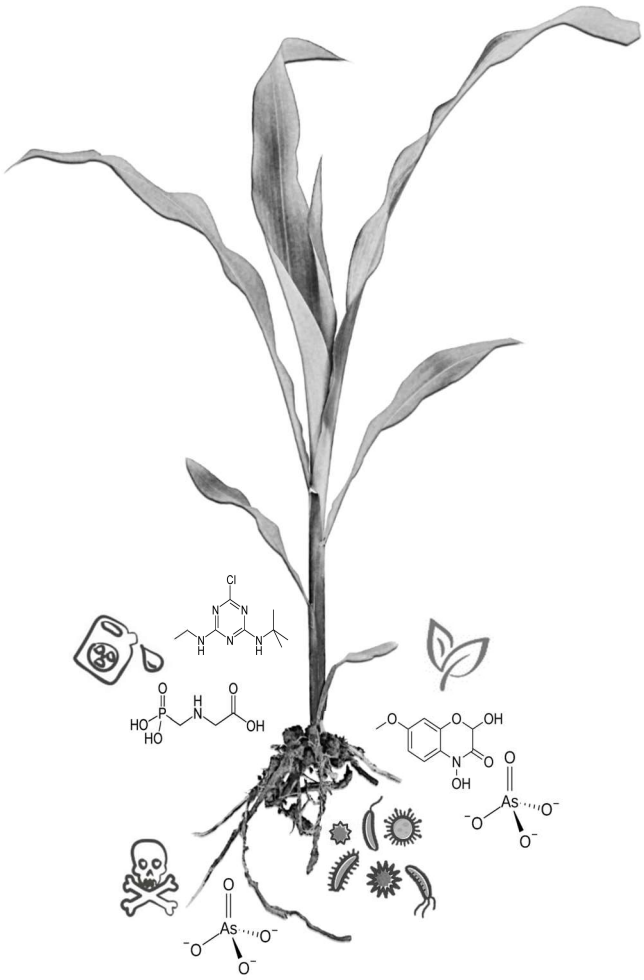
Effects of environmental chemicals on soil and plant health

Inaugural dissertation of the Faculty of Science, University of Bern

presented by
Veronica Lucia Luigina Caggìa
from Stabio TI

2022



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12th of December 2022

Promotion committee

Prof. Dr. Klaus Schlaeppi
Department of Environmental Sciences, University of Basel, Switzerland

Prof. Dr. Sandra Spielvogel
Institute of Plant Nutrition and Soil Science, Christian-Albrechts-Universität Kiel, Germany

Prof. Dr. Matthias Erb
Plant Sciences Institute, University of Bern, Switzerland

External reviewer

Prof. Dr. Marcel van der Heijden
Department of Plant and Microbial Biology, University of Zürich, Switzerland

Chairman

Prof. Dr. Willy Tinner
Plant Sciences Institute, University of Bern, Switzerland

Table of contents

Summary

The world's soils are threatened by intensive farming, industrial pollution, mining, and poor waste management that are poisoning them. All soils are under a lot of pressure, as 95% is used towards the production of food for human and animal consumption; consequently, and unsurprisingly this soil has extensive damage. Soil pollution is described as soils that contain toxic chemicals or elements, such as pollutants, can be present in high enough concentrations to constitute a risk for the environmental and human health. To understand environmental health more holistically – e.g., in the context of a food chain – we urgently need to understand decode the interactions of these environmental chemicals with soils, their intrinsic microbiomes as well as with their crops. Such understanding will allow to find possible solutions to reduce the negative impacts of contaminants in our food chain. Plants are extremely adaptable, despite their limited ability to move, and have developed multiple physiological strategies to tolerate and defend themselves against a vast array of external pressures and stresses. In recent years microbiomes are more and more recognized for their importance on environmental health. One hypothesis is that plants modulate their associated microbiomes to cope with and survive under soil contamination, for instance by enhancing degradation, transformation, immobilization, or safe storage. However, evidence for this phenomenon remains fragmented.

The aims of this thesis were to understand, taking a holistic One Health perspective, the microbiome-mediated chemical feedbacks on plant growth due to soil pollution. As contaminants we studied herbicides (Chapter I), arsenic (Chapter II) and the interaction between arsenic and benzoxazinoids (Chapter III). Soil herbicide contaminations were studied with glyphosate and terbuthylazine as chemical stresses of a healthy food chain. We found small effects on soil enzymes activities and that soil bacteria were more susceptible to herbicide contamination than fungi. However, investigation the food chain perspective, we did not find herbicide- or microbiome-mediated effects on the performance of maize plants (Chapter I). These results revealed that herbicides have a reduced impact on the surrounding environment without affecting maize growth, suggesting that herbicides present overall well-designed chemicals.

