.0019 – 0.0056). The sulfur mass fraction in *E. polonica* was again higher from the culture grown on sawdust (M = 1990 mg/kg, SD = 129 mg/kg), compared to the culture on phloem media (M = 1381 mg/kg, SD = 46 mg/kg).

Within the wood degraders, a similar pattern as to phosphorous (Figure 6) can be seen here for sulfur (Figure 7). *G. frondosa*, *P. ulmarius* and *L. edodes* show a comparable sulfur content, while *L. sulphureus* stands out with a mean value of 2700 mg/kg (SD = 95 mg/kg).

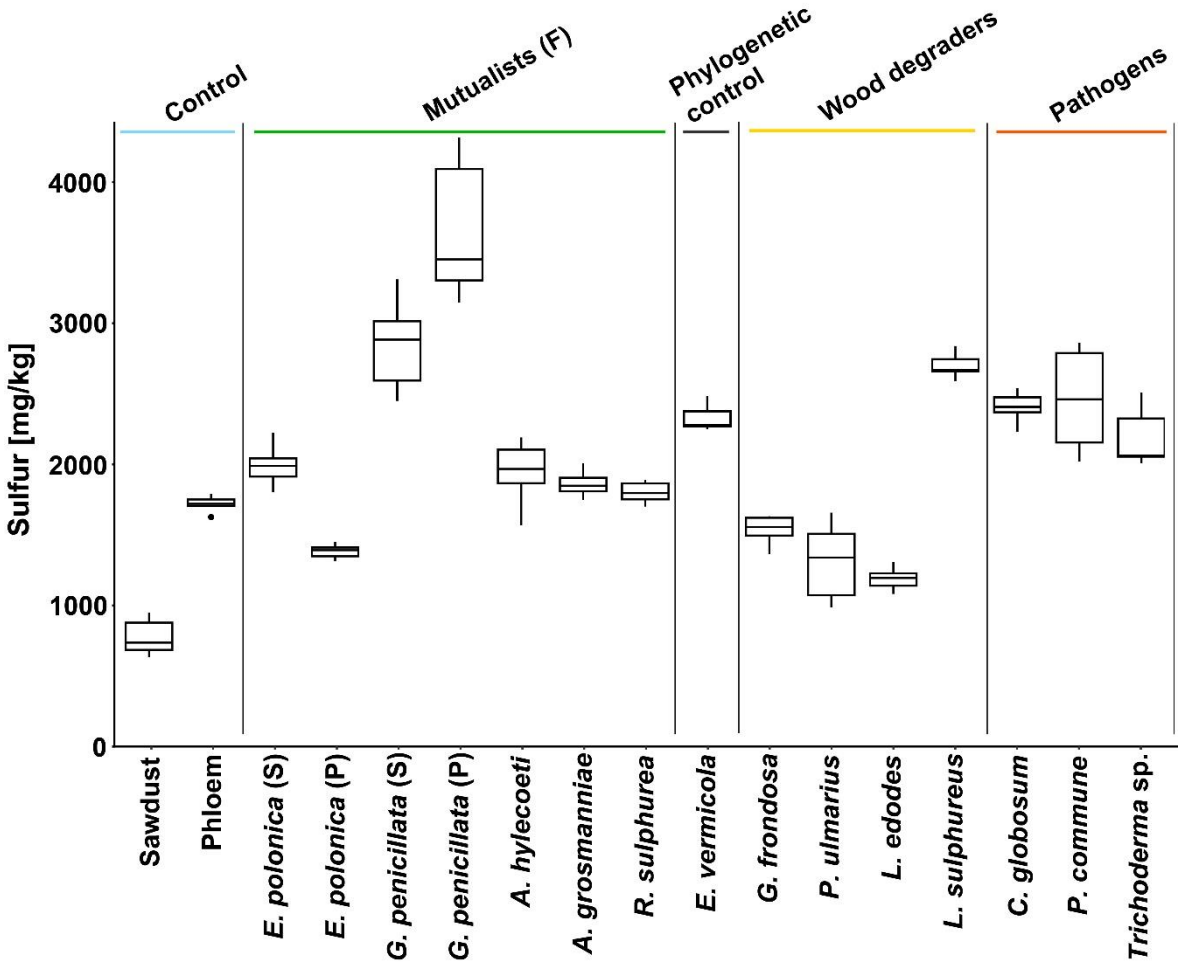

Figure 7: Sulfur (S) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

5.1.2 Evaluation of micro elements in filamentous fungi

Visualized in the next boxplot are the iron mass fractions in the filamentous fungi (Figure 8). *E. polonica* and *G. penicillata* contained a higher iron content in the sample grown on sawdust media as opposed to the sample grown on phloem. Interestingly, *E. polonica* (P) has the significantly lowest (p = 0.0036 – 0.0059) iron content of the here displayed fungi, although the sawdust and phloem controls contain an equal amount of iron. Here, the *E. polonica* (S) accumulated the most iron out of all the mutualistic fungi with 107 mg/kg (*SD* = 16 mg/kg). This was only topped by the wood-degrader *L. edodes* with 118 mg/kg (*SD* = 12 mg/kg). The pathogen *P. commune* has the third highest iron content with 86.4 mg/kg (*SD* = 4.3 mg/kg). The rest of the fungi appear to have rather a similar amount of iron ranging between 32.5 mg/kg (*SD* = 2.5 mg/kg) for *G. penicillata* (P) and 72.6 mg/kg (*SD* = 13.0 mg/kg) for *A. grosmanniae*.

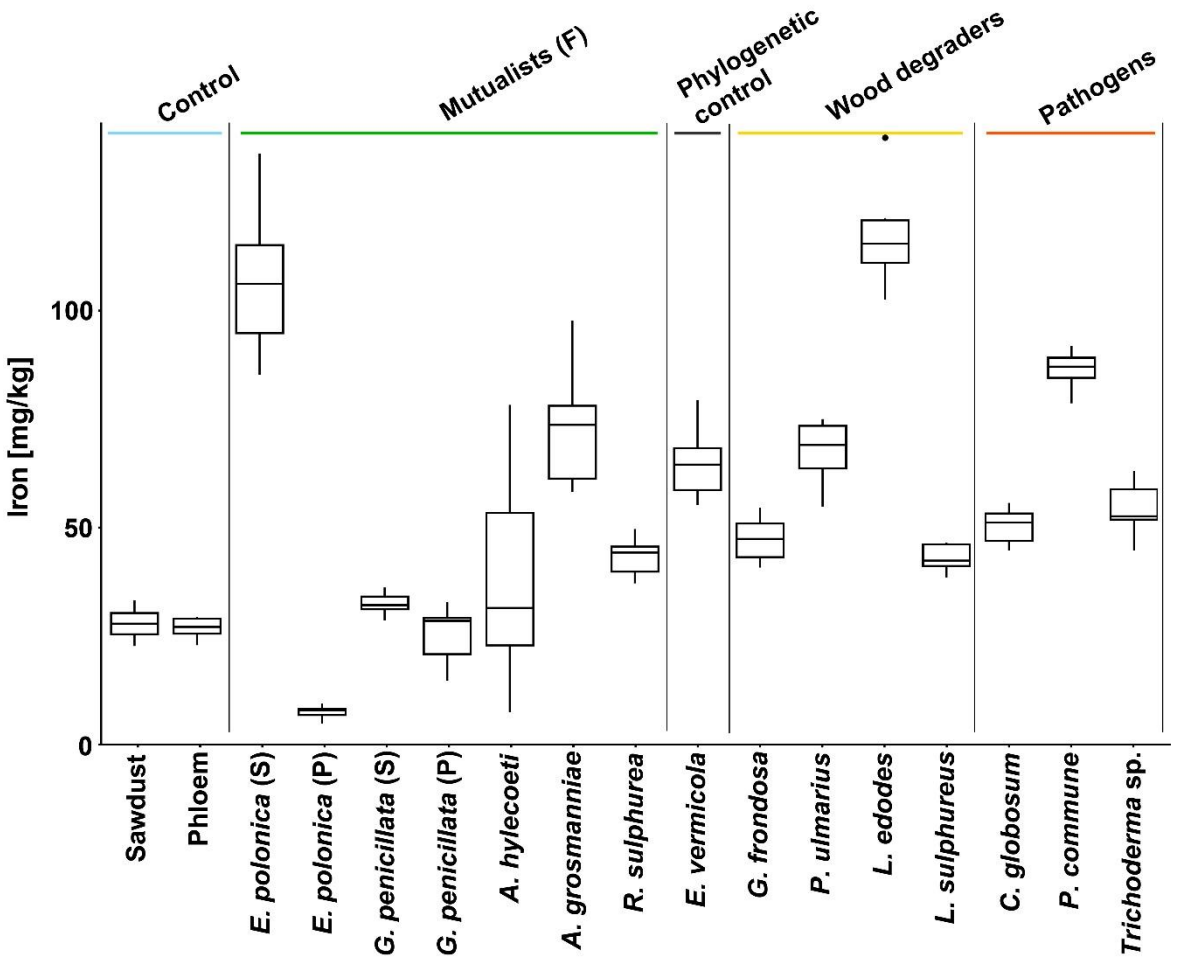

Figure 8: Iron (Fe) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

The data in Figure 9 shows a rather similar manganese content. The two wood-degrading fungi, *P. ulmarius* with 952 mg/kg (*SD* = 412 mg/kg) and *L. edodes* with 2234 mg/kg (*SD* = 252 mg/kg), stand out with their high manganese content. The mutualist, *G. penicillata* (S), contains a mean value of 847 mg/kg (*SD* = 59 mg/kg) manganese, which is with $p = 0.0019$ statistically significantly higher than the manganese content in *A. grosmanniae* with 525 mg/kg (*SD* = 105 mg/kg). Additionally, *G. penicillata* on sawdust has a significantly higher manganese mass fraction than all the pathogens (*C. globosum* $p = 0.0052$, *P. commune* $p = 0.0019$, *Trichoderma* sp. $p = 0.0030$) and two of the wood degraders (*G. frondosa* and *L. sulphureus* with $p = 0.0030$). *A. hylecoeti* showed a mean manganese content of

109 mg/kg (*SD* = 7 mg/kg). This was the lowest for all the here evaluated filamentous fungi, only the pathogen *P. commune* contained less manganese with a mean value of 85.8 mg/kg (*SD* = 11.9 mg/kg). *A. hylecoeti* accumulated significantly less manganese than all the wood degraders and pathogens (except *P. commune*) (*L. sulphureus* $p = 0.0204$, all the other pathogens and wood degraders $p = 0.0019$).

The observed pattern of *E. polonica* and *G. penicillata* accumulating more nutrients on sawdust compared to phloem media, could also be observed here for manganese.

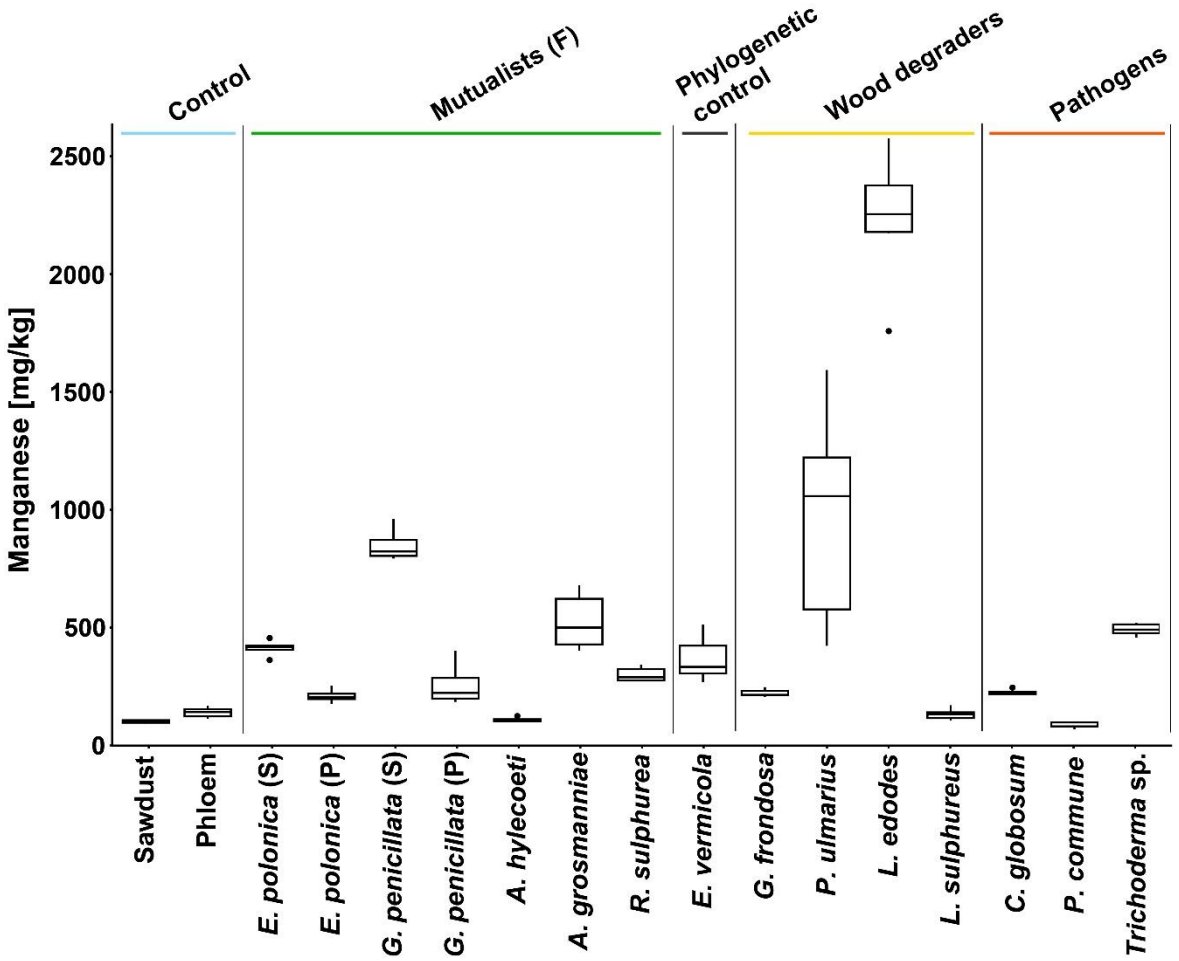

Figure 9: Manganese (Mn) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

For sodium (Na) (Figure 10) it appears that the mutualists have a generally equal or higher sodium content contrasted to the wood degraders and pathogens. The highest Na mass fraction has the mutualist *A. hylecoeti* (*M* = 1113 mg/kg, *SD* = 190 mg/kg), which is statistically significantly higher compared to all the wood degraders and pathogens ($p = 0.00067 - 0.00113$). Also, *G. penicillata* (S) contained with 814 mg/kg (*SD* = 102 mg/kg) a significantly higher sodium mass fraction than all the pathogens and wood degraders ($p = 0.00273 - 0.0219$). *A. grosmanniae* accumulated 709 mg/kg (*SD* = 124 mg/kg) sodium, which was a higher mean sodium content compared to the non-mutualistic fungi, but this was not significantly for all of them (*P. ulmarius* $p = 0.168$, *L. edodes* $p = 0.0989$, *L. sulphureus* $p = 0.0506$ and *C. globosum* $p = 0.225$).

The highest Na accumulation of the non-mutualistic fungi can be seen with the pathogen *C. globosum* with 608 mg/kg (*SD* = 18 mg/kg). Closely followed by the wood degrader *L. edodes* with a mean value of 592 mg/kg (*SD* = 47 mg/kg) sodium. The two mutualists, *E. polonica* and *G. penicillata*, cultivated on sawdust and phloem media show again a different elemental content on phloem compared to sawdust. *E. polonica* accumulated slightly more sodium from phloem (*M* = 484 mg/kg, *SD* = 15 mg/kg) rather than from sawdust (*M* = 437 mg/kg, *SD* = 56 mg/kg), while *G. penicillata* gathered 814 mg/kg (*SD* = 102 mg/kg) from sawdust and 629 mg/kg (*SD* = 69 mg/kg) from phloem.

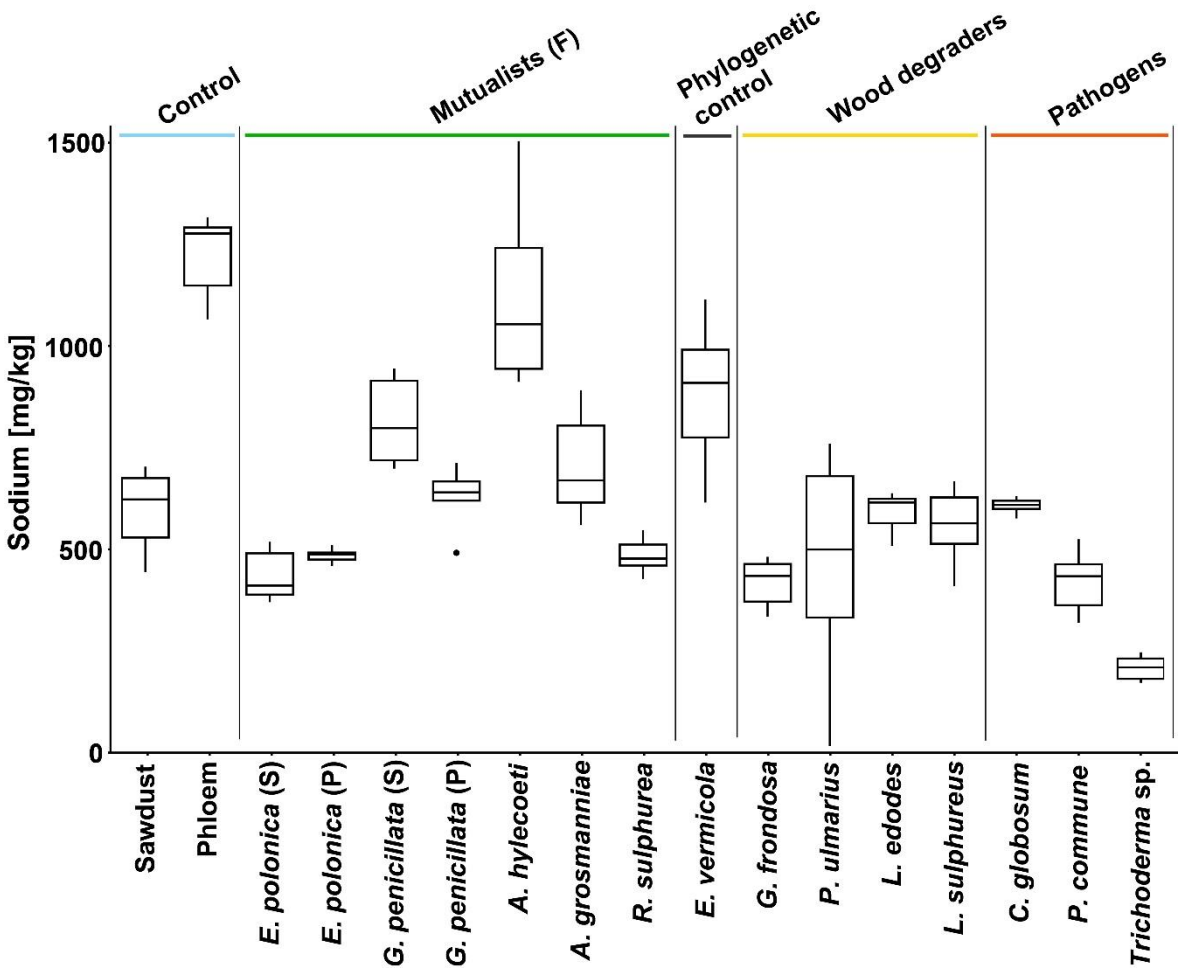

Figure 10: Sodium (Na) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

The last boxplot to be evaluated shows the zinc mass fractions (Figure 11). Here, the mutualists, *E. polonica* and *G. penicillata*, show a higher zinc adsorption from being incubated on phloem media compared to the sawdust media. *E. polonica* (S) with 82 mg/kg (*SD* = 9 mg/kg) and *E. polonica* (P) with 151 mg/kg (*SD* = 8 mg/kg). While *G. penicillata* contained 52.2 mg/kg (*SD* = 5.8 mg/kg) on sawdust and 240 mg/kg (*SD* = 40mg/kg) on phloem media. The differences of *E. polonica* between being grown on sawdust or on phloem is significant with $p = 0.002$. This is also the case for *G. penicillata* on sawdust compared to being cultivated on phloem with $p = 0.002$.

The overall zinc accumulation within the mutualists grown on sawdust do not vary greatly. Here, the zinc contents vary from a mean value of 44.7 mg/kg (*SD* = 11.0 mg/kg) from *A. grosmanniae* to 82 mg/kg (*SD* = 9 mg/kg) from *E. polonica* (S).

The pathogen *P. commune* (*M* = 129 mg/kg, *SD* = 6 mg/kg) has a significantly higher zinc accumulation than all the mutualists grown on sawdust media (p = 0.0013 – 0.0028). In contrast, the three wood degraders *G. frondosa*, *P. ulmarius* and *L. edodes* show a significantly lower zinc mass fraction than all mutualists grown on sawdust media (p = 0.0013 – 0.0377).