Taking arsenic contamination of soil as a second stress of a healthy food chain, we found a shift in soil bacterial microbial community, as a response to arsenic toxicity in soil (Chapter II). We did not detect any changes in leaves, root, and kernel microbiomes, as well as in enzyme activities when the soil was challenged with arsenic. These results illustrated the importance of microbiomes in a One Health concept. Interestingly, we discovered a clear positive impact on plant performance by root-secreted benzoxazinoids that help to cope with arsenic stress: plants exuding benzoxazinoids tolerated better soil arsenic toxicity and growth of benzoxazinoid-deficient mutant plants could be rescued by exogenous applied benzoxazinoids (Chapter III).

Hence, we confirmed the multifunctional and beneficial nature of benzoxazinoids, root exudates of grasses, to enhance plant resilience against arsenic in contaminated soils. This finding has a great agronomic potential in crop rotation systems, as it presents a tool to alleviate toxic effects due to soil arsenic contamination and therefore, ensuring better yield of crops in contaminated sites. Taken together the results of this thesis showed the importance of tackling the impact of chemicals introduced or present in the environment that potentially cause health problems to food chains.

General introduction

One Health

The modern concept of 'One Health' highlights that human well-being cannot be tackled without taking into consideration the well-being of animals, plants, soils and the environment (Banerjee & Heijden, 2022). A food chain, starting from soils to plants that represent the primary source of food for both human and animals presents a paradigm for the One Health concept. Human health relates backwards to the health of plants and the health of plants yet relates back to the foundation of our food chain: the soil. Initially, the 'One Health' concept was called 'One medicine', and was focussing on the integration of human, animal, and environmental health in light of the growing worldwide population that caused intensification of livestock production, increased urbanisation and globalization (Zinsstag et al., 2011). These rapid and violent changes clearly had disruptive consequences on the equilibrium between human and nature. In fact, global sustainability lays in the health and well-being of human, animals, and the ecosystems in which they live in (Lebel, 2003; Rapport et al., 1999). A striking example is the increasing frequency of zoonoses' outbreaks, that can infect wild and domestic animals, as well as humans, and therefore represent a worldwide threat for public health (Kahn, 2006). After a long tradition of 'One Health' mostly focusing on clinical consequences (pandemics and antimicrobial resistance), only recently it was broadened to include also soil and plant as part of the food chain (van Bruggen et al., 2019).

While pathogen spread was since a long-time part of the 'One Health' concept, the recognition of microbiomes as an integral part playing an important role in transferring and mediating health conditions through the food chain is relatively new and not much investigated factor (van Bruggen et al., 2019). Microorganisms are suspected playing an important role connecting different hosts and environments, because they connect different compartments being at their interfaces (Berg et al., 2020). Therefore, the Interdisciplinary Research Cooperation (IRC) One Health at the University of Bern started in 2018 a collaboration among nine research groups to specifically investigate possible microbiome-dependent health effects in the food chain. This interdisciplinary research cooperation aimed to elucidate how microbial communities, when being perturbated by different environmental chemicals, they affect the health along an experimental food chain consisting of soil, plant, animal, or humans (the components will be introduced in detail below). Microbial communities at different interfaces in the food chain were specifically compared when perturbated by chemical stress factors such as metalloids, herbicides, and bioactive plant secondary metabolites. Ultimately, the mission was to understand how microbiome change will affect the health of individual food chain compartments and if health effects are forwarded along the food chain. My focus within the large research project was to unravel the direct and microbiome-mediated effects of arsenic and herbicides at the soil-plant interface (**Fig. 1**).