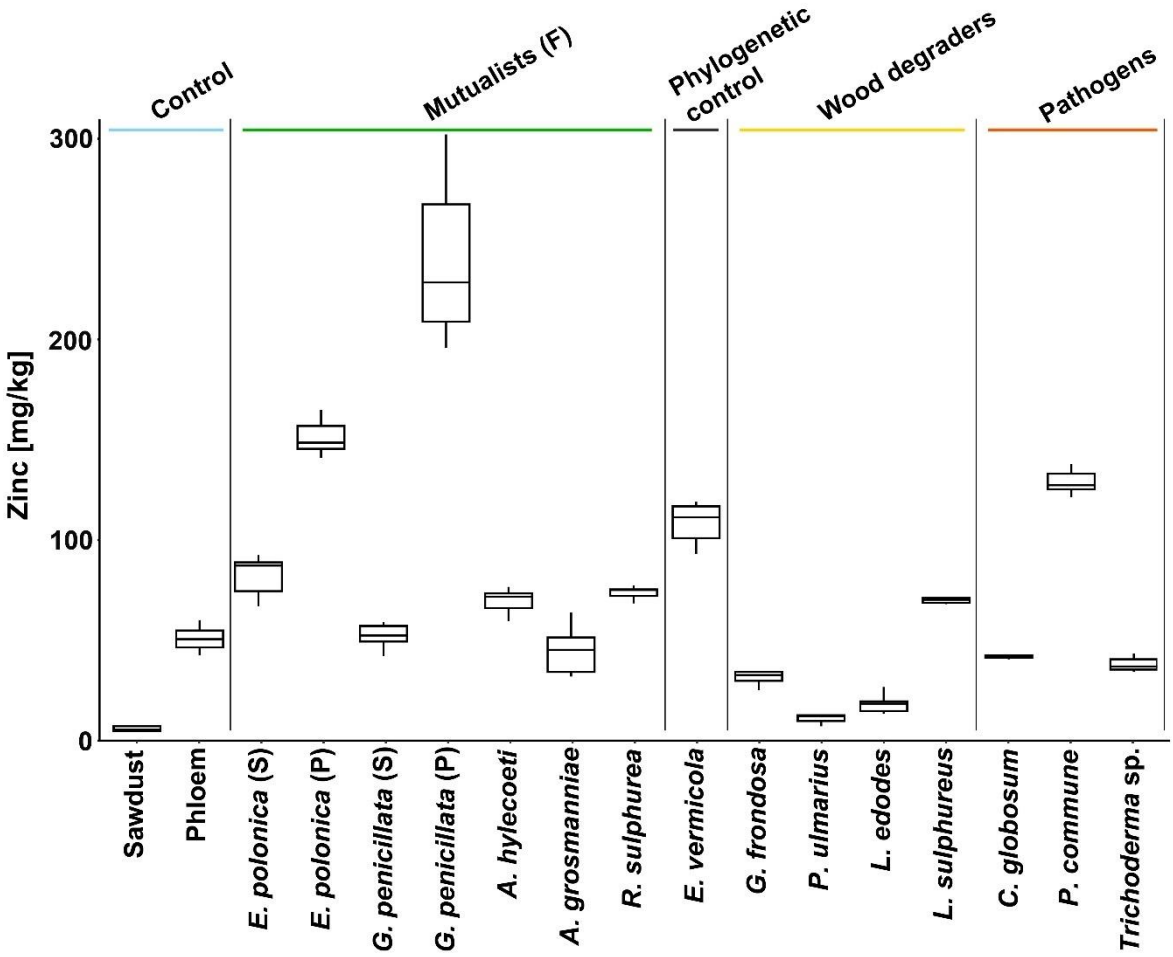

Figure 11: Zinc (Zn) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

The phylogenetic control *E. vermicola* showed for almost all examined elements a higher mass fraction than the mutualist *R. sulphurea*. The contents of the

phylogenetic control were significantly higher in Ca (p = 0.00193), Cu (p = 0.0034), Fe (p = 0.0036), K (p = 0.00189), Mg (p = 0.0021), N (p = 0.00147), Na (p = 0.00220), S (p = 0.0018), Sr (p = 0.0018) and Zn (p = 0.0020) compared to the mutualist. Moreover, *E. vermicola* has higher contents of C, Mn and P but those were not statistically significant with p = 0.6218, p = 0.2234 and p = 0.63403, respectively. The only exception from this occurrence was aluminum, were the mutualist *R. sulphurea* showed with a mean value of 163 mg/kg (*SD* = 80 mg/kg) a significantly higher (p = 0.038) Al content compared to *E. vermicola* with 43.8 mg/kg (*SD* = 25.2 mg/kg).

The heatmap in Figure 12 merely displays the elements which were analyzed with the ICP-OES system and had consequently the same unit (mg/kg). The contents of carbon and nitrogen are not shown in this diagram. This heatmap provides an overview over the mean values for the mass fractions of each element for every filamentous fungus and the controls. The color scale allows a visually aided summary of the overall elemental composition.

E. polonica showed for Al, Cu, Fe, K, Mg, Mn, P and S higher element accumulation after the cultivation on sawdust media. Even tough for Al, K, Mg, Mn, P and S the elemental content was higher in the phloem media compared to the sawdust media. A similar occurrence can be observed for *G. penicillata*. Here, this mutualist accumulated higher contents of Al, Ca, Mg, Mn, Na and Sr from the sawdust media, even though the phloem media contained more of those elements.

The heatmap (Figure 12) does not show a remarkable elemental accumulation for the mutualistic fungi compared to the wood degraders or pathogens. Although, there is a slight overall increase of the aluminum, calcium, magnesium, sodium and potassium contents in the mutualists compared to the wood degraders and pathogens.

An interesting aspect is the high content of potassium within the phylogenetic control *E. vermicola*. As mentioned above, this fungus accumulated surprisingly more elemental components than the mutualistic close relative *R. sulphurea*.

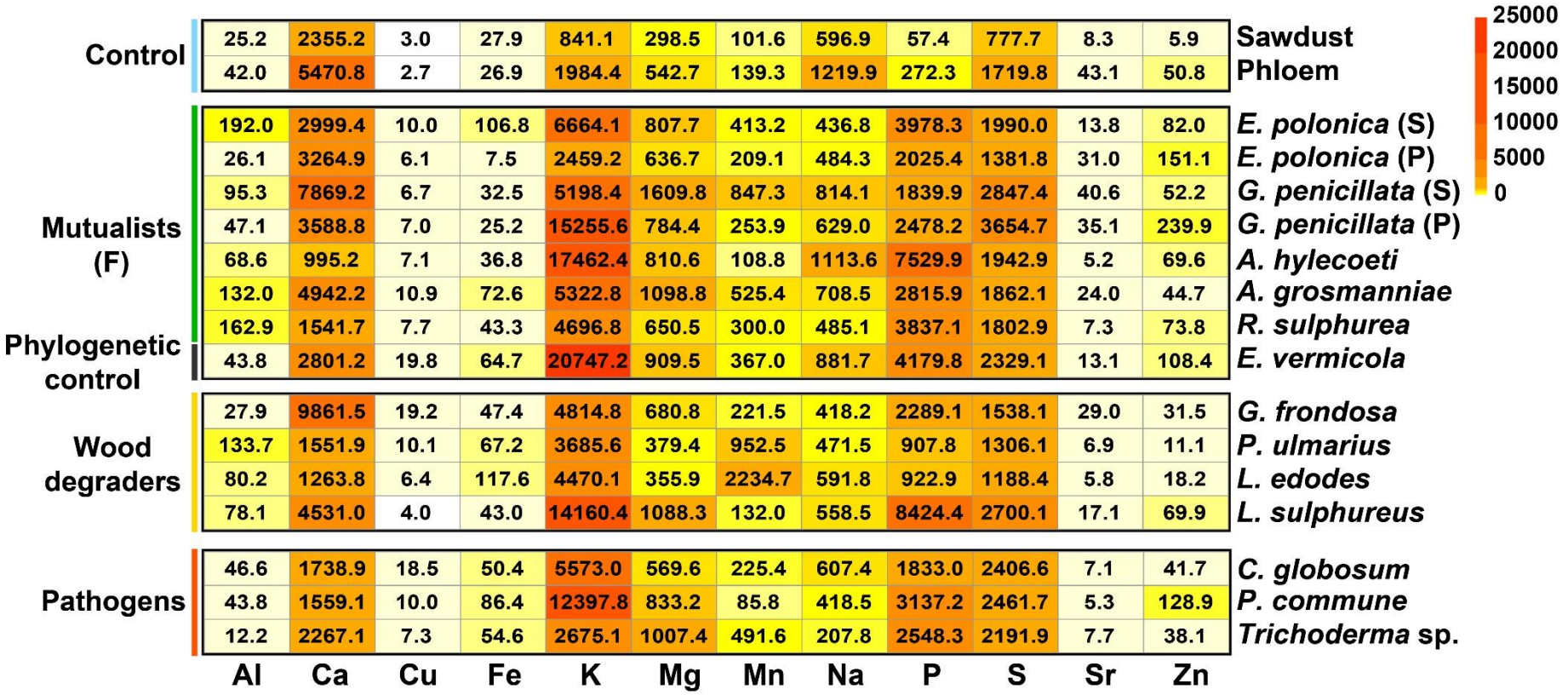

Figure 12: Overview of the elemental contents of the filamentous fungi. Numbers shown are mean values in mg/kg for the elements analyzed with the ICP-OES system. Color scale on the top right indicating a visually aided comparison for the accumulation of elements, reaching from low mass fractions (white) to high mass fractions (red). (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

5.2 Measured elemental contents in yeasts

The yeasts examined were isolated from nests of either the ambrosia beetle *Xyleborinus saxesenii* or the bark beetle *Ips typographus* (see Table 1) prior to this project (Lehenberger et al. unpublished data). The function of yeasts in association with bark and ambrosia beetles is not yet fully uncovered. For this reason, the elemental composition of selected yeasts was additionally analyzed to investigate their nutritional value after growing on sawdust (and phloem media). The boxplots in Figure 17 to Figure 30 and the heatmap in Figure 31 in the Supplements show the elemental contents of the analyzed yeasts in comparison to the filamentous fungi.

There are no big variations of the elemental compounds separating the yeasts from one another (Figure 31). The two yeasts that stand out are the *Candida* sp., which contains with a mean value of 8457 mg/kg (*SD* = 804 mg/kg) twice as much potassium as all the other yeasts (Figure 31). The other yeast, *C. japonica*, had the most contents of copper (*M* = 33.3 mg/kg, *SD* = 17.0 mg/kg), magnesium (*M* = 1685 mg/kg, *SD* = 143 mg/kg), manganese (*M* = 387 mg/kg, *SD* = 30 mg/kg), sodium (*M* = 556 mg/kg, *SD* = 60 mg/kg), phosphorous (*M* = 9525 mg/kg, *SD* = 705 mg/kg) and sulfur (*M* = 3156 mg/kg, *SD* = 130 mg/kg).

Compared to the filamentous fungi, yeasts represent a greater phosphorous and zinc source (Figure 31). Furthermore, the nitrogen content in yeasts were the highest of all the analyzed organisms. For bark beetle associated yeasts, the nitrogen contents reached from *Y. mexicana* (It12 (S)) with 3.47 % (*SD* = 0.12 %) to *C. mississippiensis* (S) with 3.97 % (*SD* = 0.24 %). The associated yeasts of the ambrosia beetles reached nitrogen contents of 5.18 % (*SD* = 0.10 %) with the yeast *C. japonica* (Figure 25, Table 13). A more detailed comparison of the yeasts with the filamentous fungi is provided through the boxplots from Figure 17 to Figure 30 and the *p*-values are listed in Table 28 to Table 41 in the Supplements.

The occurrence that the organisms grown on phloem and sawdust media accumulated more nutrients while growing on sawdust media can also be observed here. The contents of aluminum, copper, iron, and sulfur are higher within the yeasts grown on sawdust compared to the same yeasts grown on phloem. However, the phloem either contains an equal or a higher amount from each element (Figure 31).

6 Discussion

6.1 Elemental nutritional value of filamentous fungi

The aims of this thesis were to determine whether filamentous mutualistic fungi of bark and ambrosia beetles accumulate more elements than other related (ecologically or phylogenetically) filamentous fungi grown on the same substrate. Here, two mutualistic fungi of the bark beetle *Ips typographus* and tree mutualistic fungi of three ambrosia beetle species *Elateroides dermestoides*, *Xylosandrus germanus*, and *Xyleborinus saxesenii* were examined. Additionally, the phylogenetic control fungus *Esteya vermicola* was included to this study as it is the closest non-mutualistic sister species to the ambrosia beetle fungus *Raffaelea sulphurea*. Furthermore, four common wood-degrading fungi and three ubiquitous fungal pathogens were examined, where the later are common inhabitants of bark and ambrosia beetle nests.

The results summarized in the heatmap (Figure 12) clearly show, that mutualistic fungi are able to accumulate nutrients and enrich them within their mycelium. These findings confirm the assumption that mutualistic fungi are of nutritional higher value compared to xylem or phloem (Filipiak und Weiner 2017; Bracewell und Six 2015). Further, it underlines the importance of these fungi to fulfill the nutritional requirement for their beetle hosts (Ayres et al. 2000). However, the ability to accumulate higher amounts of essential elements is not restricted to mutualistic fungi. The wood-degrading fungi, the pathogens as well as phylogenetic control showed increased elemental contents compared to the controls (phloem and xylem) (Figure 12).

The results provide no clear evidence that mutualistic fungi generally contained more elements than the other tested fungi. An increased elemental accumulation only appears for some mutualists and only for a few elements. A reason for partially increased elemental content in certain fungi is unknown. It can be suggested that these fungi possibly have a higher requirement for some elements due to metabolic reasons. The pure elemental results give too little information about the purpose of an increased accumulation of certain elements. A view on the molecular composition could provide more detailed information.

What can be taken into consideration is the social behavior of the beetles. For instance, the ship timber beetle *Elateroides dermestoides*, the host of the fungal mutualist *A. hylecoeti*, must survive on its own within the larval chamber (Batra und Francke-Grosmann 1961; Egger 1974). The female ship timber beetle lays eggs in a tree and dies shortly afterwards. The larva develops alone over the next two to three years within the tree and lives closely together with the fungal mutualist (Egger 1974). The fungal partner must accumulate more nutrients within hyphae compared to other mutualistic fungi in ambrosia beetle systems with higher sociality. In these systems, more beetles are taking care about the fungal cultivars and can easily provide more substrate by increasing the nest chambers. This way, these beetles and larvae have the chance to consume higher amounts of fungal biomass compared to the *E. dermestoides – A. hylecoeti* system. This theory could be confirmed for the elements K, Na and P, were *A. hylecoeti* accumulated significantly more of those elements than the other mutualists grown on sawdust media. *A. hylecoeti* also showed a high total nitrogen content with 2.75 % (*SD* = 0.09 %), where only the bark beetle mutualist *E. polonica* accumulated more nitrogen (*M* = 3.01 %, *SD* = 0.13 %) when cultured on sawdust medium.

Following this hypothesis, the mutualists *E. polonica* and *G. penicillata* should show the second highest nutrient content. The associated bark beetle, *Ips typographus*, performs brood care to a stronger extent than the ship timber beetle. The female beetle lays the eggs in short larval galleries all along the main gallery and seals those larval galleries. Once hatched, the larvae will expand the individual galleries in a horizontal direction (Kirkendall et al. 2015; Six und Wingfield 2011). It is very noticeable that the two mutualistic fungi of *Ips typographus* complete each other in their elemental composition after the incubation on sawdust media. While *E. polonica* contained the highest elemental content of all the mutualists grown on sawdust for Al, C, Fe, N and Zn, *G. penicillata* accumulated the most of Ca, Mg, Mn, S and Sr. In summary, those two mutualists cover a broad range of elemental nutrients and showed the highest content for ten out of the total fourteen examined elements of the here tested filamentous mutualistic fungi.

The last two mutualistic fungi, *R. sulphurea* and *A. grosmanniae* are associated with facultative eusocial ambrosia beetles (Biedermann et al. 2013; Hoffmann 1941; Weber und McPherson 1984). In the nests of *X. saxesenii* the fungus *R. sulphurea* is thought to be the main mutualist and the beetle *X. germanus* is associated with *A. grosmanniae*. Those beetle species live together with approximately 40 - 80 larvae and beetles maintaining the nest. This includes cleaning each other, transporting waste out of the nest and reducing the amount of pathogenic or antagonistic fungi (Biedermann und Taborsky 2011). Additionally, the larvae and beetles continuously enlarge the nest and enable the mutualistic fungi to access new nutrients in the wood. This occurrence leads to the suggestion that those mutualistic fungi do not have to be able to extract great amounts of nutrients out of the wood by themselves because the beetles make sure to provide more nutrients by continuously expanding the nest. On the other hand, a brood with many members acquires a greater supply of nutrients. Besides, if wood is chewed and therefore fragmented by the beetles, the enzymes of the fungi gain better access for degradation (Douglas 2009), which could lead to a higher nutrient accumulation in the fungi. Those arguments pose a contrary view on this hypothesis. However, the results generally support the hypothesis about the dependence of elemental accumulation on the social behavior of the beetles. Overall, the ambrosia beetle mutualists revealed an inferior ability to gather nutrients in their cells. Possibly, they are less efficient in degrading wood than bark beetle fungi.

An astonishing phenomenon could be observed between the two mutualistic fungi *E. polonica* and *G. penicillata*. Those fungi were cultivated on phloem and sawdust media in order to test their nutrient accumulation from their habitat substrate (phloem) and to compare their accumulation with other fungi grown on sawdust. This approach led to an interesting insight. *E. polonica* accumulated more of Al, K, N, P, S, Mg and Mn from the sawdust, even though the phloem contained those elements in a greater amount (Figure 5, Figure 12). The other mutualist, *G. penicillata* showed this behavior for Al, Ca, Mg, Mn, Na and Sr (Figure 12). A possible explanation could be the that the fungi do not accumulate nutrients in a big manner, when the substrate they had grown on provides it in a great amount. Alternatively, those elements could be present in the phloem in a form that the fungi

are unable to break down. This assumption could already been marginally observed by Six und Elser (2019). They revealed that a mutualistic fungus was able to transport nutrients better from the sapwood (xylem) to the bark were the beetles feed, compared to an antagonistic fungus. Hence, mutualist might gather the elements from the xylem instead of the phloem in natural systems as well. The ability to translocate elemental nutrients is important for the spore production. With the intention of dispersal by their beetle symbiont, the spores must be produced in the feeding chambers, so that the spores can latch onto the exoskeleton or the mycetangia of adult beetles (Six und Elser 2020).
So, an explanation for the different elemental accumulation of the mutualist bark beetle fungi could be a combination of the tree possible reasons mentioned above. The fungi are able to gather nutrients better from the xylem and are capable to transport them to the beetles' feeding galleries, where their spores have the greatest success to latch onto their vector.

The differences in the elemental accumulation between the closely related *E. vermicola* and the mutualist *R. sulphurea* also support the suggestion that the mutualistic fungi do not stand out by being generally more nutritious than other fungi. *Esteya vermicola* is a nematophagous fungi and was first discovered in Taiwan in 1999 (Liou et al. 1999). Interestingly, *E. vermicola* showed in ten out of the total fourteen analyzed elements a significantly higher accumulation than the closely related mutualist *R. sulphurea*. For the three elements C, Mn and P, the phylogenetic control showed a higher elemental content too, but this was not statistically significant (see Table 15, Table 21 and Table 24 in the Supplements). These results show that closely related fungal species can differ in their elemental profile even if they are grown under the exact same conditions.

The fungi tested in this project were the main associates of each individual beetle. However, the nests not only contain just one fungus or yeast. A big variety of different yeasts, bacteria, filamentous fungi, nematodes, and even mites can be found living together with the brood (Kirkendall et al. 2015). For this reason, the cultivation of the filamentous fungi and yeasts on the sawdust and phloem media alone might have altered their behavior in accumulating nutrients. For instance, the absence of potential competitors for the nutrients might have reduced their uptake.

In contrast to that, the lack of other members of the symbiosis with a positive effect onto the filamentous mutualistic fungi could have had an influence on the here gathered results. Liu et al. (2020) showed in their research that symbiotic bacteria of the bark beetle *Dendroctonus valens* influenced the beetle-fungus-mutualism. Through volatile ammonia emitted by the bacteria, the mutualistic fungus *Leptographium procerum* had a faster carbohydrate consumption. Additionally to that, the ammonia also induced the fungus to produce amylase and secret it as an exoenzyme (Liu et al. 2020). This enzyme catalyzed the breakdown of starch in the phloem to glucose, which is a good carbon source for beetle, fungus and bacteria. This paper showed that the organisms are able to influence one another and hence, their behavior can change depending on the surrounding microbiome.

The comparison between the mutualistic fungi and the wood-degrading fungi must be made with caution because they belong to different phyla (Table 1). The mutualists are all ascomycetes, while the wood-degraders are altogether basidiomycetes. Moreover, the comparison of the filamentous fungi with the yeasts is challenging, given the different morphological attributes. The yeasts are unicellular organisms and cannot penetrate the wood (Six 2013). For filamentous ambrosia fungi, it could already been shown that they are capable of growing a few millimeters into the wood (Francke-Grosmann 1966). However, the yeasts might also participate in the nutritional support of the beetle by being incorporated into fungal mycelia or transported by the beetle within the galleries.

It must also be considered that the cultivation was performed under laboratory conditions. The natural environment would not have been suitable to obtain the answers for the research question. The very low sample weighs were additionally a challenging aspect of this thesis. Even after a growth time of four weeks, the fungi and yeasts did not develop a lot of biomass. Further, the obtained biomass lost a big portion of its weight through freeze drying due to the loss of water, leaving a total sample weight ranging from 1 mg to 36 mg. The low weights were acceptable for the elemental analyzer vario EL III with a standard sample weight during routine analysis of 25 mg. For the ICP-OES, a routine sample weight of 500 mg is used, thus the weights of the fungi and yeast samples were too low and adjustments in the calibrations were needed. However, the boxplots show an overall small range in

the measured data of the replicates for each fungus. This shows a good analytical performance of the analyzers even with such little sample weights. The ICP-OES system worked well with the diluted calibration standards and control samples. The here used methods and analyzers were suitable to evaluate the samples and to obtain the needed data for the question of this thesis. Nonetheless, the provision of more biomass is recommended.

The determination of the limit of quantification with ICP-OES system delivered the results shown in Table 10 in the Supplements. To hold enough replicates to perform statistical analysis with the measured data, some of the values below the limit of quantification were still used.

Table 11 shows how many data points were below the limit of quantification. This means a data point below the given concentration cannot be certified with a certain security, but the Tukey method did not mark those values as outliers. This could be explained by the generally low element contents in the samples.

The obtained results show that it cannot be generalized that mutualistic fungi contain over all more elemental nutrients than other filamentous fungi. So consequently, there must be other reasons why certain bark and ambrosia beetles mainly appear with the same fungal symbionts. One reason could be their possible advanced nutrient transportation (Six und Elser 2019; Filipiak und Weiner 2017; Six und Elser 2020). Here, the investigated elemental composition just allows a view on the fundamental nutritional value of the fungi. It must be investigated in more detail how the nutritional accumulation is occurring on a molecular level, examining produced sugars, vitamins, sterols, lipids, and other biologically important molecules. This should provide a more detailed overview over the nutritional value of mutualistic fungi in comparison to non-mutualistic fungi.

With recent new discoveries, the possibility of a multipartite symbiosis gets strengthened (Ibarra-Juarez et al. 2020; Douglas 2009). Bacteria, isolated from beetle galleries, are able to degrade more plant polymers compared to the fungal symbionts. Furthermore, Ibarra-Juarez et al. (2020) stated that almost all of the bacteria were able to synthesize essential amino acids, suggesting this was possible due to their ability of fixating atmospheric nitrogen. In addition, the bacteria could

produce vitamins and cofactors which could not be produced by the fungi. Ibarra-Juarez et al. (2020) hypothesized that yeasts and bacteria initially degrade the walls of the nests which support the following fungal growth and that those unicellular organisms are involved in the symbiosis in a higher degree than previously assumed. More work in this field is needed with a special focus on the interactions between the microbial symbionts of bark and ambrosia beetles.

6.2 Elemental nutritional value of yeasts

Six (2013) states, that the impact of yeasts on the survival of the bark beetles remains unclear. In this thesis, some yeasts were examined for their potential nutritional value for the beetles. Yeasts are unicellular organisms and are unable to grow into the wood in contrast to filamentous fungi (Six 2013; Francke-Grosmann 1966). However, this feature is very important for the beetles to survive in the tree. The It12 and It13 strain *Yamadazyma mexicana* and the yeast *C. mississippiensis* were isolated from a nest of the bark beetle *Ips typographus*. The remaining yeasts, *Y. mexicana* (Xs1), *Candida* sp. and *C. japonica* were isolated from a nest of *X. saxesenii*. As shown in the heatmap (Figure 31 in the Supplements) yeasts do not have vast differences between their elemental accumulation. Given that they were isolated from three different nest, the three strains of *Yamadazyma mexicana* show approximately the same elemental accumulation. Only *Y. mexicana* from *X. saxesenii* contained more magnesium, manganese, potassium, and zinc (Figure 31), which could lead to the assumption that this might be a close relative to the species *Y. mexicana* with slightly different metabolic characteristics. The results in Figure 31, Table 12 and Table 13 show that the yeasts had a much greater phosphorous, nitrogen, aluminum and zinc accumulation compared to the filamentous fungi. Hence, the yeasts propose a good phosphorous and nitrogen source for the beetles, which could be obtained by consuming yeast cells that live within the mycelia.