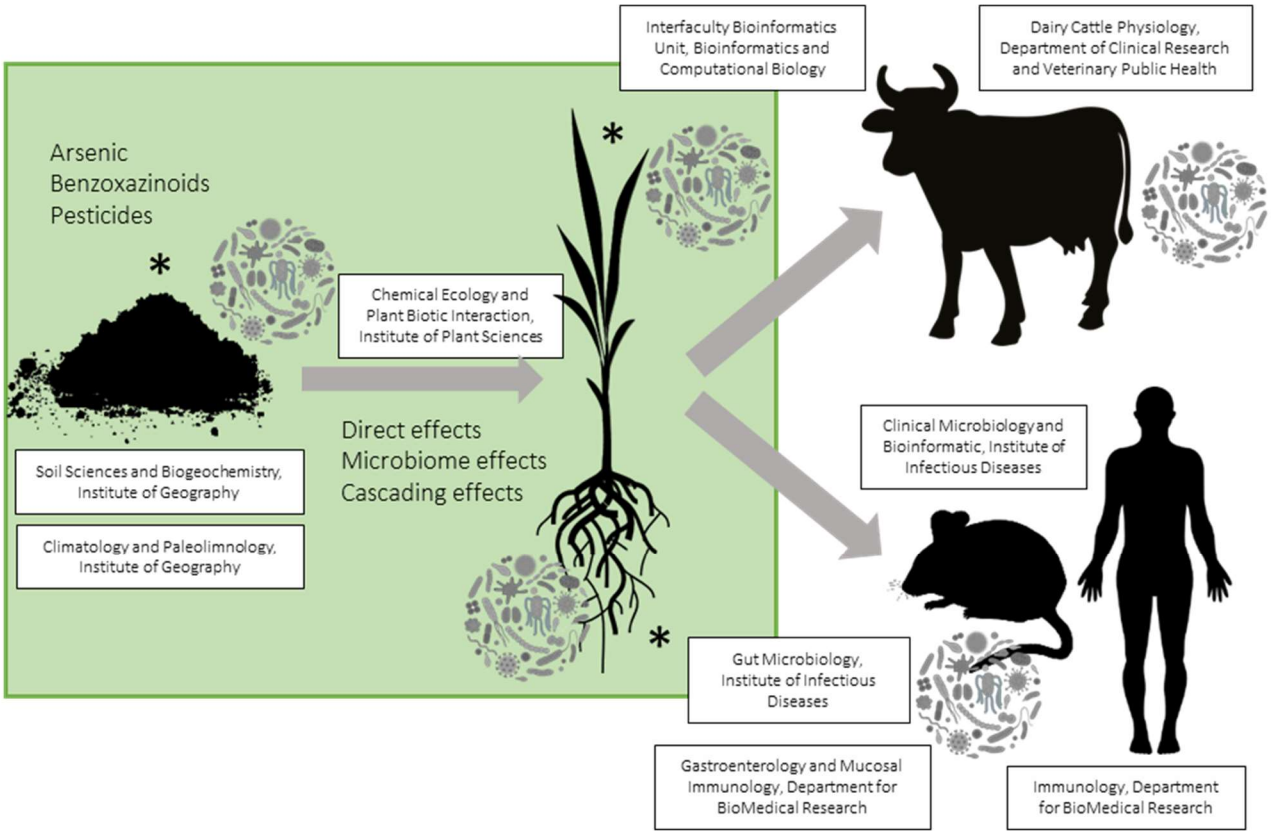


Figure 1. The representation of the entire One Health project of the University of Bern. Nine research groups, from different faculties and with different expertise, aimed to integrate sustainability and health in a multidisciplinary project that investigates an experimental food chain and how selected environmental chemicals have direct effects, or trigger microbiome and cascading effects on the next compartment in a food chain. My contribution to the IRC (*) was on the soil-plant interface, studying the effects of the three environmental chemicals on the soil functioning., soil and plant microbiomes and plant performance.

Agricultural perspective

The global human population is growing relentlessly and is anticipated to reach 9.7 billion people by 2050 (Garcia et al., 2020). Consequently, we are still facing an increasing demand in food production. The increased pressure for food production has already had a major impact on natural resources such as soil erosion and nutrients stock, increasing pollution and loss of biodiversity (Johnson et al., 2014; Smith, 2013). The current dual challenge in food production, therefore, is not only to produce more in terms of quantity but to also ensure food quality and safety, along with the sustainability in the food production (Tilman et al., 2011). Here the concept of One Health steps in to create initiatives for sustainable food systems, that ensures food security and nutrition without compromising the same possibilities for future generations (FAO, 2017a). Of course this transition to implement and make successful the 'One Health' view needs extensive collaboration between the scientific, engineering and humanity fields to compel a global policy and implementation (Garcia et al., 2020).

Our goal as a scientific community is to provide knowledge, evidence, and potential solutions by investigating and understanding the functioning and characteristics of biotic and abiotic elements interacting in the environment.

Interactions between microorganisms and the plant can cover a wide range, from mutualistic to parasitic. Several studies have identified plant-associated microbes to be beneficial for the host plant (Bhattacharyya & Jha, 2012; Porras-Alfaro & Bayman, 2011; Vorholt, 2012). Such beneficial interactions nurture the idea of exploiting them for a more sustainable agriculture. However, soil-plant interactions as well as interactions among microorganisms are very complex, and it is still difficult to define sets of microbial taxa, with genomic and functional characteristics, that can be used in sustainable agroecosystems (Toju et al., 2018). For this, fundamental science still needs a transition into applied and combined methods to create more realistic scenarios (Schlaeppi & Bulgarelli, 2015). In heading towards a Green Revolution that is respectful and compatible with the environment and safe to humans, microbes interacting and protecting the plant represent a strong potential to enhance food quality and safety through plant health management and therefore constitute innovative solutions to foster sustainability in agroecosystems (Qiu et al., 2019). The knowledge generated from our study contribute to the understanding of the 'One Health' concept, provide new insights on microbial interfaces and how to sustain health in presence of different environmental chemicals.