Interestingly the yeasts *Y. mexicana* and *C. mississippiensis* grown on phloem (P) and sawdust (S) media show the same difference in the elemental mass fraction from sawdust compared to phloem as the filamentous mutualists. Overall, the yeasts accumulated more of the elements Al, K, Mg, Mn, P, S and Zn from the sawdust

media, even though phloem contained those elements in a higher mass fraction (see Figure 31). This strengthens the assumption, that phloem might contain these elements in a form which is unavailable for the organisms. On the other hand, the yeast also might not need to accumulate this element in high quantities because the media contains a great amount of it.

As mentioned above, the weight of the samples was a challenging aspect of the analysis. The yeasts were particularly difficult to harvest from the cellophane foil because of their slimy consistency, which resulted in only 1 – 7 mg freeze dried biomass. This might also be one of the reasons for the rather big range in the elemental contents of the yeasts. A better harvesting method is needed for future work in the determination of the elemental composition of yeasts. Perhaps a liquid culture approach could provide more biomass. Although, a liquid culture would not represent the natural conditions.

The role of the yeasts within the symbiosis might not be of nutritional relevance. For instance, Leufvén et al. (1984) discovered that yeasts could play a role in the communication of the bark beetle *Ips typographus* through volatile molecules. The yeasts were able to convert cis-verbenol into verbenone. Cis-verbenol functions as a pheromone which attracts more beetles to a tree. Verbenone inhibits this attraction and might be informing the beetles that the tree has already been occupied in a large scale (Hofstetter et al. 2015; Leufvén et al. 1984). Furthermore, yeasts are suggested to use toxins of the tree as a carbon source and therefor contribute to a detoxification process within the tree (Davis 2015).

As already mentioned above, more work with the symbionts of the bark and ambrosia beetles is needed. The interactions between the microbial symbionts are of crucial importance to investigate. As studies from recent years have revealed, the symbiosis might not be restricted to a beetle and a fungal partner. Many organisms, such as bacteria, yeasts and filamentous fungi might be involved (Ibarra-Juarez et al. 2020) and more research is needed to fully understand their functions and possible influence on the beetle nutrition.

7 Conclusion and outlook

In conclusion, the here tested mutualistic filamentous fungi do not represent a commonly nutrient enriched group compared to non-mutualistic fungi. An increased elemental content could be observed for a few elements, but not collectively for all the mutualists. The dissimilar elemental accumulation could be attributed to the different social behavior of the beetles. For instance, the mutualistic fungus *A. hylecoeti* showed the highest accumulation of the macro elements potassium, phosphorous and sodium of the tested filamentous mutualists. This represents an advantage for the associated larva, which lives alone in a gallery and cannot rely on brood care of adult beetles (Egger 1974). Additionally, the results confirmed that the beetles benefit from the presence of mutualistic fungi. Studies have shown that the food uptake is reduced in the presence of a fungal associate, which was also reflected by shorter galleries (Ayres et al. 2000). However, the analysis revealed that even fungi not involved in the symbiosis showed higher nutrient contents compared to the substrate alone. Then the question arises why a beetle is associated with a certain fungus species, additionally supported through the selectivity of the mycetangia (Bracewell und Six 2015). A possible reason might be found on the molecular level, with the focus on beneficial secondary metabolites or nutritional molecules. The missing nutritional advantage observed for the here tested filamentous fungi supports the proposals of a more complex relationship, involving several organisms (Douglas 2009; Ibarra-Juarez et al. 2020). The obtained data supports those claims through the remarkably higher nitrogen, phosphorous and zinc content in yeasts, compared to the filamentous fungi. As Ibarra-Juarez et al. (2020) hypothesized, yeasts and bacteria could provide and initial degradation step, which is subsequently followed by fungal colonization.

More focus is needed on the various symbiotic organisms occurring in bark and ambrosia beetle galleries and chambers. This thesis refuted the claim of nutritionally advanced mutualistic fungi on an elemental level. However, the nutrient supplementation might highly depend on a variety of interactions. Further, the social behavior of the beetles, the available substrate and the present organisms might alter the nutrient provisioning. Perhaps the bark and ambrosia beetle outbreaks can be maintained through a better understanding of their ecosystem within the trees.

8 Acknowledgements

First, I would like to thank Dr. Gros and Dr. Leiterer for the opportunity to perform my analysis in their laboratories. Without the collaboration between the TLLLR and the Max-Planck-Institute, this project wouldn't have been possible. Additionally, I want to thank all the colleagues from the Referat 22 at the TLLLR for their professional expertise and help during my analysis.

Furthermore, I want to thank Prof. Dr. Jonathan Gershenzon and all the friendly colleagues at the Max-Planck-Institute for chemical ecology for welcoming me into their group. I had a great time and got to meet many interesting and kind people.

A very special thank you goes out to my supervisor Dr. Maximilian Lehenberger. I am beyond grateful for all the support and recommendations during my thesis, no question of mine stayed unanswered while working in your team. I also want to express my gratitude to Yu Pan, for teaching me all about the work with the fungi.

Finally, I want to thank my parents, without you I wouldn´t be where I am today.

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10 Supplements

10.1 Supplement tables of the methods

Table 5: Compositions of the culture media. Preculture media: PDA. Experimental media: sawdust and phloem media.

Media	Ingredients
Potato dextrose agar (PDA)	- 15,6 g (3.9 %) potato dextrose agar - 400 ml deionized water
Sawdust (xylem) media	- 20 g (5 %) beech wood sawdust - 8 g (2 %) plant-based agar - 400 ml deionized water
Phloem media	- 20 g (5 %) beech wood sawdust - 8 g (2 %) plant-based agar - 400 ml deionized water

Table 6: Target value and interval of C and N for the reference sample "KP_E29" for the elemental analyzer at the Thüringer Landesamt für Landwirtschaft und Ländlichen Raum (TLLLR).

Element	Target value [%]
C	42.59 ± 0.038
N	2.66 ± 0.13

Table 7: Composition of the multi-element standard solution ("Pflanzenaufschlüsse") from the Thüringer Landesamt für Landwirtschaft und Ländlichen Raum (TLLLR).

Element	Concentration [mg/l]
Ca, K	500
Al	400
P	250
Fe	200
Mg, Na, S	100
Mn	25
Sr	10
B, Co, Cu, Mo, Zn, Ti	5

Table 8: Concentrations of the evaluated elements in the calibration standards of the ICP-OES.

Element	Std. 1 [mg/l]	Std. 2 [mg/l]	Std. 3 [mg/l]	Std. 4 [mg/l]	Std. 5 [mg/l]	Std. 6 [mg/l]
P	0	0.25	0.5	2.5	5.0	12.5
S, Mg, Na	0	0.1	0.2	1.0	2.0	5.0
Zn, Cu	0	0.005	0.01	0.05	0.1	0,025
Al	0	0.4	0.8	4.0	8.0	20.0
Fe	0	0.2	0.4	2.0	4.0	10.0
K, Ca	0	0.5	1.0	5.0	10.0	25.0
Mn	0	0.025	0.05	0.25	0.5	1.25
Sr	0	0.01	0.02	0.1	0.2	0.5

Table 9: Target value and interval for the reference sample "KP208" for each element for the ICP-OES analysis at the TLLLR.

Element	Target value [mg/kg]	Interval [mg/kg]	"KP208" measured in this thesis [mg/kg]
Al	763	666 - 865	746 ± 23
Ca	19800	17460 - 22140	19151 ± 190
Cu	8.21	6.66 – 9.76	6.75 ± 0.13
Fe	528	402 - 654	563 ± 12
K	20500	17920 - 23080	20121 ± 166
Mg	3230	2790 - 3670	3137 ± 36
Mn	43.7	36,82 - 50,58	43.5 ± 1.1
Na	752	629 - 875	651 ± 11
P	2580	2204 - 2956	2472 ± 23
S	3070	2624 - 3516	3016 ± 39
Sr	49.6	43.7 – 55.5	49.5 ± 0.5
Zn	19.9	16.12 – 23.68	18.9 ± 0.3

Table 10: Determined limit of quantification for the elements measured by ICP-OES and their emitted wavelength.

Element	Emitted wavelength [nm]	Limit of quantification [mg/l]
Al	396.1	0.01
Ca	315.8	0.03
Cu	327.3	0.0005
Fe	239.5	0.003
K	769.8	0.08
Mg	279.0	0.02
Mn	257.6	0.0002
Na	589.5	0.1
P	213.6	0.02
S	180.7	0.01
Sr	407.7	0.0001
Zn	213.8	0.002

Table 11: Amount of utilized measured data points below the determined limit of quantification (LOQ) from the ICP-OES system for the creation of the boxplots and for statistical evaluations.

Control samples												
Element	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Sr	Zn
Data points < LOQ	3	0	2	0	0	0	0	0	0	0	0	0
Filamentous fungi (mutualists, phyl. control, wood degraders, pathogens)												
Element	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Sr	Zn
Data points < LOQ	12	0	1	1	0	0	0	3	0	0	0	2
Yeasts												
Element	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Sr	Zn
Data points < LOQ	7	0	0	1	0	0	0	27	0	0	0	0

10.2 Additional boxplots of the examined elements in filamentous fungi

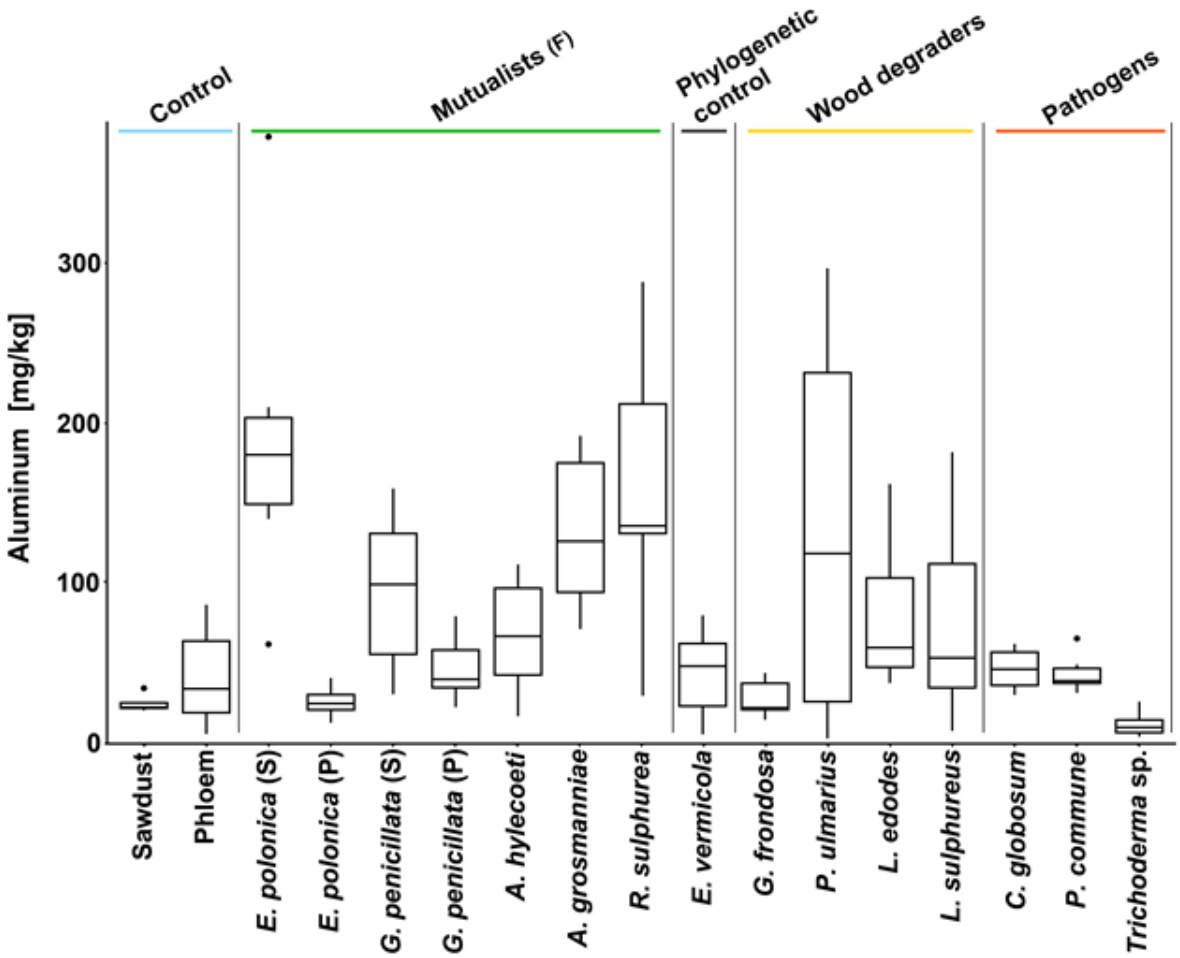

Figure 13: Aluminum (Al) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

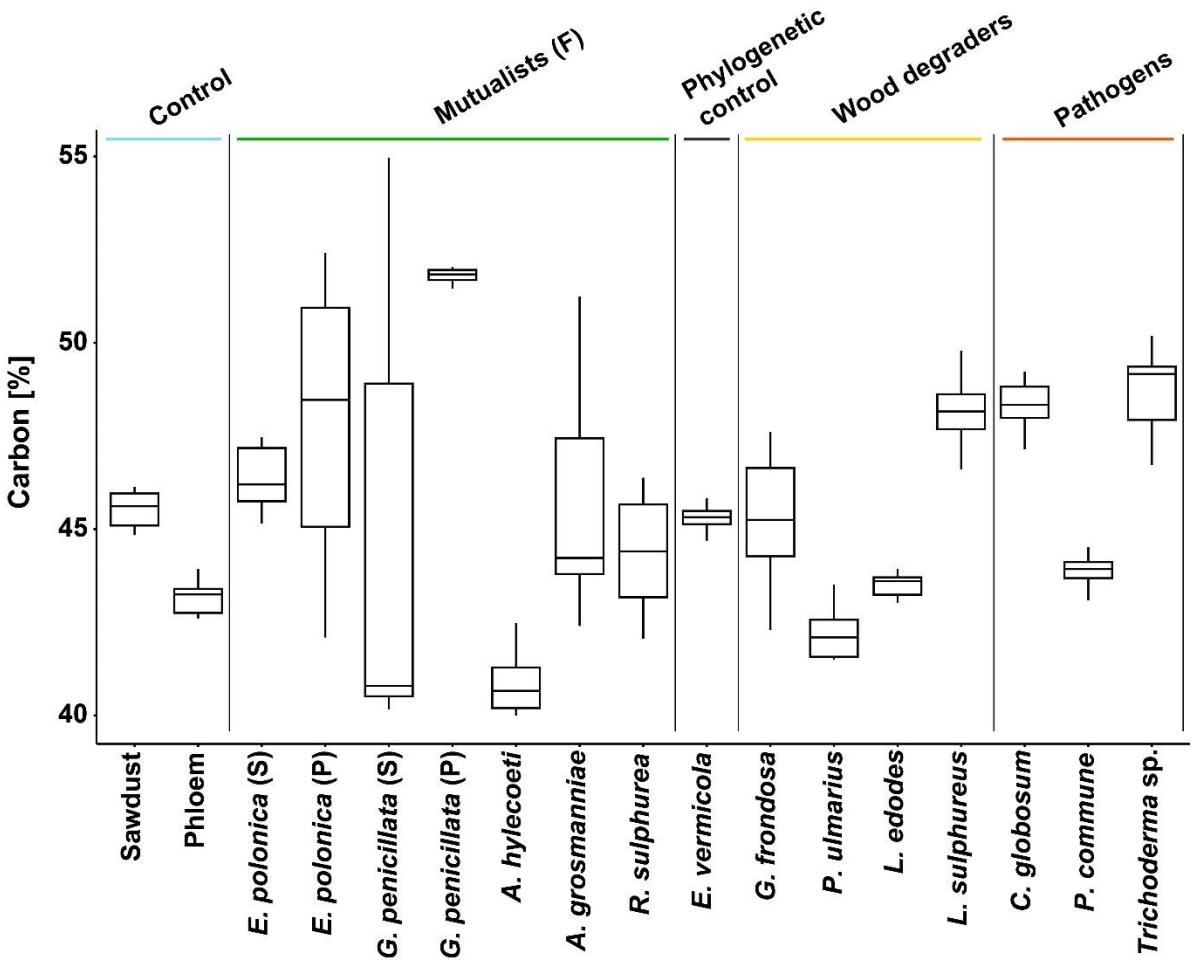

Figure 14: Carbon (C) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in a percentage (%). The analysis was carried out with an elemental analyzer. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

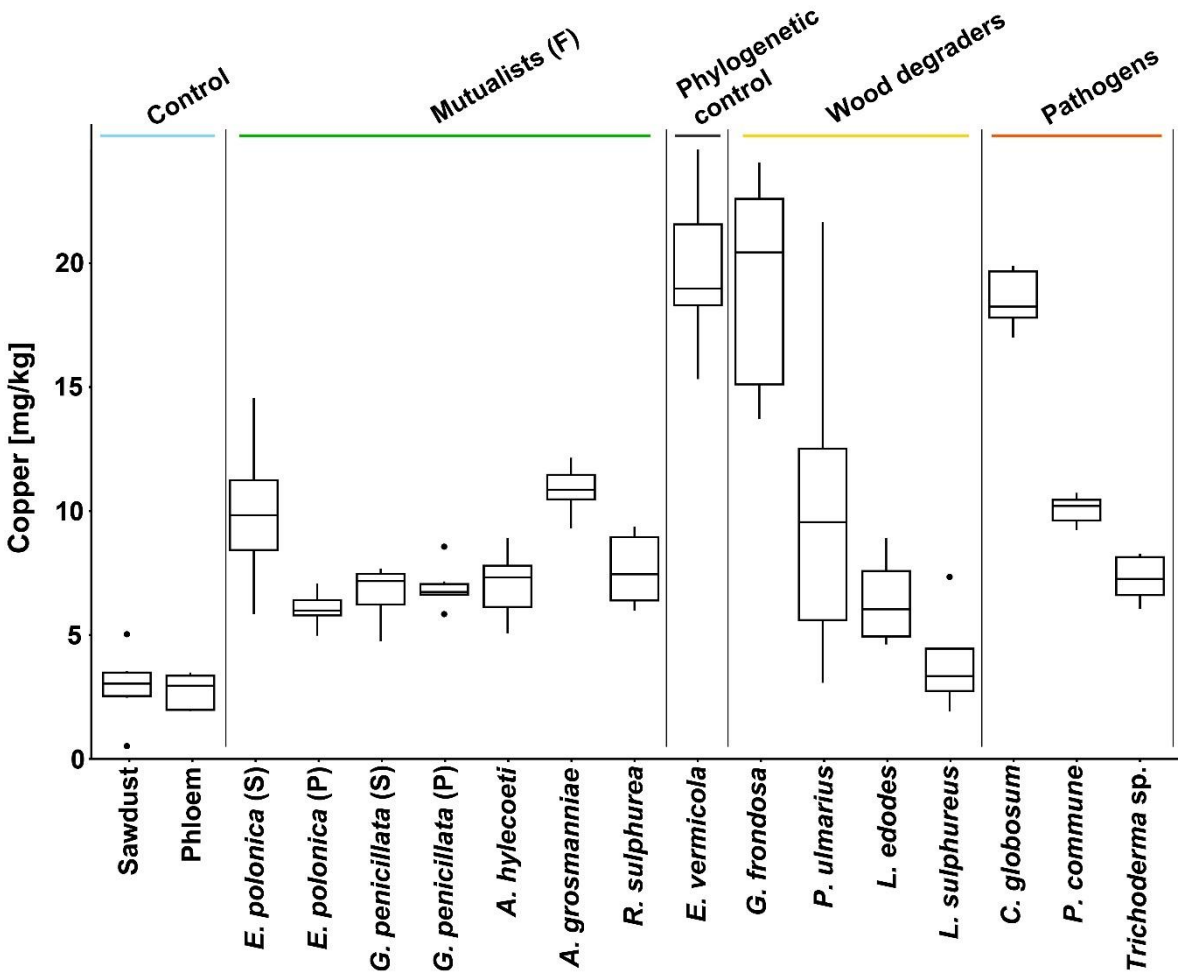

Figure 15: Copper (Cu) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

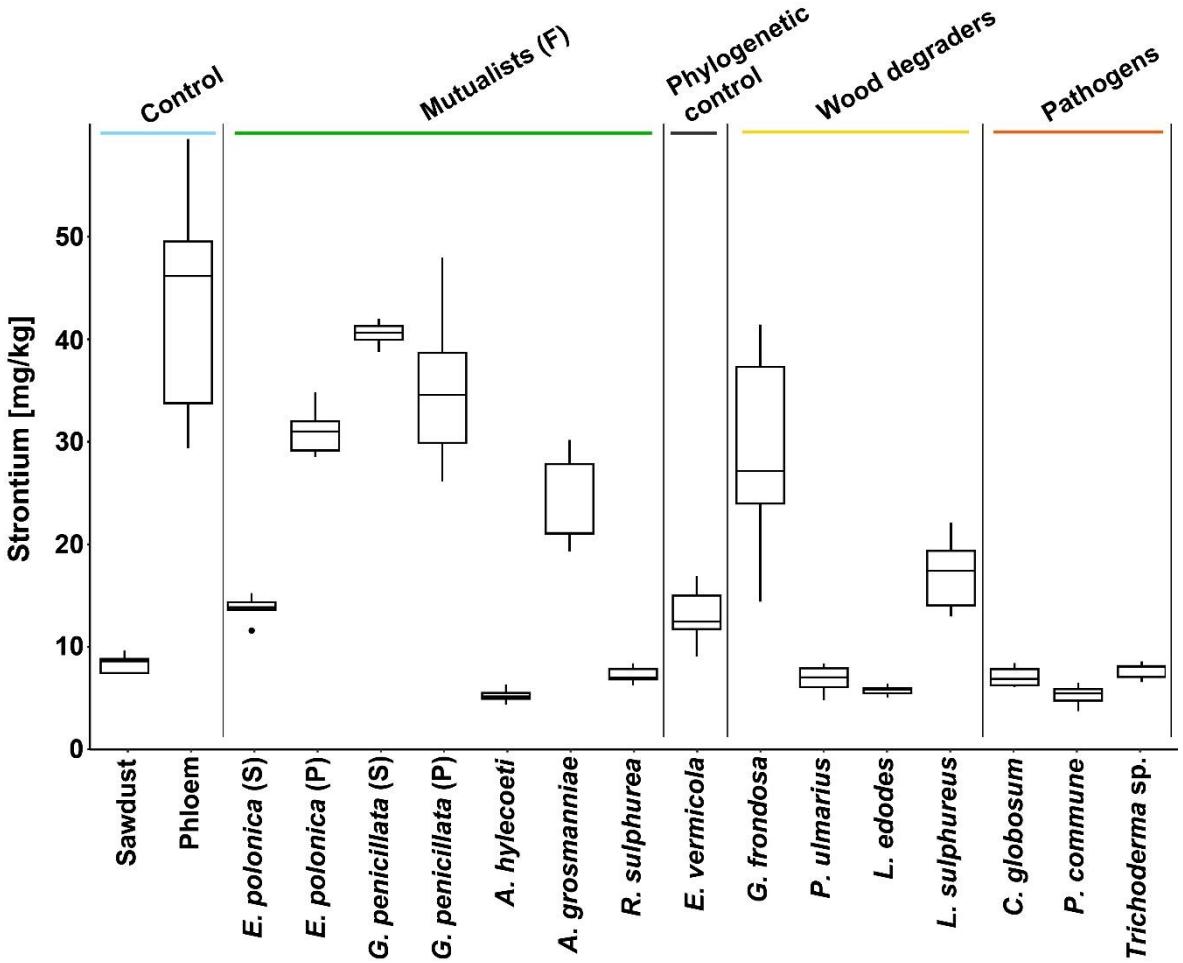

Figure 16: Strontium (Sr) content in filamentous mutualistic fungi in comparison to wood-degrading fungi and pathogens in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. (F) = Filamentous mutualists. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