Soil and plant microbiomes

In a food chain, soil presents the foundation because it allows the growth of crops that will then be consumed by both animals and humans. Since the industrialization of agriculture with the use of pesticides and increased pollution (Gowdy & Baveye, 2018; Parween & Jan, 2019), it is not surprising that the entire food chain equilibrium is disturbed and today faces great challenges (FAO, 2017b). Soil is the largest resource of microbial diversity and abundance on earth (Torsvik et al., 2002; Venter et al., 2004; Whitman et al., 1998) and diverse microbial communities can have influences on soil, plant, animal, and human health. Similar to the health of soil, microbes are also key for healthy and well-performing crops. Plant-associated microbes were discovered to assist the plant in development by boosting plant growth, suppressing pathogens and modulating plant immune system (Berendsen et al., 2012; Trivedi et al., 2020). Analogous to the commensal microbiota in human guts, that have a key function in training and enhancing major components of the host's immune system (Zheng et al., 2020), soil and plant microbiomes are also vital for plant health (Hacquard et al., 2015).

As dysbiosis of the gut microbiota is correlated with the presence of a disease and inflammatory responses (Carding et al., 2015), perturbed soil and plants microbiomes are also associated with negative health effects that can cascade to the entire food chain (Banerjee & Heijden, 2022). Therefore, for a holistic view on the health of a connected system, it is key to understand how perturbated microbiomes at the interface between system compartments affect the health of the entire system.

Applying such 'One Health' thinking to a food chain, it is essential to understand the effects of perturbated microbiomes such as e.g., between the soil and the plant to realize their ecological functions and how their resistance to different stressors works. The key for a holistic understanding of global health of an entire system is to know what happens when microbiomes between food chain compartments are perturbated.

Experimental food chain and perturbating chemicals

The Interfaculty Research Cooperation built an experimental food chain consisting of soil, maize plant, dairy cows, and mice as human model. For microbiome perturbations we chose to work with glyphosate and terbuthylazine (herbicides), arsenic (metalloid) and benzoxazinoids (plant toxins). We did preliminary tests on five different Swiss agricultural soils, and we selected the 'Q-Matte' soil as it presented no or very low traces of the studied environmental chemicals. The soil comes from Frauenkappelen where the main soil types are Haplic Cambisols and Haplic Luvisols (IUSS Working Group WRB, 2022) and has a silty loam texture.

To determine the impact of the selected environmental chemicals on soil, we decided to use soil enzymes as they respond faster than other soil variables to anthropogenic factors. Therefore, we considered soil enzyme activities as early and sensitive measure to evaluate soil quality before and after the addition of a stressor (Ladd, 1985; Miller & Dick, 1995; Theriot et al., 2013). For that, we contemplated in our study 3 to 5 enzymes involved in relevant transformation in the carbon, nitrogen and phosphorus cycles (Aon et al., 2001; Wallenstein & Burns, 2015).

As crop, we decided to work with maize (*Zea mays* L.) that is an annual grass in the family Poaceae, and it is the most abundantly produced cereal in the world, together with rice (Erenstein et al., 2022). According to the Food and Agriculture Organization of the United Nations (FAO), maize production in 2011 exceeded 883 million tons, which is greater than the respective production of wheat and rice (FAOSTAT, 2004). It can be grown in different conditions, with a wide range of temperatures, altitudes and latitudes, soil, and land type, having of course the expected variation in yield production. Corn represents for farmers an interesting possibility because of its higher yield compared to other cereals, especially when the land is scarce and the human population very dense (Shiferaw et al., 2011). Maize is used for human consumption, but the majority is used to feed livestock. For example in the US, the largest worldwide producer with 34% of the total market, 44% of the corn production is destined to animal feeding (Ceres, 2016). Maize can be processed into a variety of food and industrial products (Ranum & Pe, 2014), therefore its importance within the food chain is highly relevant. For the above-mentioned reasons and because of its agronomic and economic importance, we decided to include maize in the experimental food chain. In our research we used maize performance, by measuring plant height, chlorophyll content and yield, as parameter to evaluate the impact of the selected environmental chemical.

Herbicides

As a first stressor, an anthropogenic source of perturbation for the soil microbiome, the IRC decided to focus on herbicides. Herbicides were chosen among the many different pesticides because they represent ca. 50% of the 2 million tons that are used worldwide each year (De et al., 2014; Sharma et al., 2019). More specifically, we selected glyphosate and terbuthylazine because they belong to different chemical groups and because they differ in their mode of action on the targeted weed. Glyphosate (Gly) is considered the most successful herbicide in history (Duke & Powles, 2008) and it is known to have a relatively low negative environmental impact (Franz et al., 1997). It is applied as a post-emergence treatment (van Bruggen et al., 2018), and it is mostly taken up by the leaves of the plant and transported to the entire plant causing tissue death (Kafarski et al., 1988) due to the reduction of the production of aromatic amino acids and accumulation of shikimic acid and hydroxybenzoic acids (Becerril et al., 1989; Hernandez et al., 1999; Lydon & Duke, 1988). On the other hand, there is terbuthylazine (Tb), which is used as a pre- and post-emergence herbicide, applied directly to the soil and mainly taken up by roots (Foundation for Arable Research, 2007). Tb acts by inhibiting the Hill reaction and CO_2 sorption in the photosynthesis (Linan, 2009). Finally, the two herbicides differ in their half-life in soil with glyphosate having a shorter half-life (~30 days) compared to terbuthylazine (~70 days). However, the use of both Gly and Tb is controversial because of the possible effects on the environment, evidence for highly impacting consequences on the human health, their accumulation and degradation products formation, and finally the negative effects on non-target organisms (Bai & Ogbourne, 2016; Bottoni et al., 2013; Palma et al., 2014; Reddy et al., 2004; Tsaboula et al., 2016). We have challenged the soil microbiome with both herbicides at the recommended doses to investigate whether microbiome-mediated health effects occur on maize.