10.3 Boxplots of the examined elements in all tested organisms

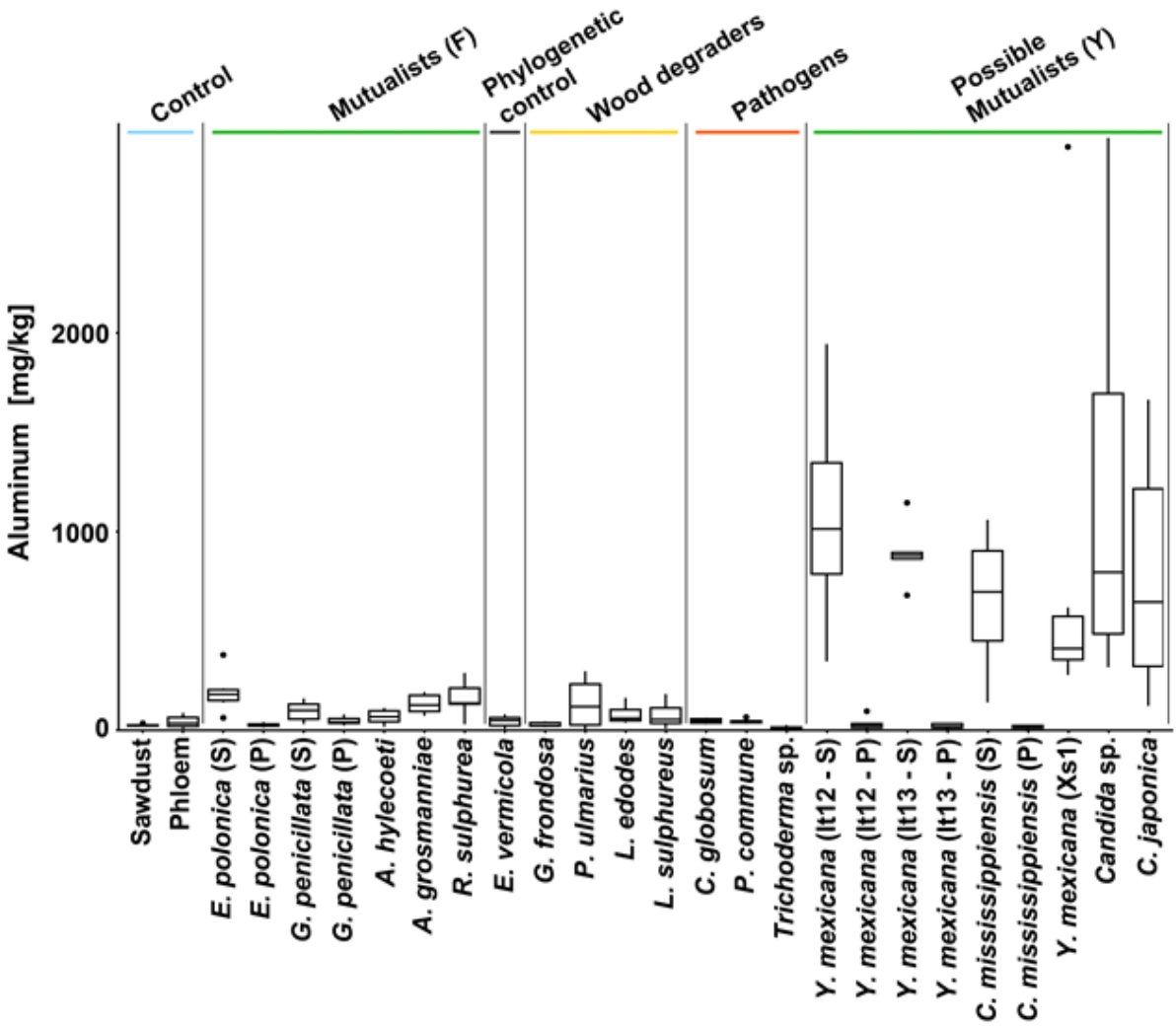

Figure 17: Aluminum (Al) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

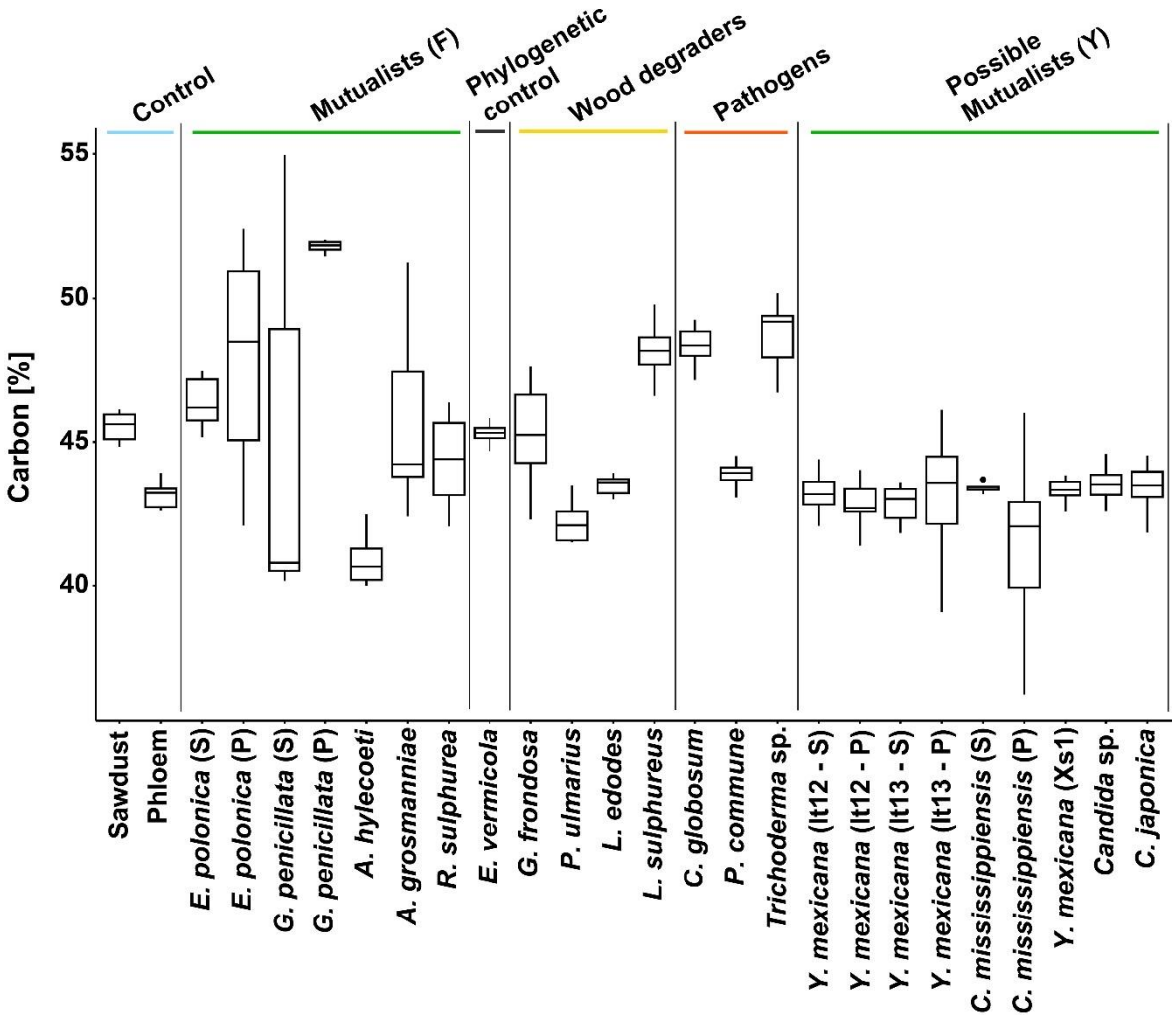

Figure 18: Carbon (C) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in a percentage (%). The analysis was carried out with an elemental analyzer. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

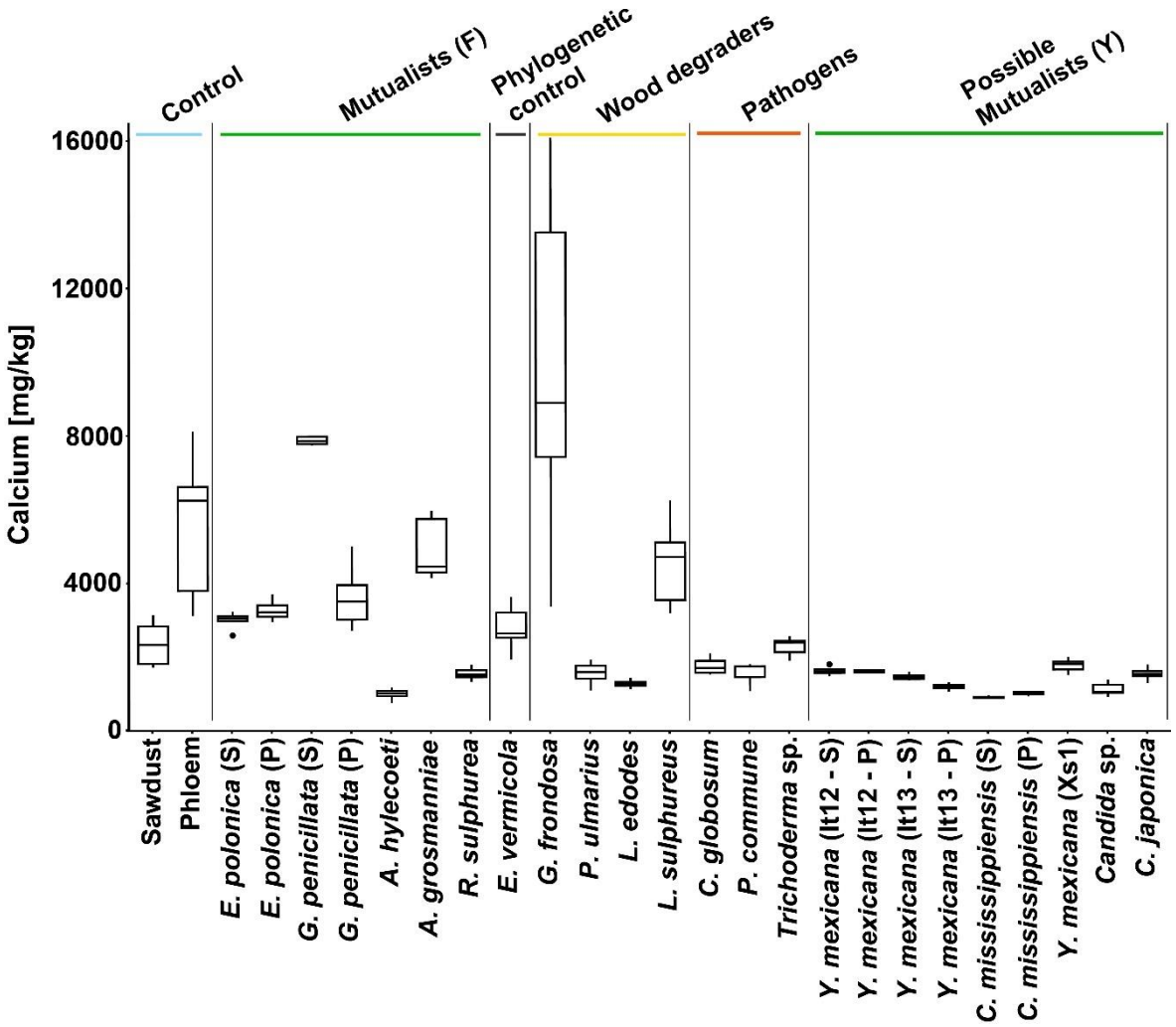

Figure 19: Calcium (Ca) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

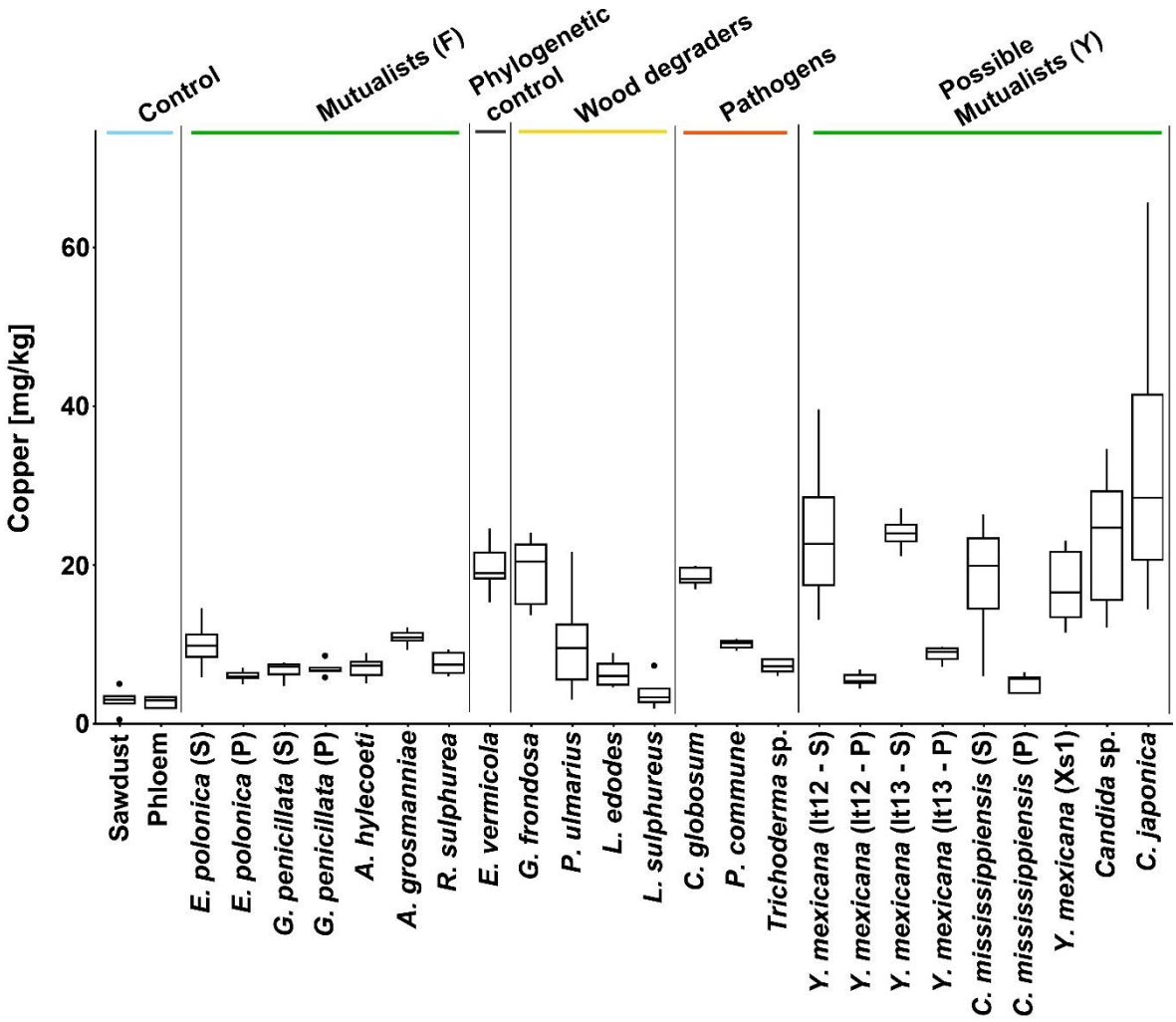

Figure 20: Copper (Cu) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

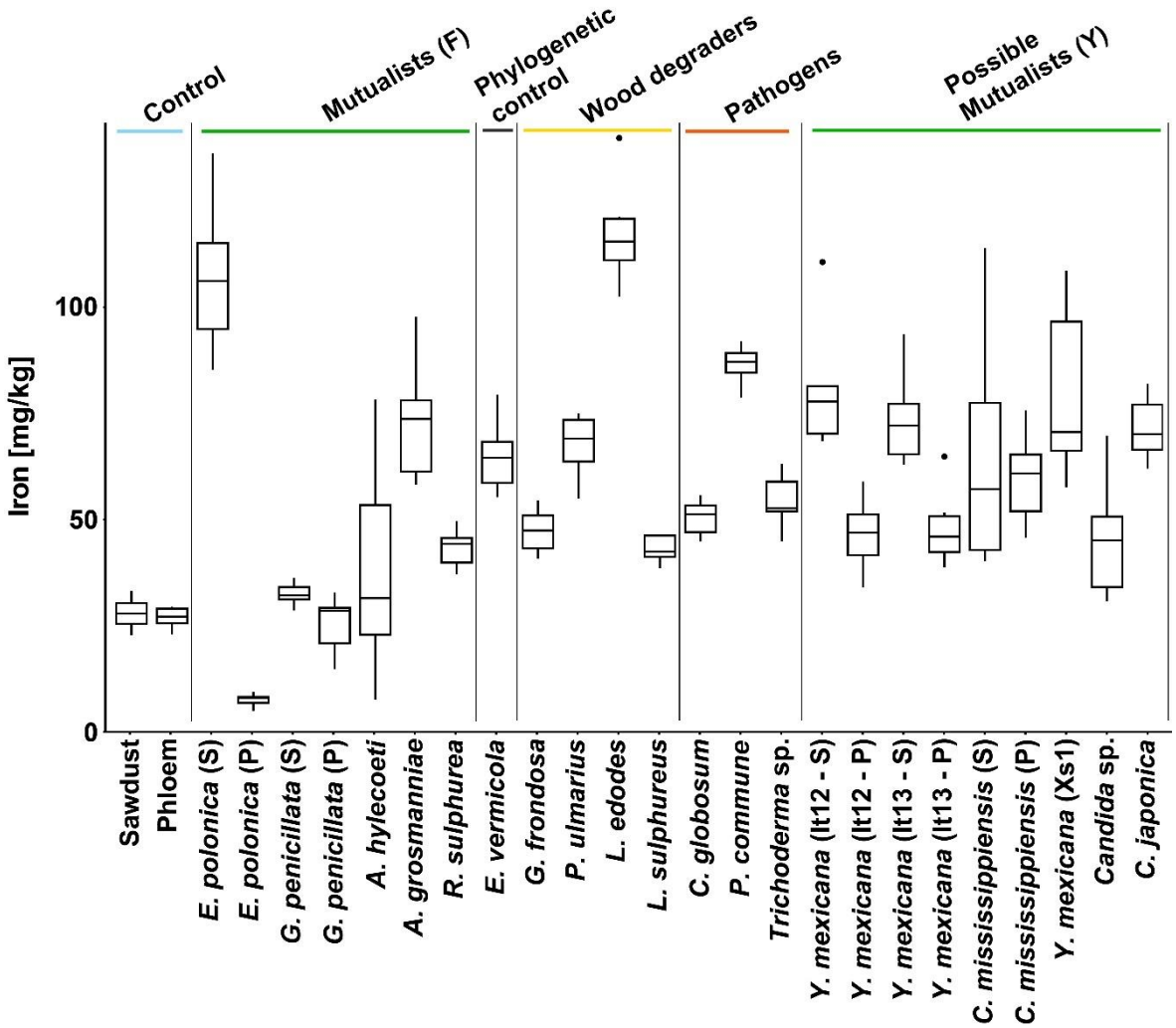

Figure 21: Iron (Fe) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

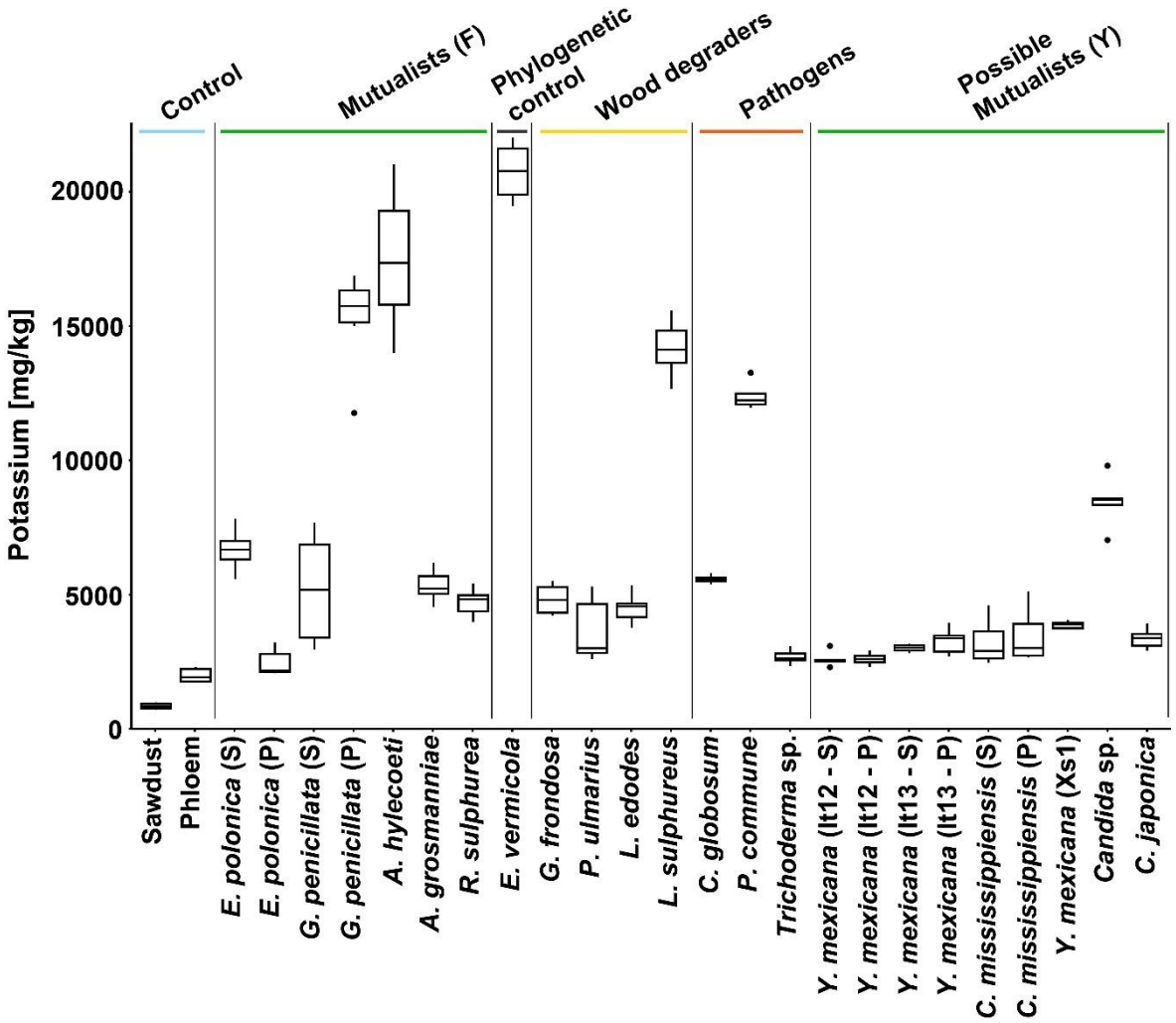

Figure 22: Potassium (K) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

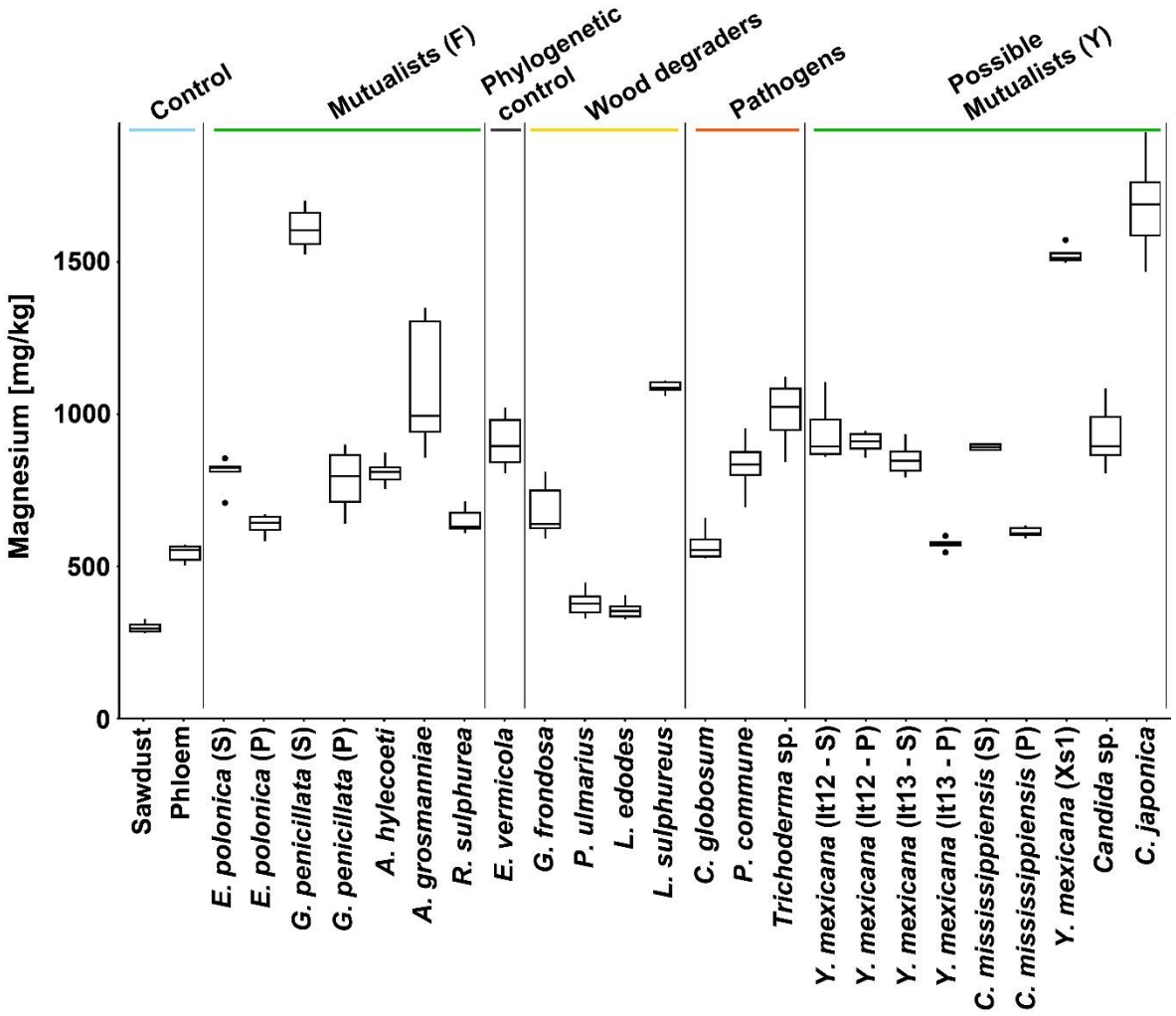

Figure 23: Magnesium (Mg) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

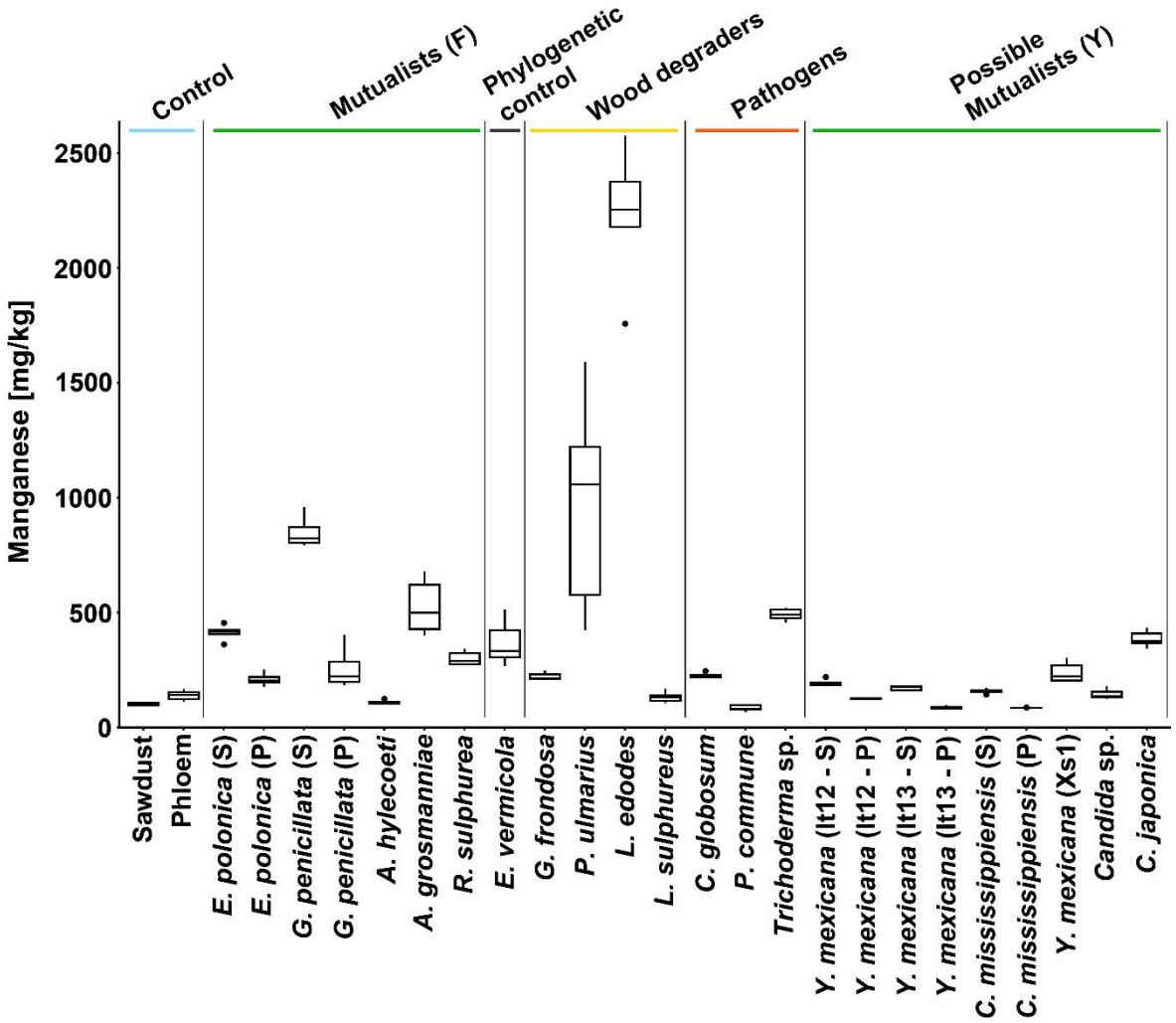

Figure 24: Manganese (Mn) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

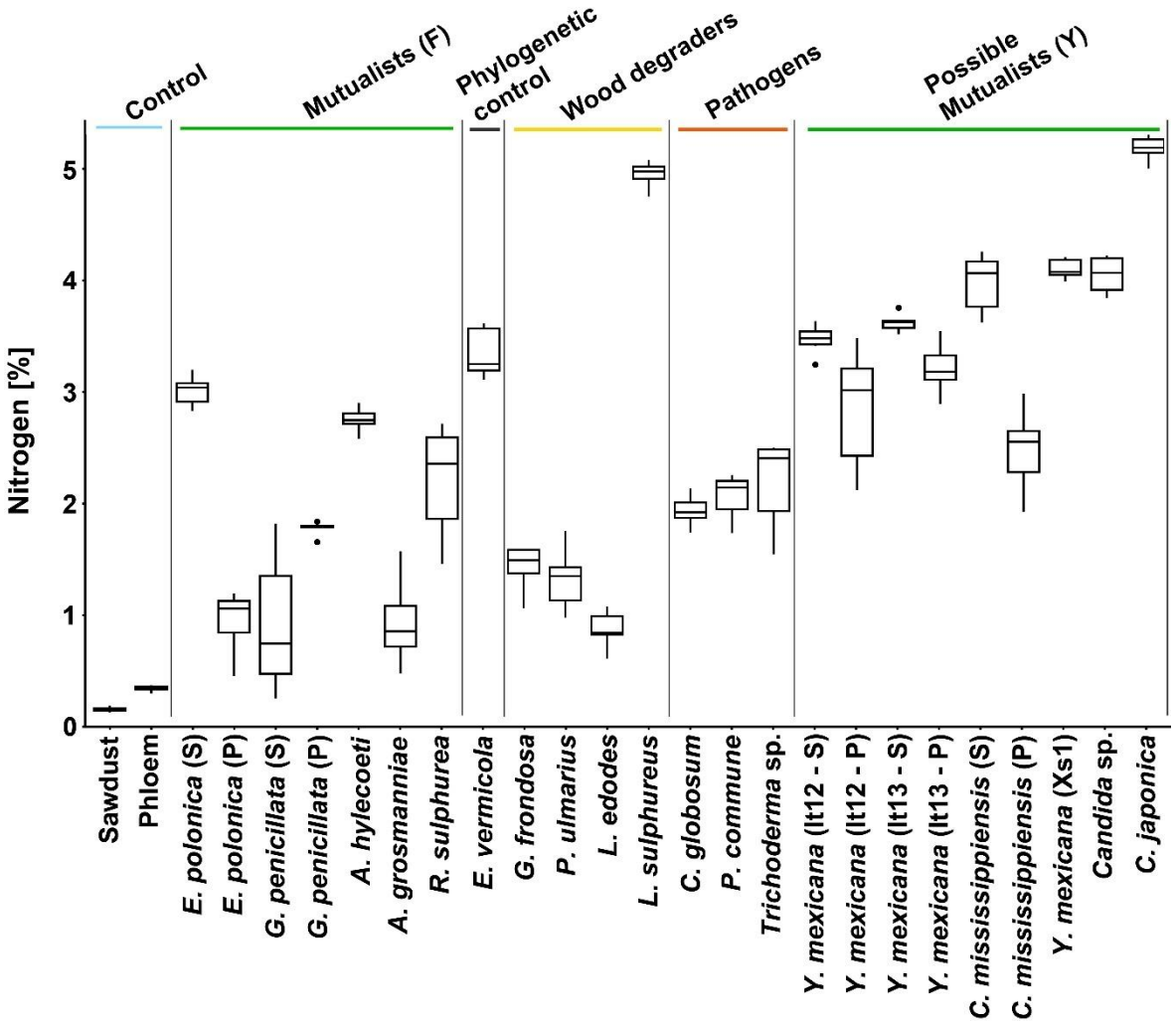

Figure 25: Nitrogen (N) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in a percentage (%). The analysis was carried out with an elemental analyzer. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