Arsenic

As a second stressor, we worked with arsenic that is a naturally occurring metalloid, widely distributed in natural environments such as water, sediment and soil, and has been proven to be harmful to many organisms in sufficient concentrations (Nriagu et al., 2007). Arsenic is released to the environment either through natural processes or because of industrial activities. It is present in combination with other elements in organic and inorganic forms, where the inorganic forms are the most toxic (FAO, 2018) and also present in higher amount in soils. The inorganic form arsenate is a chemical analogue of phosphate and therefore it is easily taken up by the plant through phosphate transporters (Cao & Ma, 2004; Meharg & Macnair, 1990; Wu et al., 2011) and enters the food chain. At toxic concentrations, arsenic interferes with plant growth and development by disrupting several parts of its metabolism, which can also lead to death (Ci et al., 2012; Møller et al., 2007; Singh et al., 2006). This toxic metalloid is not only a treat for micro- and macrofauna living in the soil and for plants but it can also have downstream effects on livestock and humans, due to crop consumption (Bhatti et

al., 2013; Nachman et al., 2018; Shibata et al., 2016). Today's recommendations and risk management of arsenic focus on drinking water, even though exposure through crop consumption has been confirmed to be a well-established route to humans (Ware, 2007; Upadhyay et al., 2019), causing a series of health problems (Chen & Costa, 2021; Cubadda et al., 2017; Pearce et al., 2012). Typical health problems of humans chronically exposed to As include skin lesions, diabetes, cardiovascular problems, lung and skin cancer (Mohammed Abdul et al., 2015). In this research, we have spiked the soil with different arsenic concentrations (AsV) to explore its effect on soil microbiome and functioning, and on maize-associated microbes and performance.

Plant secondary metabolites

As a third chemical perturbation for the rhizosphere microbiome, we decided to work with a class of plant secondary metabolites: the benzoxazinoids. In fact, these bioactive molecules are produced by the plant to interact with its environment, including communication with other organisms, above- and belowground, and to influence the rhizosphere and consequently the soil properties (Guerrieri et al., 2019). Benzoxazinoids are plant metabolites found in wild and cultivated Poaceae (Kokubo et al., 2017) and a lot of studies have been done about their role as chemical defense against insect, pathogens and neighbouring competing plants (Niemeyer, 2009). However, they were later shown to also perturbate the rhizosphere microbiome so that the performance of the next plant generation was affected (Hu et al., 2018b). Therefore, we worked with benzoxazinoids as chemical perturbation for the rhizosphere microbiome with possible downstream growth effect on plants and potentially on the entire food chain. For all these reasons, benzoxazinoids represent a highly relevant group of specialized metabolites, because involved in ecological functions with strong potential in agricultural pest management and crop rotation systems (Zhou et al., 2018). In addition, recent speculations have been done on the potential of benzoxazinoids in counteracting metal toxicity in soil, however studies have been focusing on aluminium (Poschenrieder et al., 2005; Zhao et al., 2019). Consequently, a deeper understanding of the interaction of arsenic with crops, their exuded metabolites and their associated microbiome can help to reveal novel tools towards safer food production. In this thesis, we have explored the effects of the performance of benzoxazinoid-producing and benzoxazinoid-deficient maize plants under arsenic conditions to unravel the potential of these root exudates in arsenic tolerance.

Thesis outline

The overall aim of this thesis was to understand if, to which degree and how the soil microbiome links soil and plant health. To investigate these questions, we perturbated the soil microbiome with three different chemical stresses and we assessed soil microbiome composition and functioning alongside with measuring plant performance. The three chemical stresses include bioactive plant secondary metabolites, the herbicides Gly and Tb and arsenic as a heavy metal contamination. The risk of exposure of these environmental chemicals is nowadays relatively high. Direct toxicity effects of these chemicals are well studied; however, we are often not aware of their indirect effects that are forwarded to other hosts or compartments. We applied 'One Health' thinking to the soil-plant part of the experimental food chain of the IRC One Health consortium. The research aims included three parts to investigate I) microbiome-mediated links between glyphosate or terbuthylazine impacts on soil health and their downstream consequences on plant performance, II) microbiome-mediated links between soil arsenic contaminations, their effects on soil health and their downstream consequences on plant performance and III) the interaction between root exudate and microbiome-mediated effects on soil and plant performance (**Fig. 2**).