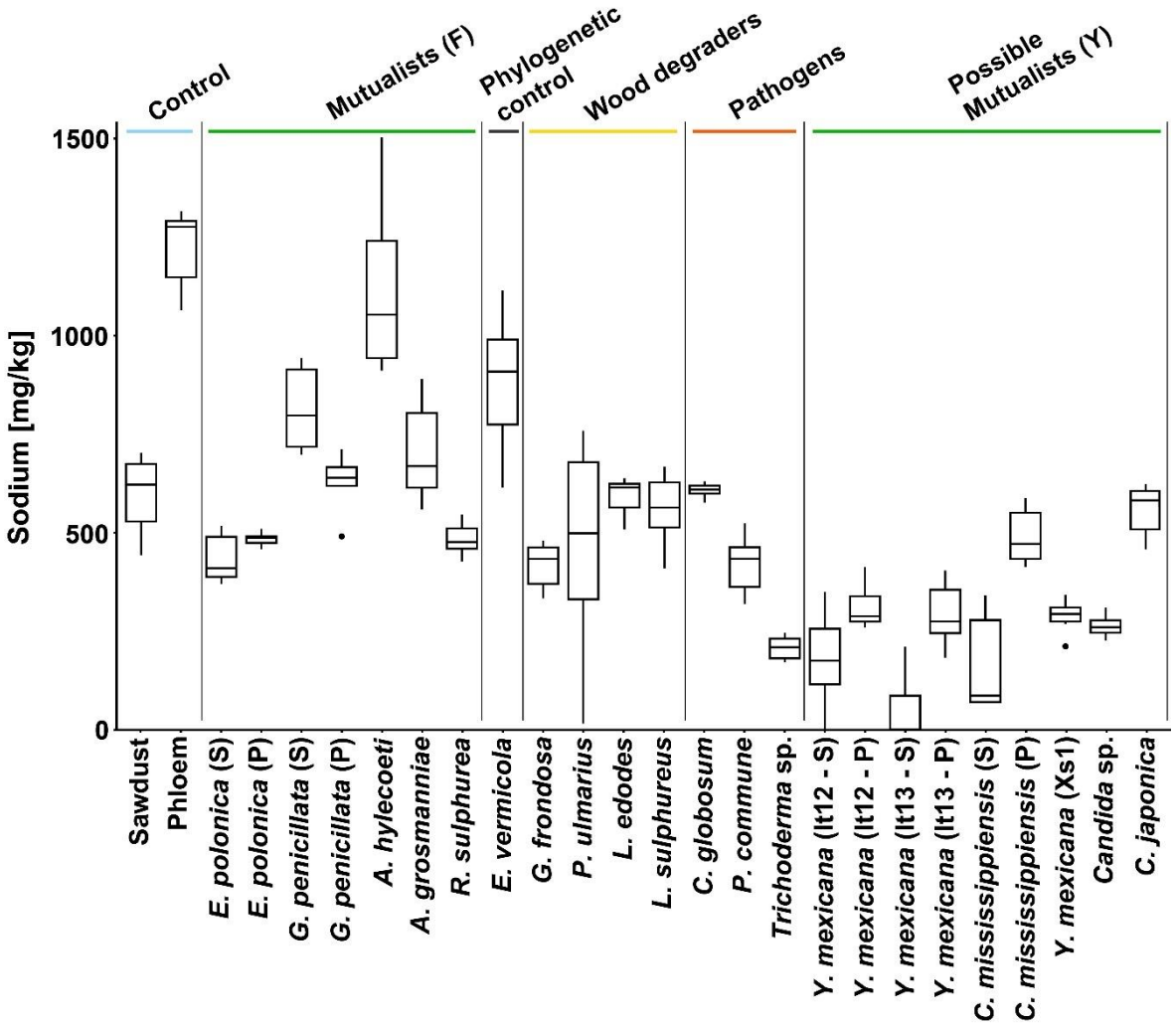

Figure 26: Sodium (Na) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

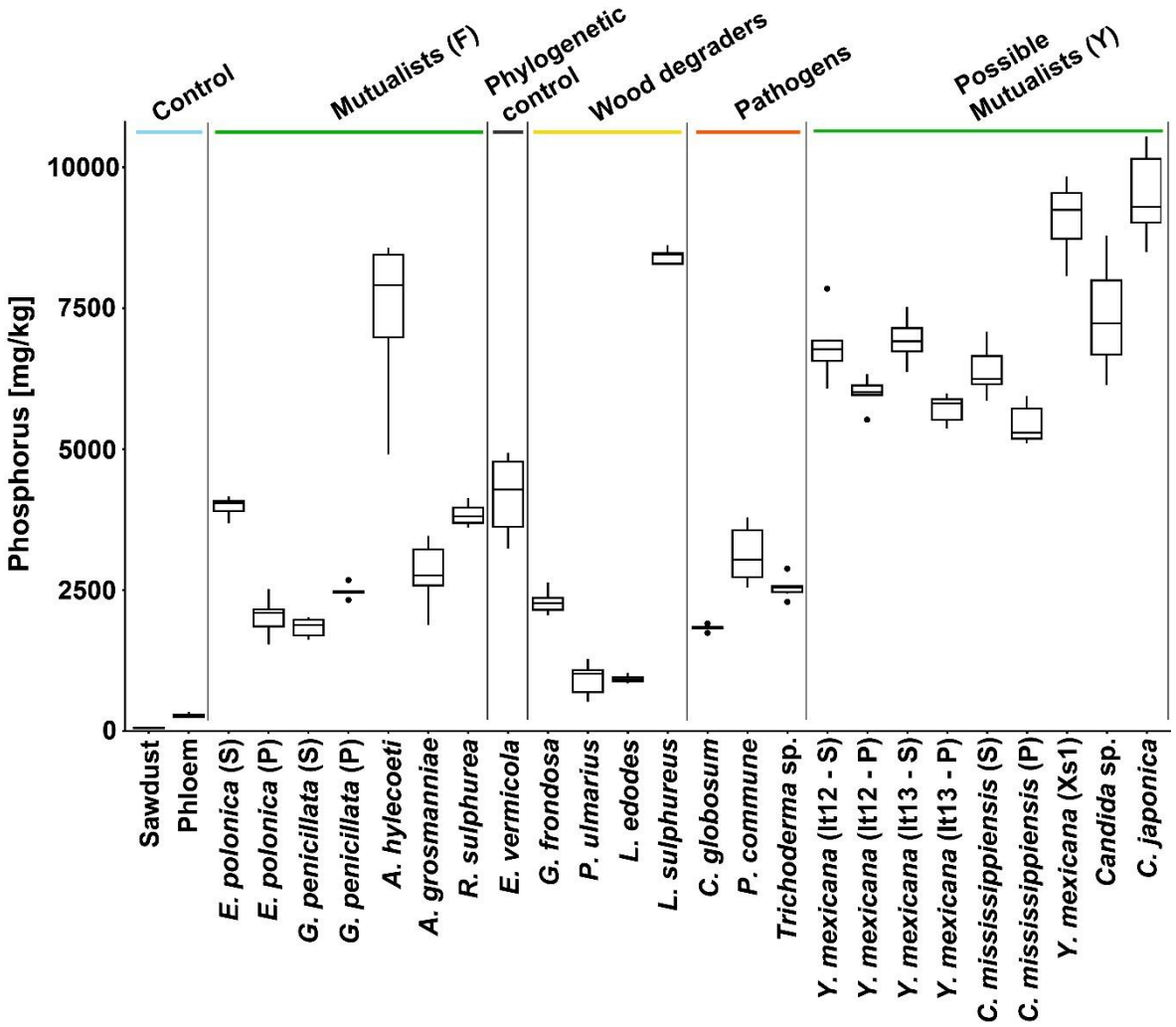

Figure 27: Phosphorus (P) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

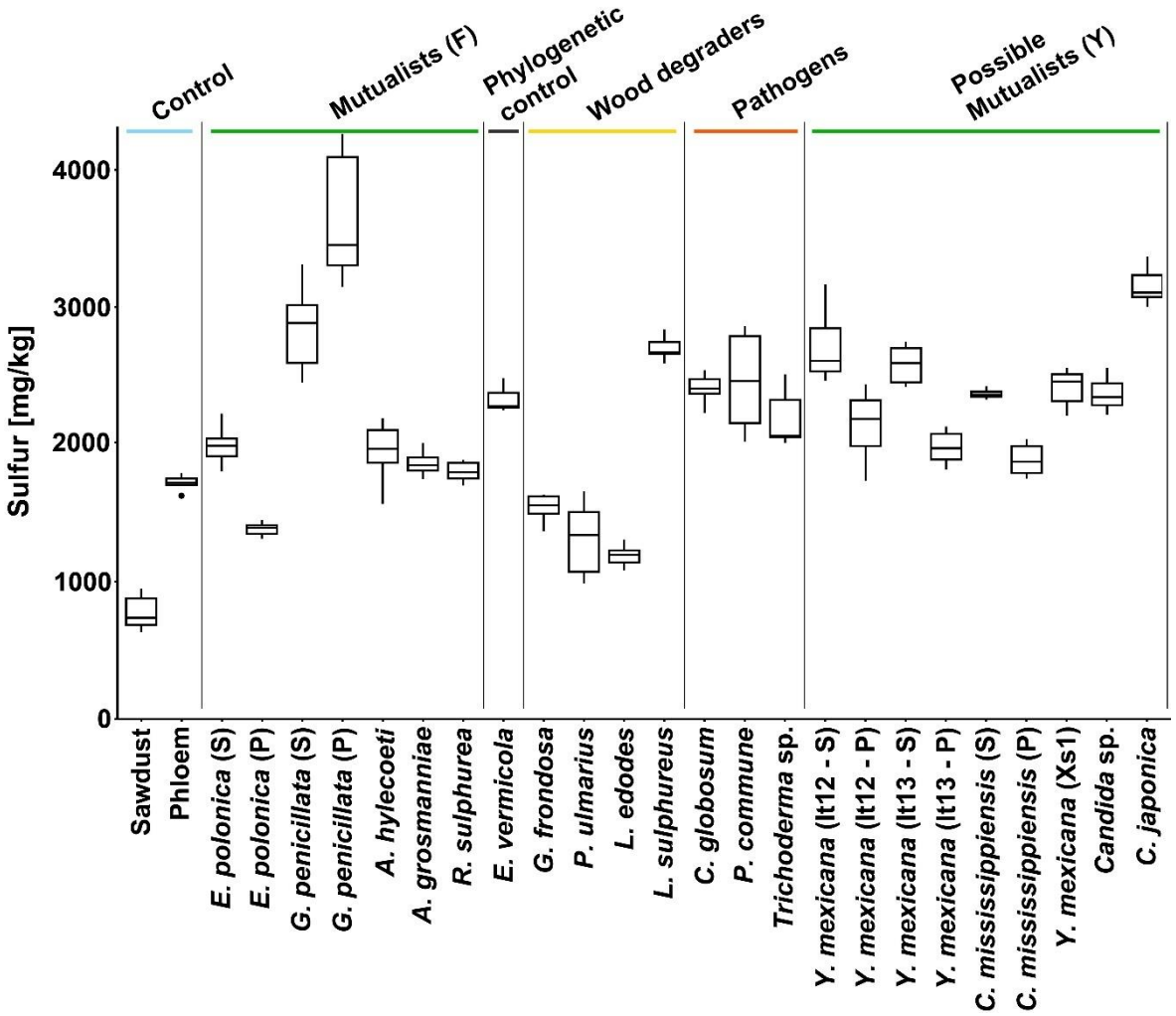

Figure 28: Sulfur (S) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

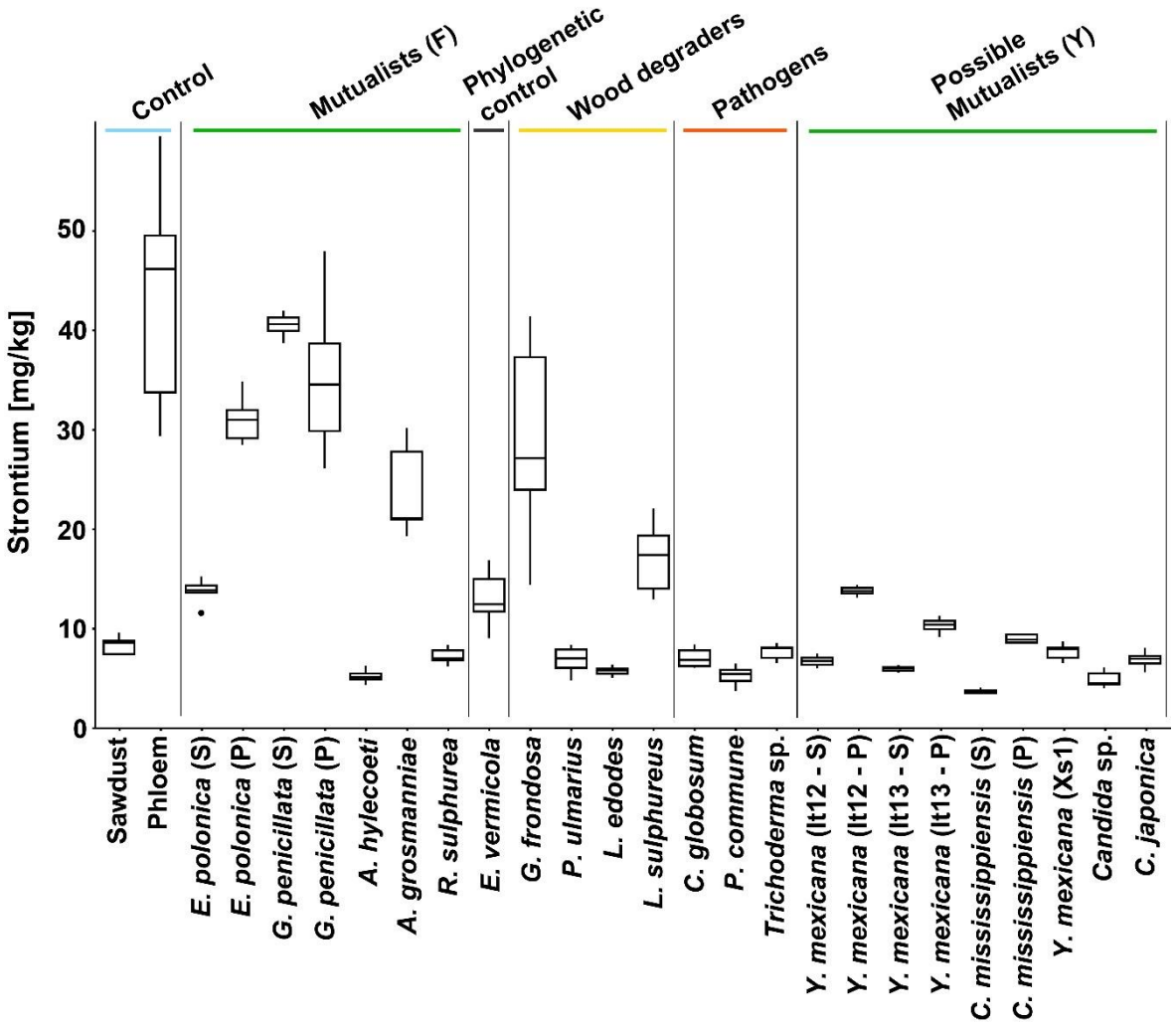

Figure 29: Strontium (Sr) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

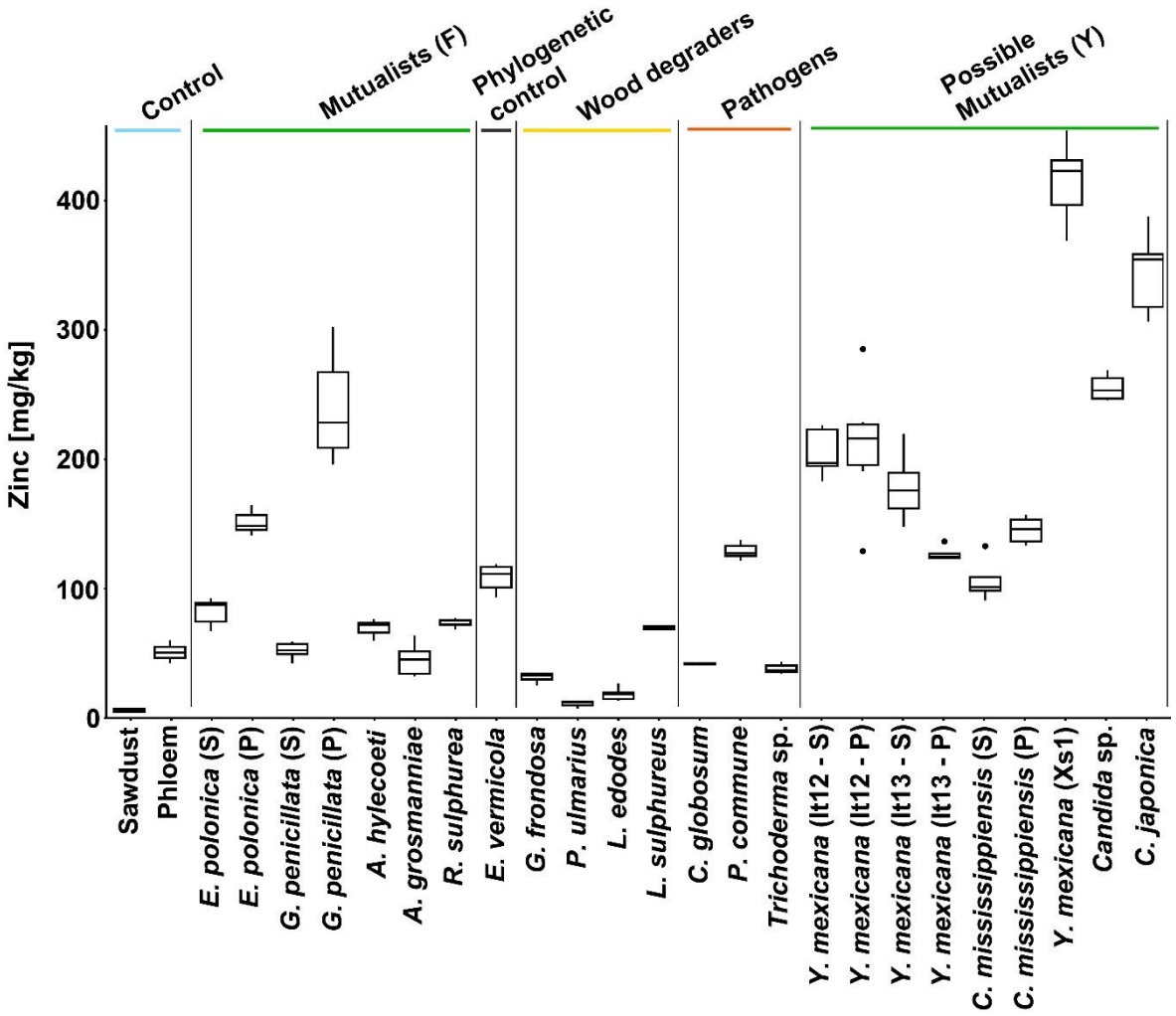

Figure 30: Zinc (Zn) content in filamentous mutualistic fungi in comparison to wood-degrading fungi, pathogens and yeasts in mg/kg. The analysis was carried out with an ICP-OES system after a HNO_3 pressure digestion. The controls are cut out fragments (10 mm diameter) of the culture media consisting of 2 % plant-based agar, water and either 5 % phloem or 5 % sawdust. The phylogenetic control is a close relative to *R. sulphurea*. It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeast. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

10.4 Overview of elemental contents in all analyzed samples

Table 12: Mean values (*M*) ± standard deviation (*SD*) of all the examined elements for all samples. For allocation of the here listed sample strains to the organism names see Table 1.

Sample strain	Al [mg/kg]	C [%]	Ca [mg/kg]	Cu [mg/kg]	Fe [mg/kg]	K [mg/kg]	Mg [mg/kg]
Sawdust	25.15 ± 5.00	45.53 ± 0.50	2355.16 ± 546.42	2.96 ± 1.35	27.90 ± 3.53	841.11 ± 99.71	298.47 ± 16.66
Phloem	41.97 ± 28.05	43.15 ± 0.44	5470.84 ± 1775.38	2.74 ± 0.67	26.88 ± 2.32	1984.36 ± 242.13	542.74 ± 25.03
331 (S)	191.95 ± 95.80	46.38 ± 0.86	2999.43 ± 201.95	9.96 ± 2.61	106.79 ± 16.22	6664.08 ± 686.24	807.69 ± 46.60
331 (P)	26.12 ± 8.39	47.85 ± 3.57	3264.86 ± 236.83	6.08 ± 0.61	7.50 ± 1.44	2459.19 ± 428.32	636.74 ± 31.08
188 (S)	95.33 ± 46.31	44.96 ± 5.49	7869.24 ± 105.19	6.73 ± 1.03	32.45 ± 2.46	5198.39 ± 1956.00	1609.80 ± 64.23
188 (P)	47.08 ± 20.53	51.80 ± 0.20	3588.85 ± 743.86	6.96 ± 0.83	25.17 ± 6.01	15255.61 ± 1676.14	784.43 ± 96.67
10	68.61 ± 32.91	40.79 ± 0.73	995.19 ± 118.73	7.07 ± 1.08	36.82 ± 19.13	17462.37 ± 2143.15	810.57 ± 34.50
339	131.95 ± 45.87	45.76 ± 2.87	4942.24 ± 763.88	10.88 ± 0.91	72.62 ± 12.98	5322.80 ± 542.10	1098.84 ± 195.31
159	162.95 ± 80.21	44.36 ± 1.50	1541.66 ± 156.02	7.66 ± 1.34	43.31 ± 4.26	4696.82 ± 457.98	650.52 ± 35.40
167	43.76 ± 25.16	45.29 ± 0.35	2801.21 ± 555.27	19.82 ± 2.82	64.72 ± 7.77	20747.21 ± 956.00	909.47 ± 81.44
207	27.90 ± 11.29	45.24 ± 1.79	9861.48 ± 4418.48	19.22 ± 4.16	47.35 ± 4.93	4814.78 ± 511.41	680.85 ± 84.03
209	133.67 ± 113.85	42.19 ± 0.69	1551.93 ± 274.35	10.08 ± 5.86	67.21 ± 7.32	3685.60 ± 1061.97	379.41 ± 40.05
211	80.23 ± 44.51	43.50 ± 0.32	1263.82 ± 90.03	6.37 ± 1.60	117.57 ± 11.68	4470.13 ± 479.31	355.93 ± 25.78
213	78.12 ± 69.52	48.16 ± 1.01	4531.03 ± 1184.57	3.96 ± 2.11	43.01 ± 3.38	14160.36 ± 1048.84	1088.30 ± 20.11
7	46.56 ± 11.94	48.32 ± 0.68	1738.92 ± 209.84	18.52 ± 1.11	50.44 ± 3.96	5572.98 ± 135.15	569.58 ± 46.61
21	43.77 ± 11.09	43.87 ± 0.44	1559.15 ± 273.89	10.04 ± 0.52	86.42 ± 4.27	12397.75 ± 463.86	833.22 ± 75.47
338	12.20 ± 7.35	48.66 ± 1.17	2267.13 ± 217.68	7.30 ± 0.85	54.61 ± 5.66	2675.05 ± 227.75	1007.41 ± 94.40
It12 (S)	1080.58 ± 510.20	43.23 ± 0.71	1602.52 ± 101.13	24.05 ± 8.84	80.82 ± 14.31	2583.21 ± 240.00	937.45 ± 90.84
It12 (P)	31.91 ± 30.48	42.83 ± 0.84	1594.14 ± 33.20	5.60 ± 0.80	46.50 ± 7.79	2597.39 ± 195.84	907.25 ± 31.83
It13 (S)	891.93 ± 148.46	42.84 ± 0.67	1444.63 ± 78.84	24.08 ± 2.15	73.81 ± 10.40	3004.47 ± 126.34	851.75 ± 47.79
It13 (P)	20.94 ± 12.18	43.15 ± 2.12	1174.18 ± 80.72	8.77 ± 0.92	48.16 ± 8.55	3238.22 ± 411.31	573.58 ± 16.11
It23 (S)	655.01 ± 316.31	43.41 ± 0.16	887.30 ± 27.79	18.24 ± 6.88	65.12 ± 26.23	3201.36 ± 761.93	891.64 ± 9.32
It23 (P)	16.93 ± 8.97	41.43 ± 2.91	989.55 ± 49.13	5.14 ± 1.09	59.81 ± 10.13	3434.60 ± 905.37	613.08 ± 15.35
Xs1	833.12 ± 946.35	43.33 ± 0.40	1744.85 ± 153.92	25.87 ± 22.19	79.57 ± 19.56	3864.87 ± 127.31	1523.45 ± 26.34
Xs21	1207.42 ± 961.80	43.54 ± 0.60	1103.50 ± 167.18	23.28 ± 8.28	45.60 ± 13.38	8457.23 ± 804.73	928.13 ± 92.39
Xs22	786.32 ± 594.81	43.43 ± 0.83	1516.48 ± 148.29	33.26 ± 16.99	71.45 ± 7.02	3346.69 ± 332.62	1685.17 ± 143.87

Table 13: Table 12 continued.

Sample strain	Mn [mg/kg]	N [%]	Na [mg/kg]	P [mg/kg]	S [mg/kg]	Sr [mg/kg]	Zn [mg/kg]
Sawdust	101.58 ± 6.16	0.1521 ± 0.0187	596.92 ± 90.39	57.40 ± 1.79	777.67 ± 113.45	8.32 ± 0.83	5.91 ± 1.38
Phloem	139.29 ± 19.85	0.3404 ± 0.0223	1219.85 ± 95.62	272.27 ± 34.04	1719.80 ± 51.54	43.12 ± 10.48	50.84 ± 6.01
331 (S)	413.21 ± 28.12	3.0107 ± 0.126	436.81 ± 56.20	3978.31 ± 151.79	1990.02 ± 129.40	13.78 ± 1.12	81.95 ± 9.06
331 (P)	209.11 ± 22.59	0.9484 ± 0.245	484.33 ± 15.42	2025.36 ± 311.25	1381.77 ± 46.92	30.96 ± 2.08	151.15 ± 8.28
188 (S)	847.27 ± 58.87	0.923 ± 0.574	814.14 ± 101.90	1839.88 ± 157.11	2847.44 ± 297.96	40.55 ± 1.06	52.21 ± 5.77
188 (P)	253.90 ± 75.98	1.7734 ± 0.0621	628.98 ± 69.03	2478.19 ± 103.88	3654.70 ± 469.34	35.12 ± 7.02	239.86 ± 39.54
10	108.78 ± 6.84	2.7540 ± 0.0906	1113.62 ± 190.48	7529.93 ± 1048.47	1942.93 ± 200.69	5.23 ± 0.49	69.64 ± 5.32
339	525.42 ± 105.61	0.9283 ± 0.346	708.49 ± 123.98	2815.92 ± 517.55	1862.06 ± 84.18	24.05 ± 4.07	44.65 ± 11.04
159	299.97 ± 26.76	2.205 ± 0.447	485.06 ± 38.12	3837.07 ± 179.26	1802.95 ± 67.20	7.29 ± 0.76	73.80 ± 3.03
167	366.99 ± 81.25	3.355 ± 0.201	881.69 ± 158.43	4179.76 ± 634.76	2329.09 ± 81.95	13.14 ± 2.63	108.41 ± 9.33
207	221.49 ± 14.85	1.432 ± 0.188	418.21 ± 55.55	2289.12 ± 189.44	1538.09 ± 94.33	28.97 ± 9.42	31.49 ± 3.42
209	952.47 ± 412.89	1.316 ± 0.241	471.52 ± 251.20	907.77 ± 259.13	1306.14 ± 256.96	6.88 ± 1.25	11.05 ± 2.17
211	2234.72 ± 252.13	0.880 ± 0.150	591.82 ± 47.27	922.94 ± 62.20	1188.40 ± 69.74	5.75 ± 0.42	18.18 ± 4.20
213	132.01 ± 22.92	4.951 ± 0.116	558.54 ± 95.21	8424.41 ± 144.07	2700.09 ± 95.18	17.15 ± 3.63	69.93 ± 1.50
7	225.38 ± 10.71	1.937 ± 0.121	607.45 ± 17.61	1832.96 ± 54.41	2406.60 ± 99.84	7.08 ± 0.91	41.74 ± 0.70
21	85.76 ± 11.92	2.063 ± 0.176	418.54 ± 69.45	3137.19 ± 464.17	2461.66 ± 329.77	5.29 ± 0.95	128.93 ± 5.66
338	491.62 ± 23.29	2.184 ± 0.370	207.79 ± 27.71	2548.25 ± 177.80	2191.85 ± 188.39	7.66 ± 0.70	38.12 ± 3.22
It12 (S)	193.91 ± 12.44	3.469 ± 0.122	181.32 ± 116.19	6823.33 ± 537.27	2713.33 ± 250.57	6.77 ± 0.51	206.04 ± 16.26
It12 (P)	125.89 ± 2.86	2.843 ± 0.499	312.60 ± 53.77	5997.50 ± 246.55	2138.62 ± 232.24	13.82 ± 0.43	210.95 ± 46.68
It13 (S)	170.71 ± 9.28	3.6203 ± 0.0734	54.61 ± 81.65	6936.17 ± 366.71	2581.77 ± 132.51	5.99 ± 0.25	178.51 ± 23.47
It13 (P)	86.85 ± 6.71	3.214 ± 0.196	294.85 ± 76.54	5717.15 ± 235.95	1978.77 ± 116.54	10.36 ± 0.66	126.77 ± 4.60
It23 (S)	158.35 ± 9.19	3.973 ± 0.243	169.24 ± 116.51	6399.56 ± 425.34	2368.42 ± 35.15	3.73 ± 0.20	106.47 ± 14.40
It23 (P)	85.10 ± 0.82	2.475 ± 0.319	490.53 ± 67.97	5440.99 ± 329.34	1887.03 ± 110.53	9.01 ± 0.41	145.28 ± 9.21
Xs1	238.91 ± 38.76	4.1069 ± 0.0795	287.72 ± 40.68	9099.42 ± 613.28	2410.51 ± 130.89	7.67 ± 0.72	415.60 ± 29.47
Xs21	145.10 ± 20.75	4.051 ± 0.1479	264.02 ± 26.94	7356.76 ± 951.68	2367.84 ± 120.32	4.94 ± 0.79	255.36 ± 9.07
Xs22	386.75 ± 30.07	5.184 ± 0.101	556.17 ± 59.92	9525.03 ± 705.14	3156.21 ± 129.67	6.90 ± 0.72	343.03 ± 27.60

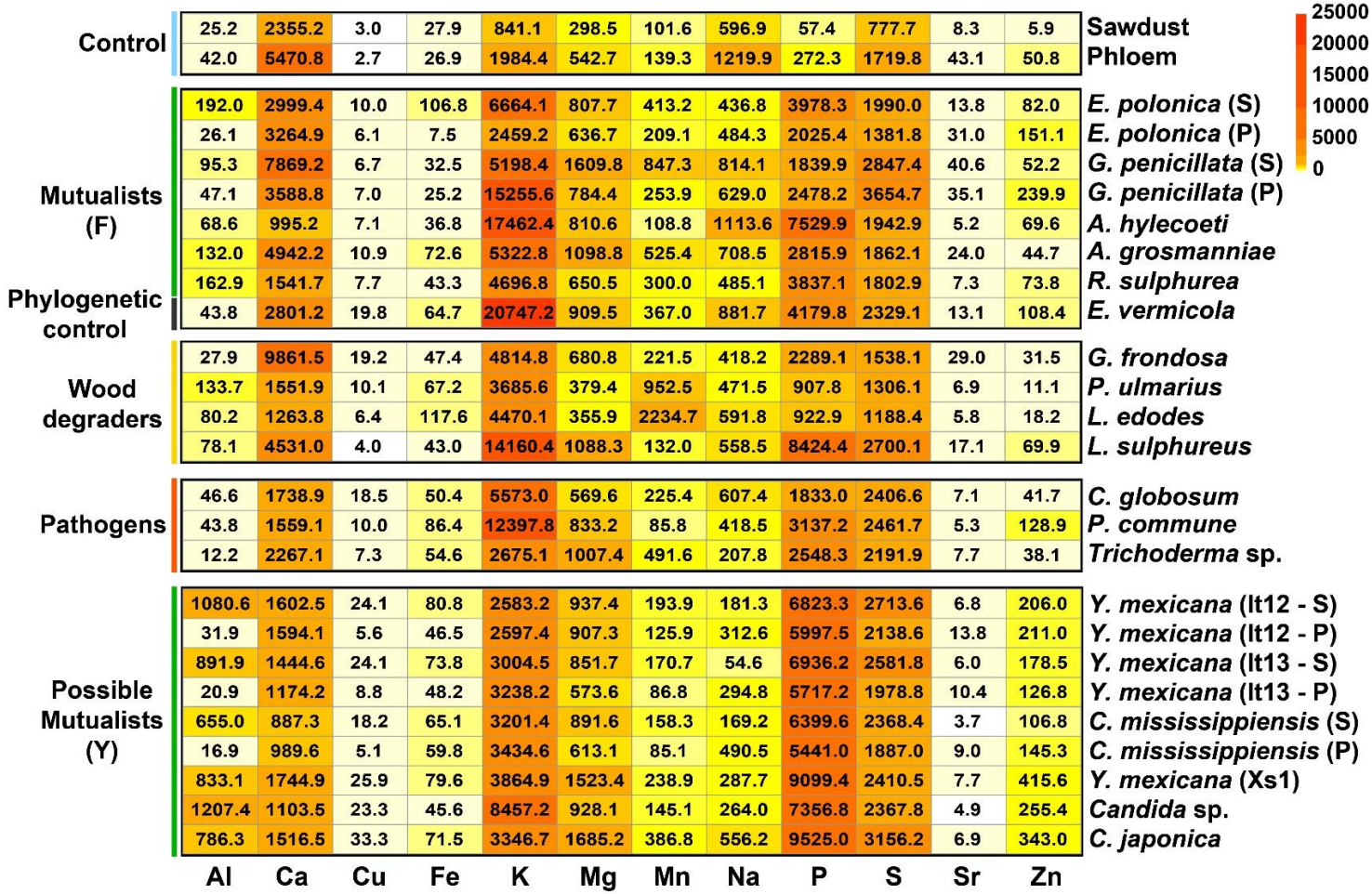

Figure 31: Overview of the elemental contents of the filamentous fungi and yeasts. Elements are listed in the bottom row. Organism names are listed on the right side and the groups are listed on the left side. Numbers shown are mean values in mg/kg for the elements analyzed with the ICP-OES system. Color scale on the top right indicating a visually aided comparison for the accumulation of elements, reaching from low mass fractions (white) to high mass fractions (red). It12, It13 = Different *Y. mexicana* strains isolated from *I. typographus*. Xs1 = *Y. mexicana* strain isolated from *X. saxesenii*. (F) = Filamentous mutualists. (Y) = Yeasts. (S) = Fungi grown on sawdust media. (P) = Fungi grown on phloem media.