In **chapter I**, we investigated the hypothesis that the application of the herbicides glyphosate and terbuthylazine negatively impacts the soil microbial communities and their functioning and that will also negatively affect plant performance. Our setup was conceived to unravel the differences between pre- and post-emergence herbicide application, which means spraying directly onto bare soil before the growth of the weed and spraying on the weed after it has grown. We tested the hypothesis that perturbations are stronger when the herbicides are sprayed directly onto soil and when soil moisture is high. We found a relevant context dependency of glyphosate and terbuthylazine presence in soil, with evidence for possible faster metabolization in higher soil moisture. We discovered that soil bacteria were affected more than fungi by the application of the two different herbicides. The shifts in community composition were strongly dependent on soil moisture; while the mode of application (soil vs. weed) did not have an effect. Overall, herbicide applications had only small impacts on soil microbiome composition and functioning, whereas the modes of application and soil moisture did not play a role at all. Finally, we found that a single application of glyphosate and terbuthylazine in recommended doses, did not affect the subsequent crop performance.

In **chapter II**, we studied the effects of different arsenic concentrations on soil communities and functioning, as well as plant-associated microbiomes and performance. Our hypothesis was that the soil microbiome, functioning, and plant performance were negatively affected by the metalloid contamination. Soil functioning, approximated by measurements of soil enzyme activities, was not affected by the increased arsenic concentrations in soil. However, we observed an increase in bacterial diversity in soil communities induced by arsenic contamination. Finally, also in different plant compartments (roots, leaves, and kernels) we did not find any changes in microbial communities exposed to arsenic. In addition, we found that the interaction of

maize plants with the native soil microorganisms decreased the arsenic translocation to the plant and we found in soil pore water that the soil microbes transformed the toxic inorganic arsenic to organic arsenic, which is less toxic (Annexes Chapter II). We further discovered that soil microbes supported corn to limit inorganic arsenic translocation to other plant tissues in highly contaminated soil. Finally, we could observe that indigenous soil microorganisms, compared to disturbed communities, are beneficial for the plant that must cope with arsenic (Annexes Chapter II).

Finally in chapter III, we explored the interaction in soil between arsenic and the main group of maize exuded plant secondary metabolites, the benzoxazinoids. Benzoxazinoids were found earlier to possibly mitigate aluminium toxicity in plants and therefore, we hypothesized that they help the plant growing in arsenic-contaminated soil, too. Our results showed that a benzoxazinoid-exuding maize plant coped better with arsenic contamination compared to the *bx1* mutant, defective in benzoxazinoids because of a mutation in the first gene of its biosynthesis. While the two genotypes grew equally under arsenic-free conditions, *bx1* grew smaller and with less biomass than the wildtype in arsenic-contaminated soil. We also had evidence that the external addition of benzoxazinoids purified from the wildtype, can rescue the *bx1* mutant conferring arsenic tolerance. Concerning the bacterial and fungal communities, we found a small yet significant shift in microbiome composition respectively. Interestingly, we also discovered that the second plant generation planted in benzoxazinoid-conditioned soil, could still benefit from the positive effects of benzoxazinoids in arsenic contaminated soil; wildtype maize grows higher in wildtype-conditioned soil than in *bx1*-conditioned soil. Taken together our findings reveal the significance of including soil and plant in the 'One Health' framework and the relevance of addressing the influence of different environmental chemicals in the health of these two compartments.