10.5 *P*-values for the examined elements in filamentous fungi

Table 14: *P*-values of aluminum for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Al and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.570	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.021	0.021	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	1.000	0.590	0.017	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.030	0.159	0.098	0.021	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.056	0.763	0.021	0.118	0.118	-	-	-	-	-	-	-	-	-	-	-
f_10	0.078	0.289	0.021	0.057	0.407	0.470	-	-	-	-	-	-	-	-	-	-
g_339	0.021	0.017	0.536	0.017	0.468	0.021	0.065	-	-	-	-	-	-	-	-	-
h_159	0.021	0.028	0.582	0.017	0.303	0.056	0.028	0.763	-	-	-	-	-	-	-	-
h1_167	0.508	1.000	0.021	0.407	0.209	1.000	0.320	0.017	0.038	-	-	-	-	-	-	-
i_207	1.000	0.672	0.017	0.902	0.030	0.260	0.065	0.017	0.021	0.451	-	-	-	-	-	-
j_209	0.570	0.518	0.622	0.518	0.988	0.518	0.701	0.988	0.763	0.518	0.536	-	-	-	-	-
k_211	0.021	0.303	0.074	0.017	0.792	0.303	0.801	0.198	0.209	0.384	0.030	0.792	-	-	-	-
l_213	0.273	0.570	0.118	0.273	0.763	0.763	1.000	0.253	0.273	0.672	0.400	0.763	0.763	-	-	-
m_7	0.056	0.902	0.021	0.030	0.253	0.808	0.407	0.017	0.088	1.000	0.098	0.536	0.303	0.763	-	-
n_21	0.030	0.808	0.021	0.065	0.253	0.902	0.407	0.017	0.088	0.989	0.253	0.536	0.253	0.763	0.988	-
o_338	0.118	0.159	0.017	0.088	0.017	0.017	0.017	0.017	0.017	0.088	0.098	0.303	0.017	0.082	0.017	0.017

Table 15: *P*-values of carbon for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: C and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.0026	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.1135	0.0026	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.5485	0.0482	0.6218	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.7645	0.7645	0.7645	0.3527	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.0029	0.0029	0.0029	0.0602	0.0602	-	-	-	-	-	-	-	-	-	-	-
f_10	0.0009	0.0009	0.0009	0.0011	0.3988	0.0011	-	-	-	-	-	-	-	-	-	-
g_339	0.7645	0.0628	0.5485	0.4153	0.6218	0.0029	0.0011	-	-	-	-	-	-	-	-	-
h_159	0.3527	0.2956	0.0628	0.1937	0.7645	0.0029	0.0011	0.5485	-	-	-	-	-	-	-	-
h1_167	0.4153	0.0026	0.0859	0.3527	0.7645	0.0029	0.0009	0.7645	0.6218	-	-	-	-	-	-	-
i_207	0.8676	0.0840	0.3971	0.3285	0.7645	0.0048	0.0021	0.9593	0.6218	1.0000	-	-	-	-	-	-
j_209	0.0026	0.0628	0.0026	0.0140	0.7645	0.0029	0.0044	0.0140	0.0326	0.0026	0.0156	-	-	-	-	-
k_211	0.0029	0.2586	0.0029	0.0602	0.7645	0.0048	0.0011	0.2033	0.5485	0.0029	0.1039	0.0096	-	-	-	-
l_213	0.0026	0.0026	0.0088	0.9219	0.5485	0.0029	0.0009	0.1487	0.0026	0.0026	0.0156	0.0026	0.0029	-	-	-
m_7	0.0029	0.0029	0.0096	1.0000	0.4695	0.0048	0.0011	0.1135	0.0029	0.0029	0.0092	0.0029	0.0048	0.7240	-	-
n_21	0.0029	0.0602	0.0029	0.0602	0.7645	0.0048	0.0011	0.4695	0.7645	0.0029	0.1973	0.0051	0.2586	0.0029	0.0048	-
o_338	0.0026	0.0026	0.0209	0.9219	0.2412	0.0029	0.0009	0.1135	0.0026	0.0026	0.0156	0.0026	0.0029	0.4866	0.5485	0.0029

Table 16: *P*-values of calcium for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Ca and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.00233	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.06118	0.00675	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.00980	0.04726	0.20138	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.00399	0.05932	0.00647	0.00399	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.01476	0.11312	0.15458	0.48044	0.00399	-	-	-	-	-	-	-	-	-	-	-
f_10	0.00067	0.00067	0.00122	0.00067	0.00193	0.00067	-	-	-	-	-	-	-	-	-	-
g_339	0.00193	0.48044	0.00233	0.00193	0.00399	0.00980	0.00067	-	-	-	-	-	-	-	-	-
h_159	0.00638	0.00193	0.00233	0.00193	0.00399	0.00193	0.00067	0.00193	-	-	-	-	-	-	-	-
h1_167	0.28442	0.00980	0.47677	0.14652	0.00399	0.06271	0.00067	0.00193	0.00193	-	-	-	-	-	-	-
i_207	0.00233	0.04403	0.00392	0.01121	0.46259	0.01829	0.00122	0.06118	0.00233	0.00675	-	-	-	-	-	-
j_209	0.02868	0.00233	0.00392	0.00233	0.00647	0.00233	0.00233	0.00233	0.95933	0.00233	0.00392	-	-	-	-	-
k_211	0.00193	0.00193	0.00233	0.00193	0.00399	0.00193	0.00166	0.00193	0.00399	0.00193	0.00233	0.08609	-	-	-	-
l_213	0.00233	0.55417	0.01177	0.06118	0.00647	0.25903	0.00122	0.86099	0.00233	0.01829	0.03333	0.00392	0.00233	-	-	-
m_7	0.06118	0.00233	0.00392	0.00233	0.00647	0.00233	0.00122	0.00233	0.15458	0.00675	0.00392	0.33676	0.00233	0.00392	-	-
n_21	0.01476	0.00193	0.00233	0.00193	0.00399	0.00193	0.00193	0.00193	0.92185	0.00193	0.00233	1.00000	0.14652	0.00233	0.55417	-
o_338	1.00000	0.00193	0.00233	0.00193	0.00399	0.00193	0.00067	0.00193	0.00193	0.03333	0.00233	0.00399	0.00193	0.00233	0.01121	0.00193

Table 17: *P*-values of copper for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Cu and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.7704	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.0035	0.0034	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.0053	0.0034	0.0186	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.0081	0.0035	0.0346	0.2385	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.0053	0.0035	0.0742	0.1029	0.8304	-	-	-	-	-	-	-	-	-	-	-
f_10	0.0034	0.0028	0.0252	0.0795	0.5975	0.7149	-	-	-	-	-	-	-	-	-	-
g_339	0.0053	0.0035	0.4566	0.0035	0.0053	0.0053	0.0034	-	-	-	-	-	-	-	-	-
h_159	0.0035	0.0034	0.2199	0.0566	0.5311	0.5975	0.5209	0.0053	-	-	-	-	-	-	-	-
h1_167	0.0035	0.0034	0.0034	0.0034	0.0035	0.0035	0.0028	0.0035	0.0034	-	-	-	-	-	-	-
i_207	0.0053	0.0035	0.0086	0.0035	0.0053	0.0053	0.0034	0.0053	0.0035	0.9522	-	-	-	-	-	-
j_209	0.0232	0.0081	0.8229	0.3391	0.5975	0.4566	0.5209	0.6946	0.5389	0.0186	0.0142	-	-	-	-	-
k_211	0.0142	0.0034	0.0280	1.0000	0.7704	0.5975	0.4173	0.0035	0.2199	0.0034	0.0035	0.4075	-	-	-	-
l_213	0.8229	0.7007	0.0092	0.1442	0.0458	0.1141	0.0150	0.0081	0.0280	0.0053	0.0081	0.1029	0.0702	-	-	-
m_7	0.0081	0.0053	0.0053	0.0053	0.0081	0.0081	0.0034	0.0081	0.0053	0.5244	0.7149	0.0702	0.0053	0.0142	-	-
n_21	0.0035	0.0034	0.8229	0.0034	0.0035	0.0035	0.0028	0.1393	0.0053	0.0034	0.0035	0.8229	0.0034	0.0053	0.0053	-
o_338	0.0035	0.0034	0.0405	0.0280	0.5311	0.5975	0.5975	0.0035	0.7607	0.0034	0.0035	0.5975	0.4075	0.0458	0.0053	0.0034

Table 18: *P*-values of iron for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Fe and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.7203	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.0036	0.0036	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.0039	0.0039	0.0036	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.0768	0.0114	0.0036	0.0039	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.4730	0.9522	0.0036	0.0036	0.0432	-	-	-	-	-	-	-	-	-	-	-
f_10	0.5788	0.5788	0.0036	0.0039	0.9049	0.2914	-	-	-	-	-	-	-	-	-	-
g_339	0.0036	0.0036	0.0041	0.0036	0.0036	0.0036	0.0036	-	-	-	-	-	-	-	-	-
h_159	0.0039	0.0039	0.0036	0.0039	0.0039	0.0036	0.3215	0.0036	-	-	-	-	-	-	-	-
h1_167	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0042	0.3483	0.0036	-	-	-	-	-	-	-
i_207	0.0039	0.0039	0.0036	0.0039	0.0039	0.0036	0.2806	0.0036	0.2723	0.0036	-	-	-	-	-	-
j_209	0.0059	0.0059	0.0041	0.0059	0.0059	0.0041	0.0174	0.6633	0.0059	0.7721	0.0059	-	-	-	-	-
k_211	0.0039	0.0039	0.2677	0.0039	0.0039	0.0036	0.0036	0.0036	0.0039	0.0036	0.0039	0.0059	-	-	-	-
l_213	0.0059	0.0059	0.0041	0.0059	0.0059	0.0041	0.3573	0.0041	1.0000	0.0041	0.2046	0.0108	0.0059	-	-	-
m_7	0.0039	0.0039	0.0036	0.0039	0.0039	0.0036	0.1749	0.0036	0.0504	0.0041	0.4219	0.0114	0.0039	0.0625	-	-
n_21	0.0039	0.0039	0.0432	0.0039	0.0039	0.0036	0.0036	0.0623	0.0039	0.0041	0.0039	0.0059	0.0039	0.0059	0.0039	-
o_338	0.0036	0.0036	0.0036	0.0036	0.0036	0.0036	0.0664	0.0143	0.0110	0.0330	0.0861	0.0225	0.0036	0.0132	0.3950	0.0036

Table 19: *P*-values of potassium for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: K and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.00189	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.00189	0.00189	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.00189	0.11312	0.00189	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.00196	0.00196	0.55844	0.00337	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.00196	0.00196	0.00196	0.00196	0.00327	-	-	-	-	-	-	-	-	-	-	-
f_10	0.00067	0.00067	0.00067	0.00067	0.00122	0.11612	-	-	-	-	-	-	-	-	-	-
g_339	0.00196	0.00196	0.01018	0.00196	1.00000	0.00327	0.00122	-	-	-	-	-	-	-	-	-
h_159	0.00189	0.00189	0.00189	0.00189	1.00000	0.00196	0.00067	0.11588	-	-	-	-	-	-	-	-
h1_167	0.00189	0.00189	0.00189	0.00189	0.00196	0.00196	0.00197	0.00196	0.00189	-	-	-	-	-	-	-
i_207	0.00196	0.00196	0.00196	0.00196	1.00000	0.00327	0.00122	0.33676	0.75866	0.00196	-	-	-	-	-	-
j_209	0.00189	0.00189	0.00189	0.04559	0.15331	0.00196	0.00067	0.04246	0.18235	0.00189	0.11588	-	-	-	-	-
k_211	0.00189	0.00189	0.00189	0.00189	1.00000	0.00196	0.00067	0.06118	0.48419	0.00189	0.55844	0.22881	-	-	-	-
l_213	0.00196	0.00196	0.00196	0.00196	0.00327	0.14840	0.00603	0.00327	0.00196	0.00196	0.00327	0.00196	0.00196	-	-	-
m_7	0.00196	0.00196	0.01018	0.00196	1.00000	0.00327	0.00122	0.42186	0.00337	0.00196	0.01061	0.00196	0.00196	0.00327	-	-
n_21	0.00337	0.00337	0.00337	0.00337	0.00555	0.09643	0.00189	0.00555	0.00337	0.00337	0.00555	0.00337	0.00337	0.01061	0.00555	-
o_338	0.00189	0.00189	0.00189	0.41325	0.00337	0.00196	0.00067	0.00196	0.00189	0.00189	0.00196	0.06271	0.00189	0.00196	0.00196	0.00337

Table 20: *P*-values of magnesium for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Mg and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.0021	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.0021	0.0021	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.0021	0.0021	0.0034	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.0021	0.0021	0.0034	0.0034	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.0021	0.0021	0.8419	0.0264	0.0021	-	-	-	-	-	-	-	-	-	-	-
f_10	0.0021	0.0021	0.8165	0.0021	0.0021	0.8868	-	-	-	-	-	-	-	-	-	-
g_339	0.0021	0.0021	0.0021	0.0021	0.0021	0.0035	0.0021	-	-	-	-	-	-	-	-	-
h_159	0.0021	0.0021	0.0035	0.6572	0.0021	0.0212	0.0021	0.0021	-	-	-	-	-	-	-	-
h1_167	0.0021	0.0021	0.1149	0.0021	0.0021	0.0616	0.0223	0.1441	0.0021	-	-	-	-	-	-	-
i_207	0.0021	0.0021	0.0187	0.8366	0.0034	0.0839	0.0196	0.0021	0.8419	0.0035	-	-	-	-	-	-
j_209	0.0021	0.0021	0.0034	0.0034	0.0034	0.0021	0.0021	0.0021	0.0021	0.0021	0.0034	-	-	-	-	-
k_211	0.0021	0.0021	0.0021	0.0021	0.0021	0.0021	0.0021	0.0021	0.0021	0.0021	0.0021	0.3234	-	-	-	-
l_213	0.0035	0.0035	0.0057	0.0057	0.0057	0.0035	0.0021	0.7839	0.0035	0.0035	0.0057	0.0057	0.0035	-	-	-
m_7	0.0021	0.6572	0.0034	0.0482	0.0034	0.0060	0.0021	0.0021	0.0175	0.0021	0.0482	0.0034	0.0021	0.0057	-	-
n_21	0.0021	0.0021	0.5729	0.0021	0.0021	0.5729	0.3425	0.0139	0.0021	0.2891	0.0104	0.0021	0.0021	0.0035	0.0021	-
o_338	0.0021	0.0021	0.0035	0.0021	0.0021	0.0035	0.0021	0.6572	0.0021	0.0839	0.0021	0.0021	0.0021	0.2960	0.0021	0.0090

Table 21: *P*-values of manganese for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Mn and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.0019	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.0019	0.0019	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.0019	0.0019	0.0019	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.0019	0.0019	0.0030	0.0019	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.0019	0.0019	0.0032	0.3975	0.0019	-	-	-	-	-	-	-	-	-	-	-
f_10	0.1553	0.0030	0.0019	0.0019	0.0019	0.0019	-	-	-	-	-	-	-	-	-	-
g_339	0.0019	0.0019	0.0576	0.0019	0.0019	0.0019	0.0019	-	-	-	-	-	-	-	-	-
h_159	0.0019	0.0019	0.0019	0.0019	0.0019	0.1794	0.0019	0.0019	-	-	-	-	-	-	-	-
h1_167	0.0019	0.0019	0.3829	0.0019	0.0019	0.0300	0.0019	0.0300	0.2234	-	-	-	-	-	-	-
i_207	0.0019	0.0019	0.0030	0.3829	0.0030	0.9522	0.0019	0.0019	0.0019	0.0019	-	-	-	-	-	-
j_209	0.0019	0.0019	0.0055	0.0019	0.7473	0.0019	0.0019	0.1076	0.0019	0.0051	0.0019	-	-	-	-	-
k_211	0.0019	0.0019	0.0030	0.0019	0.0030	0.0019	0.0019	0.0019	0.0019	0.0019	0.0030	0.0019	-	-	-	-
l_213	0.0055	0.5500	0.0030	0.0019	0.0030	0.0019	0.0204	0.0019	0.0019	0.0019	0.0030	0.0019	0.0030	-	-	-
m_7	0.0032	0.0032	0.0052	0.2181	0.0052	1.0000	0.0019	0.0032	0.0032	0.0032	0.3496	0.0032	0.0052	0.0052	-	-
n_21	0.0429	0.0019	0.0019	0.0019	0.0019	0.0019	0.0019	0.0019	0.0019	0.0019	0.0019	0.0019	0.0019	0.0019	0.0032	-
o_338	0.0019	0.0019	0.0030	0.0019	0.0030	0.0019	0.0019	0.9522	0.0019	0.0164	0.0030	0.0819	0.0030	0.0030	0.0052	0.0019

Table 22: *P*-values of nitrogen for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: N and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.00147	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.00180	0.00180	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.00147	0.00147	0.00180	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.00147	0.22881	0.00180	0.55538	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.00343	0.00343	0.00555	0.00343	0.05577	-	-	-	-	-	-	-	-	-	-	-
f_10	0.00049	0.00049	0.00198	0.00049	0.00049	0.00147	-	-	-	-	-	-	-	-	-	-
g_339	0.00147	0.00147	0.00180	0.90152	0.90152	0.00343	0.00049	-	-	-	-	-	-	-	-	-
h_159	0.00147	0.00147	0.00180	0.00147	0.00539	0.22706	0.00147	0.00180	-	-	-	-	-	-	-	-
h1_167	0.00147	0.00147	0.00587	0.00147	0.00147	0.00343	0.00049	0.00147	0.00147	-	-	-	-	-	-	-
i_207	0.00180	0.00180	0.00320	0.00991	0.25488	0.00555	0.00113	0.02665	0.00991	0.00180	-	-	-	-	-	-
j_209	0.00147	0.00147	0.00180	0.03128	0.22881	0.00630	0.00049	0.06112	0.00539	0.00147	0.39190	-	-	-	-	-
k_211	0.00147	0.00147	0.00180	0.40054	0.72640	0.00343	0.00049	0.81679	0.00147	0.00147	0.00587	0.00337	-	-	-	-
l_213	0.00180	0.00180	0.00320	0.00180	0.00180	0.00555	0.00113	0.00180	0.00180	0.00180	0.00320	0.00180	0.00180	-	-	-
m_7	0.00147	0.00147	0.00180	0.00147	0.00180	0.03553	0.00049	0.00147	0.34281	0.00147	0.00180	0.00180	0.00147	0.00180	-	-
n_21	0.00147	0.00147	0.00180	0.00147	0.00180	0.03553	0.00049	0.00147	0.40054	0.00147	0.00180	0.00180	0.00147	0.00180	0.18848	-
o_338	0.00147	0.00147	0.00180	0.00147	0.00539	0.22706	0.00049	0.00180	0.72640	0.00147	0.00991	0.00337	0.00147	0.00180	0.22881	0.40054

Table 23: *P*-values of sodium for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Na and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.00220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.00750	0.00220	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.03877	0.00220	0.38224	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.00447	0.00273	0.00273	0.00273	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.85451	0.00273	0.00803	0.00803	0.01385	-	-	-	-	-	-	-	-	-	-	-
f_10	0.00067	0.15822	0.00067	0.00067	0.00611	0.00113	-	-	-	-	-	-	-	-	-	-
g_339	0.20962	0.00220	0.00220	0.00220	0.17645	0.51314	0.00067	-	-	-	-	-	-	-	-	-
h_159	0.05366	0.00220	0.25794	1.00000	0.00273	0.01321	0.00067	0.00220	-	-	-	-	-	-	-	-
h1_167	0.01174	0.00447	0.00220	0.00220	0.43659	0.03346	0.04144	0.07435	0.00220	-	-	-	-	-	-	-
i_207	0.01321	0.00273	0.59151	0.02186	0.00447	0.00447	0.00113	0.00273	0.09887	0.00273	-	-	-	-	-	-
j_209	0.59151	0.00220	0.51647	0.85451	0.02186	0.51314	0.00067	0.16765	0.91497	0.01174	0.51314	-	-	-	-	-
k_211	0.78877	0.00273	0.00447	0.00447	0.00447	0.29174	0.00113	0.09887	0.00803	0.01321	0.00447	0.78877	-	-	-	-
l_213	0.59151	0.00273	0.03346	0.09887	0.00447	0.29174	0.00113	0.05059	0.17645	0.00803	0.03877	0.85451	0.85451	-	-	-
m_7	0.85451	0.00273	0.00273	0.00273	0.00447	0.22540	0.00113	0.22540	0.00273	0.00803	0.00447	0.78877	0.94417	0.46588	-	-
n_21	0.00750	0.00220	0.85451	0.05366	0.00273	0.00447	0.00067	0.00220	0.12976	0.00220	0.85451	0.51647	0.00447	0.03346	0.00273	-
o_338	0.00220	0.00220	0.00220	0.00220	0.00273	0.00273	0.00067	0.00220	0.00220	0.00220	0.00273	0.09887	0.00273	0.00273	0.00273	0.00220