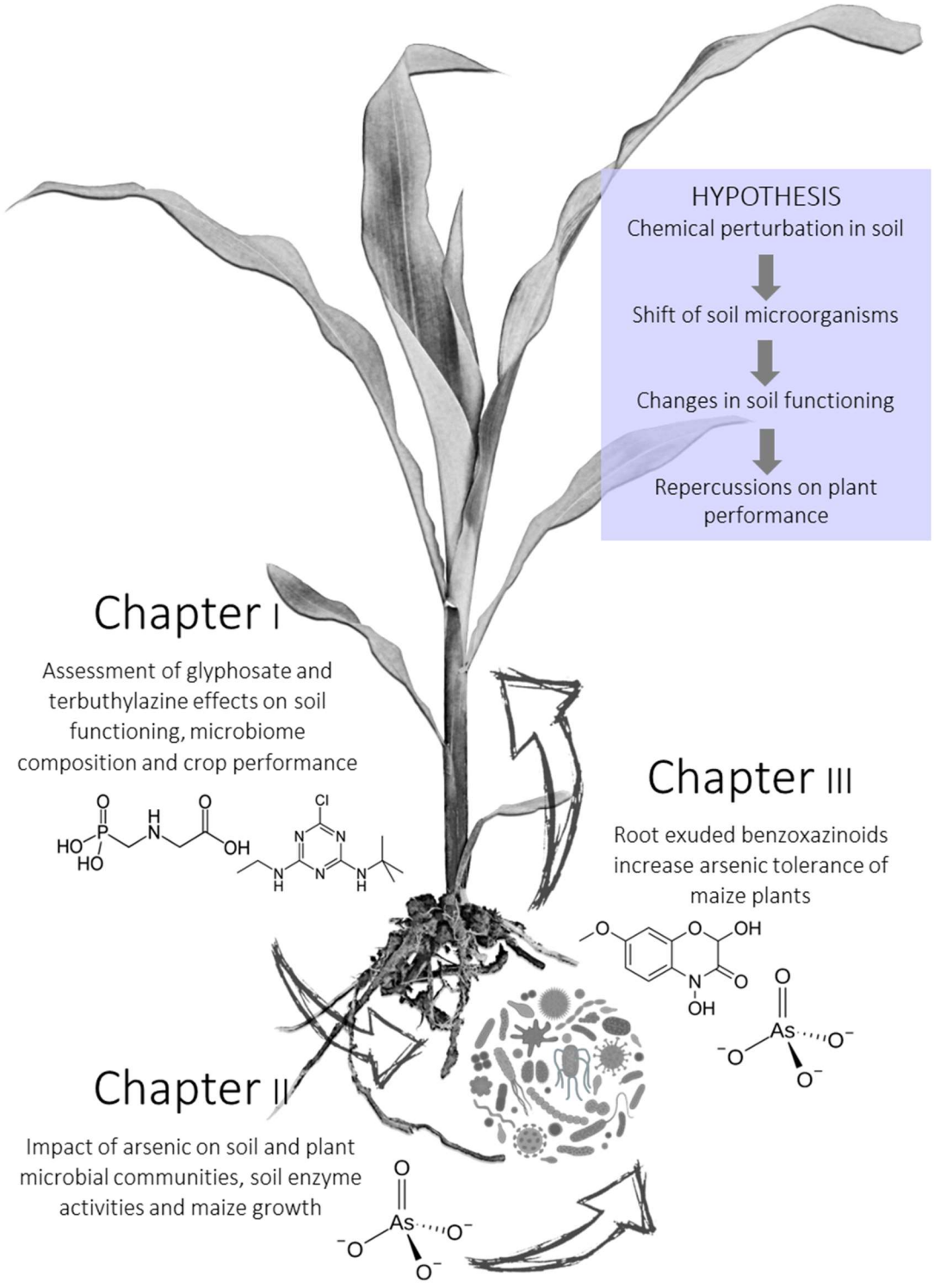


Figure 2. Within the One Health project, here is a visual summary of the thesis outline divided in the three chapters focusing on the soil-plant compartments. The thesis is based on the observation of the effects of herbicides (Chapter I) and arsenic (Chapter II) on soil microbiomes and functioning as well as the effect on plant performance, and we integrated the effects of both arsenic and benzoxazinoids in the soil-plant systems (chapter III). The hypothesis of the cascading effects of chemical perturbation in soil within the One Health concept is depicted in the violet box.

Chapter I

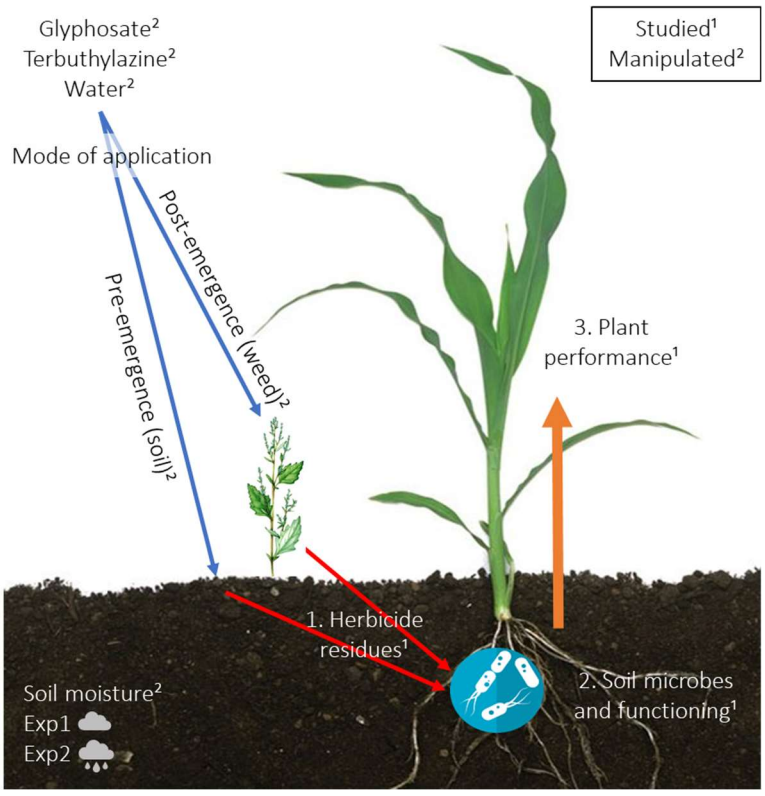
Assessment of glyphosate and terbuthylazine effects on soil functioning, microbiome composition and crop performance