Table 24: *P*-values of phosphorous for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: P and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.00223	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.00223	0.00223	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.00223	0.00223	0.00223	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.00350	0.00223	0.00223	0.19194	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.00350	0.00223	0.00223	0.02510	0.00350	-	-	-	-	-	-	-	-	-	-	-
f_10	0.00105	0.00077	0.00077	0.00077	0.00105	0.00105	-	-	-	-	-	-	-	-	-	-
g_339	0.00223	0.00223	0.00223	0.02015	0.00957	0.19194	0.00077	-	-	-	-	-	-	-	-	-
h_159	0.00223	0.00223	0.17943	0.00223	0.00223	0.00223	0.00077	0.00223	-	-	-	-	-	-	-	-
h1_167	0.00223	0.00223	0.72098	0.00223	0.00223	0.00223	0.00105	0.00350	0.63403	-	-	-	-	-	-	-
i_207	0.00350	0.00223	0.00223	0.19194	0.00350	0.07180	0.00105	0.05764	0.00223	0.00223	-	-	-	-	-	-
j_209	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00077	0.00223	0.00223	0.00223	0.00223	-	-	-	-	-
k_211	0.00350	0.00223	0.00223	0.00223	0.00350	0.00350	0.00105	0.00223	0.00223	0.00223	0.00350	0.94522	-	-	-	-
l_213	0.00540	0.00350	0.00350	0.00350	0.00540	0.00540	0.11379	0.00350	0.00350	0.00350	0.00540	0.00350	0.00540	-	-	-
m_7	0.00540	0.00350	0.00350	0.28003	0.93763	0.00540	0.00223	0.00608	0.00350	0.00350	0.00540	0.00350	0.00540	0.00939	-	-
n_21	0.00223	0.00223	0.00223	0.00223	0.00223	0.00566	0.00077	0.27340	0.00834	0.02015	0.00566	0.00223	0.00223	0.00350	0.00350	-
o_338	0.00350	0.00223	0.00223	0.00566	0.00350	0.60659	0.00105	0.30613	0.00223	0.00223	0.07180	0.00223	0.00350	0.00540	0.00540	0.02510

Table 25: *P*-values of sulfur for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: S and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.0019	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.0018	0.0019	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.0018	0.0019	0.0018	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.0019	0.0033	0.0019	0.0019	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.0019	0.0033	0.0019	0.0019	0.0105	-	-	-	-	-	-	-	-	-	-	-
f_10	0.0013	0.0375	0.8431	0.0013	0.0018	0.0018	-	-	-	-	-	-	-	-	-	-
g_339	0.0018	0.0058	0.1085	0.0018	0.0019	0.0019	0.2448	-	-	-	-	-	-	-	-	-
h_159	0.0018	0.0586	0.0209	0.0018	0.0019	0.0019	0.0518	0.4036	-	-	-	-	-	-	-	-
h1_167	0.0018	0.0019	0.0018	0.0018	0.0034	0.0019	0.0013	0.0018	0.0018	-	-	-	-	-	-	-
i_207	0.0019	0.0105	0.0019	0.0262	0.0033	0.0033	0.0043	0.0019	0.0019	0.0019	-	-	-	-	-	-
j_209	0.0018	0.0058	0.0018	0.4731	0.0019	0.0019	0.0018	0.0018	0.0018	0.0018	0.3133	-	-	-	-	-
k_211	0.0018	0.0019	0.0018	0.0018	0.0019	0.0019	0.0013	0.0018	0.0018	0.0018	0.0019	0.5470	-	-	-	-
l_213	0.0034	0.0056	0.0034	0.0034	0.4484	0.0056	0.0018	0.0034	0.0034	0.0034	0.0056	0.0034	0.0034	-	-	-
m_7	0.0019	0.0033	0.0019	0.0019	0.0182	0.0033	0.0018	0.0019	0.0019	0.3133	0.0033	0.0019	0.0019	0.0056	-	-
n_21	0.0018	0.0019	0.0086	0.0018	0.0825	0.0019	0.0035	0.0018	0.0018	0.8168	0.0019	0.0018	0.0018	0.5464	0.9452	-
o_338	0.0018	0.0019	0.0601	0.0018	0.0058	0.0019	0.0417	0.0018	0.0018	0.1794	0.0019	0.0018	0.0018	0.0034	0.1121	0.1794

Table 26: *P*-values of strontium for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Sr and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.0018	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.0018	0.0018	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.0018	0.0460	0.0018	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.0018	0.7308	0.0030	0.0018	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.0018	0.2891	0.0018	0.3483	0.0606	-	-	-	-	-	-	-	-	-	-	-
f_10	0.0012	0.0012	0.0018	0.0012	0.0018	0.0012	-	-	-	-	-	-	-	-	-	-
g_339	0.0018	0.0032	0.0018	0.0055	0.0018	0.0224	0.0012	-	-	-	-	-	-	-	-	-
h_159	0.0224	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	-	-	-	-	-	-	-	-
h1_167	0.0018	0.0018	0.4730	0.0018	0.0018	0.0018	0.0012	0.0018	0.0018	-	-	-	-	-	-	-
i_207	0.0018	0.0846	0.0114	0.6376	0.0761	0.3919	0.0018	0.3919	0.0018	0.0063	-	-	-	-	-	-
j_209	0.0846	0.0018	0.0030	0.0018	0.0030	0.0018	0.0341	0.0018	0.6376	0.0018	0.0030	-	-	-	-	-
k_211	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0427	0.0018	0.0018	0.0018	0.0018	0.1159	-	-	-	-
l_213	0.0018	0.0018	0.2700	0.0018	0.0030	0.0018	0.0018	0.0281	0.0018	0.0606	0.0327	0.0030	0.0018	-	-	-
m_7	0.0428	0.0018	0.0030	0.0018	0.0030	0.0018	0.0018	0.0018	0.6376	0.0018	0.0030	0.7043	0.0109	0.0030	-	-
n_21	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.6237	0.0018	0.0018	0.0018	0.0018	0.0606	0.5640	0.0018	0.0183	-
o_338	0.1453	0.0018	0.0018	0.0018	0.0018	0.0018	0.0012	0.0018	0.6376	0.0018	0.0018	0.3919	0.0018	0.0018	0.3260	0.0018

Table 27: *P*-values of zinc for the filamentous fungi. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Zn and Fungi

	0_Sawdust	a_Phloem	b_331	c_331Phloem	d_188	e_188Phloem	f_10	g_339	h_159	h1_167	i_207	j_209	k_211	l_213	m_7	n_21
a_Phloem	0.0020	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
b_331	0.0020	0.0020	-	-	-	-	-	-	-	-	-	-	-	-	-	-
c_331Phloem	0.0028	0.0020	0.0020	-	-	-	-	-	-	-	-	-	-	-	-	-
d_188	0.0028	0.9452	0.0020	0.0028	-	-	-	-	-	-	-	-	-	-	-	-
e_188Phloem	0.0020	0.0020	0.0020	0.0020	0.0020	-	-	-	-	-	-	-	-	-	-	-
f_10	0.0013	0.0013	0.0144	0.0013	0.0013	0.0013	-	-	-	-	-	-	-	-	-	-
g_339	0.0020	0.3272	0.0020	0.0020	0.1875	0.0020	0.0020	-	-	-	-	-	-	-	-	-
h_159	0.0028	0.0020	0.1875	0.0028	0.0028	0.0020	0.0876	0.0020	-	-	-	-	-	-	-	-
h1_167	0.0020	0.0020	0.0020	0.0020	0.0020	0.0020	0.0013	0.0020	0.0020	-	-	-	-	-	-	-
i_207	0.0028	0.0020	0.0020	0.0028	0.0028	0.0020	0.0013	0.0377	0.0028	0.0020	-	-	-	-	-	-
j_209	0.0097	0.0020	0.0020	0.0028	0.0028	0.0020	0.0013	0.0020	0.0028	0.0020	0.0028	-	-	-	-	-
k_211	0.0020	0.0020	0.0020	0.0020	0.0020	0.0020	0.0013	0.0020	0.0020	0.0020	0.0029	0.0020	-	-	-	-
l_213	0.0028	0.0020	0.0377	0.0028	0.0028	0.0020	0.5048	0.0020	0.0695	0.0020	0.0028	0.0028	0.0020	-	-	-
m_7	0.0049	0.0057	0.0031	0.0049	0.0190	0.0031	0.0020	0.7606	0.0049	0.0031	0.0049	0.0049	0.0031	0.0049	-	-
n_21	0.0028	0.0020	0.0020	0.0028	0.0028	0.0020	0.0013	0.0020	0.0028	0.0020	0.0028	0.0028	0.0020	0.0028	0.0049	-
o_338	0.0020	0.0020	0.0020	0.0020	0.0029	0.0020	0.0013	0.6293	0.0020	0.0020	0.0053	0.0020	0.0020	0.0020	0.0778	0.0020

10.6 *P*-values for the examined elements in yeasts

Table 28: *P*-values of aluminum for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Al and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.5793	-	-	-	-	-	-	-	-	-
b_It12	0.0085	0.0053	-	-	-	-	-	-	-	-
b1_It12Phloem	0.8221	0.6418	0.0066	-	-	-	-	-	-	-
c_It13	0.0146	0.0066	0.8221	0.0085	-	-	-	-	-	-
c1_It13Phloem	0.6418	0.4195	0.0053	0.8511	0.0066	-	-	-	-	-
d_It23	0.0085	0.0053	0.4864	0.0066	0.5026	0.0053	-	-	-	-
d1_It23Phloem	0.5784	0.2561	0.0085	0.6418	0.0146	0.7321	0.0085	-	-	-
e_XS1	0.0085	0.0053	0.2994	0.0066	0.1459	0.0053	0.6890	0.0085	-	-
f_XS21	0.0066	0.0053	0.8038	0.0053	0.8143	0.0053	0.6418	0.0066	0.5161	-
g_XS22	0.0066	0.0053	0.5161	0.0053	0.5105	0.0053	0.9452	0.0066	0.8038	0.5968

Table 29: *P*-values of carbon for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: C and Yeast

	0_Sawdust	a_Phloem	p_It12	p1_It12Phloem	q_It13	q1_It13Phloem	r_It23	r1_It23Phloem	s_XS1	t_XS21
a_Phloem	0.0053	-	-	-	-	-	-	-	-	-
p_It12	0.0053	0.9221	-	-	-	-	-	-	-	-
p1_It12Phloem	0.0092	0.7544	0.7372	-	-	-	-	-	-	-
q_It13	0.0053	0.8682	0.7544	0.9998	-	-	-	-	-	-
q1_It13Phloem	0.0962	0.8682	0.9722	0.7372	0.8318	-	-	-	-	-
r_It23	0.0174	0.6746	0.6746	0.6746	0.6134	0.9722	-	-	-	-
r1_It23Phloem	0.0677	0.4701	0.5336	0.6134	0.6793	0.6134	0.6134	-	-	-
s_XS1	0.0053	0.8682	0.8682	0.6793	0.6134	0.9221	1.0000	0.4460	-	-
t_XS21	0.0053	0.6793	0.7544	0.6134	0.4701	1.0000	0.9028	0.3642	0.7372	-
u_XS22	0.0053	0.6746	0.7544	0.6134	0.5336	1.0000	0.7544	0.4701	0.8318	0.9722

Table 30: *P*-values of calcium for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Ca and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.0027	-	-	-	-	-	-	-	-	-
b_It12	0.0063	0.0027	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0027	0.0027	1.0000	-	-	-	-	-	-	-
c_It13	0.0027	0.0027	0.0314	0.0189	-	-	-	-	-	-
c1_It13Phloem	0.0027	0.0027	0.0027	0.0027	0.0027	-	-	-	-	-
d_It23	0.0039	0.0039	0.0061	0.0061	0.0061	0.0039	-	-	-	-
d1_It23Phloem	0.0027	0.0027	0.0039	0.0039	0.0039	0.0039	0.0113	-	-	-
e_XS1	0.0621	0.0027	0.1544	0.0841	0.0063	0.0027	0.0039	0.0027	-	-
f_XS21	0.0027	0.0027	0.0027	0.0027	0.0039	0.2743	0.0129	0.3727	0.0027	-
g_XS22	0.0039	0.0027	0.2526	0.1987	0.3060	0.0027	0.0039	0.0027	0.0314	0.0039

Table 31: *P*-values of copper for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Cu and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.8038	-	-	-	-	-	-	-	-	-
b_It12	0.0054	0.0054	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0082	0.0054	0.0054	-	-	-	-	-	-	-
c_It13	0.0150	0.0111	0.9312	0.0150	-	-	-	-	-	-
c1_It13Phloem	0.0054	0.0054	0.0054	0.0054	0.0150	-	-	-	-	-
d_It23	0.0054	0.0054	0.5674	0.0149	0.3367	0.0940	-	-	-	-
d1_It23Phloem	0.0257	0.0058	0.0082	0.8543	0.0243	0.0082	0.0149	-	-	-
e_XS1	0.0054	0.0054	0.4815	0.0054	0.2357	0.0054	0.8654	0.0082	-	-
f_XS21	0.0054	0.0054	1.0000	0.0054	0.9312	0.0054	0.3959	0.0082	0.6746	-
g_XS22	0.0054	0.0054	0.5323	0.0054	0.7279	0.0054	0.1430	0.0058	0.2423	0.4575

Table 32: *P*-values of iron for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Fe and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.8011	-	-	-	-	-	-	-	-	-
b_It12	0.0057	0.0057	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0057	0.0057	0.0057	-	-	-	-	-	-	-
c_It13	0.0057	0.0057	0.4710	0.0057	-	-	-	-	-	-
c1_It13Phloem	0.0057	0.0057	0.0057	0.8839	0.0170	-	-	-	-	-
d_It23	0.0057	0.0057	0.3388	0.4053	0.5674	0.4710	-	-	-	-
d1_It23Phloem	0.0057	0.0057	0.0269	0.0601	0.1050	0.1463	0.9372	-	-	-
e_XS1	0.0057	0.0057	0.4710	0.0058	0.8824	0.0099	0.4053	0.1963	-	-
f_XS21	0.0170	0.0057	0.0170	0.8203	0.0170	0.9372	0.4053	0.1963	0.0269	-
g_XS22	0.0057	0.0057	0.3388	0.0057	0.9372	0.0099	0.4710	0.1050	0.8824	0.0269

Table 33: *P*-values of potassium for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: K and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.0032	-	-	-	-	-	-	-	-	-
b_It12	0.0032	0.0041	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0032	0.0032	0.6349	-	-	-	-	-	-	-
c_It13	0.0032	0.0032	0.0366	0.0129	-	-	-	-	-	-
c1_It13Phloem	0.0032	0.0032	0.0125	0.0125	0.5101	-	-	-	-	-
d_It23	0.0032	0.0032	0.1219	0.1650	0.8333	0.5872	-	-	-	-
d1_It23Phloem	0.0032	0.0032	0.0219	0.0565	1.0000	0.7583	0.4710	-	-	-
e_XS1	0.0032	0.0032	0.0041	0.0041	0.0041	0.0125	0.1650	0.4710	-	-
f_XS21	0.0032	0.0032	0.0041	0.0041	0.0041	0.0032	0.0041	0.0041	0.0041	-
g_XS22	0.0032	0.0032	0.0080	0.0041	0.0985	0.6558	0.5101	0.5872	0.0125	0.0032

Table 34: *P*-values of magnesium for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Mg and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.0029	-	-	-	-	-	-	-	-	-
b_It12	0.0029	0.0029	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0029	0.0029	0.9726	-	-	-	-	-	-	-
c_It13	0.0029	0.0029	0.1089	0.0776	-	-	-	-	-	-
c1_It13Phloem	0.0029	0.0277	0.0043	0.0043	0.0043	-	-	-	-	-
d_It23	0.0083	0.0083	1.0000	0.5238	0.2886	0.0125	-	-	-	-
d1_It23Phloem	0.0029	0.0029	0.0043	0.0043	0.0043	0.0064	0.0125	-	-	-
e_XS1	0.0045	0.0045	0.0064	0.0064	0.0064	0.0064	0.0203	0.0064	-	-
f_XS21	0.0029	0.0029	0.8839	0.8839	0.2070	0.0029	1.0000	0.0029	0.0045	-
g_XS22	0.0029	0.0029	0.0029	0.0029	0.0029	0.0029	0.0083	0.0029	0.0586	0.0029

Table 35: *P*-values of manganese for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Mn and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.0019	-	-	-	-	-	-	-	-	-
b_It12	0.0028	0.0028	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0019	0.2207	0.0028	-	-	-	-	-	-	-
c_It13	0.0042	0.0347	0.0066	0.0042	-	-	-	-	-	-
c1_It13Phloem	0.0066	0.0019	0.0028	0.0019	0.0042	-	-	-	-	-
d_It23	0.0042	0.2179	0.0066	0.0042	0.1069	0.0042	-	-	-	-
d1_It23Phloem	0.0079	0.0079	0.0119	0.0079	0.0190	0.4198	0.0190	-	-	-
e_XS1	0.0019	0.0019	0.0104	0.0019	0.0042	0.0019	0.0042	0.0079	-	-
f_XS21	0.0019	0.8048	0.0028	0.0205	0.1167	0.0019	0.2778	0.0079	0.0019	-
g_XS22	0.0019	0.0019	0.0028	0.0019	0.0042	0.0019	0.0042	0.0079	0.0019	0.0019

Table 36: *P*-values of nitrogen for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: N and Yeast

	0_Sawdust	a_Phloem	p_It12	p1_It12Phloem	q_It13	q1_It13Phloem	r_It23	r1_It23Phloem	s_XS1	t_XS21
a_Phloem	0.0015	-	-	-	-	-	-	-	-	-
p_It12	0.0015	0.0015	-	-	-	-	-	-	-	-
p1_It12Phloem	0.0015	0.0015	0.0164	-	-	-	-	-	-	-
q_It13	0.0015	0.0015	0.0714	0.0015	-	-	-	-	-	-
q1_It13Phloem	0.0015	0.0015	0.0392	0.2207	0.0028	-	-	-	-	-
r_It23	0.0015	0.0015	0.0028	0.0015	0.0253	0.0015	-	-	-	-
r1_It23Phloem	0.0015	0.0015	0.0015	0.1779	0.0015	0.0015	0.0015	-	-	-
s_XS1	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.6434	0.0015	-	-
t_XS21	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015	0.8048	0.0015	0.7235	-
u_XS22	0.0015	0.0015	0.0027	0.0015	0.0027	0.0015	0.0015	0.0015	0.0015	0.0015

Table 37: *P*-values of sodium for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Na and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.0032	-	-	-	-	-	-	-	-	-
b_It12	0.0032	0.0032	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0032	0.0032	0.1063	-	-	-	-	-	-	-
c_It13	0.0061	0.0061	0.1527	0.0077	-	-	-	-	-	-
c1_It13Phloem	0.0032	0.0032	0.1063	0.6630	0.0080	-	-	-	-	-
d_It23	0.0053	0.0053	0.6630	0.2208	0.1574	0.2415	-	-	-	-
d1_It23Phloem	0.0783	0.0032	0.0032	0.0052	0.0077	0.0032	0.0077	-	-	-
e_XS1	0.0032	0.0032	0.2208	0.7121	0.0077	0.9452	0.2208	0.0052	-	-
f_XS21	0.0032	0.0032	0.2741	0.1729	0.0077	0.6630	0.4714	0.0052	0.3547	-
g_XS22	0.3565	0.0032	0.0032	0.0032	0.0061	0.0032	0.0053	0.1430	0.0032	0.0032

Table 38: *P*-values of phosphorus for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: P and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.0027	-	-	-	-	-	-	-	-	-
b_It12	0.0035	0.0027	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0035	0.0027	0.0108	-	-	-	-	-	-	-
c_It13	0.0035	0.0027	0.5887	0.0035	-	-	-	-	-	-
c1_It13Phloem	0.0035	0.0027	0.0035	0.0776	0.0035	-	-	-	-	-
d_It23	0.0061	0.0037	0.3548	0.2714	0.0963	0.0212	-	-	-	-
d1_It23Phloem	0.0035	0.0027	0.0035	0.0108	0.0035	0.2017	0.0108	-	-	-
e_XS1	0.0027	0.0027	0.0027	0.0027	0.0027	0.0027	0.0037	0.0027	-	-
f_XS21	0.0027	0.0027	0.3871	0.0064	0.5437	0.0027	0.1215	0.0027	0.0094	-
g_XS22	0.0027	0.0027	0.0027	0.0027	0.0027	0.0027	0.0037	0.0027	0.3973	0.0037

Table 39: *P*-values of sulfur for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: S and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.0023	-	-	-	-	-	-	-	-	-
b_It12	0.0023	0.0023	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0023	0.0080	0.0023	-	-	-	-	-	-	-
c_It13	0.0023	0.0040	0.5539	0.0080	-	-	-	-	-	-
c1_It13Phloem	0.0023	0.0023	0.0023	0.2797	0.0023	-	-	-	-	-
d_It23	0.0093	0.0134	0.0093	0.2577	0.0244	0.0093	-	-	-	-
d1_It23Phloem	0.0023	0.0203	0.0023	0.0878	0.0040	0.2577	0.0134	-	-	-
e_XS1	0.0023	0.0023	0.0152	0.0328	0.1576	0.0023	0.4359	0.0023	-	-
f_XS21	0.0023	0.0023	0.0104	0.1500	0.0427	0.0023	0.7879	0.0023	0.6315	-
g_XS22	0.0023	0.0023	0.0229	0.0023	0.0023	0.0023	0.0093	0.0023	0.0023	0.0023

Table 40: *P*-values of strontium for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Sr and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.0020	-	-	-	-	-	-	-	-	-
b_It12	0.0060	0.0027	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0020	0.0020	0.0027	-	-	-	-	-	-	-
c_It13	0.0038	0.0038	0.0571	0.0038	-	-	-	-	-	-
c1_It13Phloem	0.0027	0.0020	0.0027	0.0020	0.0038	-	-	-	-	-
d_It23	0.0038	0.0038	0.0060	0.0038	0.0097	0.0038	-	-	-	-
d1_It23Phloem	0.3727	0.0027	0.0038	0.0027	0.0060	0.0060	0.0060	-	-	-
e_XS1	0.1711	0.0020	0.0571	0.0020	0.0038	0.0020	0.0038	0.0098	-	-
f_XS21	0.0020	0.0020	0.0060	0.0020	0.0790	0.0020	0.0063	0.0027	0.0020	-
g_XS22	0.0130	0.0020	0.8357	0.0020	0.0347	0.0020	0.0038	0.0027	0.1356	0.0038

Table 41: *P*-values of zinc for the yeasts. Data was calculated using the Kruskal-Wallis-Test followed by a pairwise Wilcoxon Post-Hoc test in R (Version 4.2.2). For allocation of the here listed sample strains to the organism names see Table 1.

data: Zn and Yeast

	0_Sawdust	a_Phloem	b_It12	b1_It12Phloem	c_It13	c1_It13Phloem	d_It23	d1_It23Phloem	e_XS1	f_XS21
a_Phloem	0.0031	-	-	-	-	-	-	-	-	-
b_It12	0.0031	0.0031	-	-	-	-	-	-	-	-
b1_It12Phloem	0.0033	0.0031	0.8357	-	-	-	-	-	-	-
c_It13	0.0033	0.0031	0.0385	0.1830	-	-	-	-	-	-
c1_It13Phloem	0.0033	0.0031	0.0031	0.0052	0.0033	-	-	-	-	-
d_It23	0.0052	0.0035	0.0035	0.0099	0.0052	0.0854	-	-	-	-
d1_It23Phloem	0.0033	0.0031	0.0031	0.0687	0.0170	0.0099	0.0052	-	-	-
e_XS1	0.0033	0.0031	0.0031	0.0033	0.0033	0.0033	0.0052	0.0033	-	-
f_XS21	0.0033	0.0031	0.0031	0.0687	0.0033	0.0033	0.0052	0.0033	0.0033	-
g_XS22	0.0031	0.0031	0.0031	0.0031	0.0031	0.0031	0.0035	0.0031	0.0035	0.0031