Veronica Caggìa[1], Jan Waelchli[2], Aurea Chiaia-Hernandez[3], Lutz Weihermüller[5], Martin Grosjean[3], Sandra Spielvogel[4,5] and Klaus Schlaeppi[1,2]

[1] Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland
[2] Department of Environmental Sciences, University of Basel, Bernoullistrasse 32, 4056 Basel, Switzerland
[3] Institute of Geography, University of Bern, Hallerstrasse 12, CH-3012 Bern, Switzerland
[4] Institute of Plant Nutrition and Soil Science, Christian-Albrechts-Universität, Hermann-Rodewald-Strasse 2, D-24118 Kiel, Germany
[5] Institute of Bio- and Geosciences, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany

Abstract

Herbicides are widely used for weed's control in agriculture, though their fate and impact on non-target organisms like soil microbes and their functioning remain relatively unknown. A further complication is that herbicide effects vary depending on how they are applied and due to abiotic conditions like soil moisture. In this study we tested the hypothesis that glyphosate and terbuthylazine application impact the soil microbial communities and their functioning to a stronger degree when they are directly sprayed onto soil and when soil moisture is high. In our experiments, we measured a high context dependency of glyphosate and terbuthylazine bioavailability in soil and found evidence for rapid metabolization at high soil moisture. We found that the soil bacterial rather than the fungal community was mainly affected by a single application of the two tested herbicides of contrasting chemistry and activity. The identified shifts in community composition were independent of the modes of herbicide application (directly on soil vs spray on a weed) but strongly dependent on soil moisture. We further found that herbicide applications only had a small impact on soil functioning, which was approximated with analyses of soil enzyme activities. Finally, we also assessed the post-application performance of the subsequent crop and found that the herbicides did not affect maize height, chlorophyll content and biomass. Overall, our study revealed that a single application of herbicides in recommended doses had minor effects on the soil microbiome with a high temporal and soil moisture dependency. The latter finding points to the key research need of solving the context-dependency of rapid herbicide degradation in soil to avoid repercussions on non-target organisms and soil functioning.



Keywords: glyphosate and terbuthylazine, enzymes activity, soil microbiome, maize performance, pre- and post-emergence herbicide application.

Introduction

The application of pesticides, including herbicides to fight weeds, has been a major practice introduced with the intensification of agriculture with the green revolution. Weed management is crucial to ensure crop productivity, since weeds are the most important biotic constraint to agricultural production worldwide (Chauhan, 2020). In fact, weeds take up 30-40% of the applied nutrients from soil, thereby, competing with the crops for the consumption of the applied fertilizer (Retta et al., 1991). Technically, herbicides are beneficial for crop production, albeit they can also pose negative effects to the environment or the health of the consumer. Herbicides differ from each other due to their modes of action and how they are applied, whether as pre- or post-emergence treatments. Therefore, it is expected that herbicides impact non-targeted organisms differently and at different magnitudes (Helander et al., 2018; Qi et al., 2020; Thiour-Mauprivez et al., 2019).

Glyphosate (N-(phosphonomethyl)glycine) is a commonly deployed, non-selective broad-spectrum herbicide that is widely used since the past decades as the most successful herbicide in history (Duke & Powles, 2008) and with generally low negative environmental effects (Franz et al., 1997; Vereecken, 2005). It is a typical post-emergence herbicide and is also applied after planting glyphosate-resistant crops (Van Bruggen et al., 2018). Glyphosate is taken up by the leaves (only little by roots) and transported to the entire plant, causing tissue death (Kafarski et al., 1988). The translocation of glyphosate from leaves to plant roots is also responsible for its residues in soil (Laitinen et al., 2007). Glyphosate inhibits the plant enzyme 5-enolpyruvylshikimic-3-phosphate synthase, which causes a reduction in the biosynthesis of aromatic amino acids (tryptophan, tyrosine and phenylalanine) and the accumulation of shikimic acid and hydroxybenzoic acids (Becerril et al., 1989; Hernandez et al., 1999; Lydon & Duke, 1988). Glyphosate has a relatively short half-life in soil and water of ~30 days in temperate climate (Duke, 2020). Soil microbes convert glyphosate into aminomethylphosphonic acid (AMPA), which is more persistent than glyphosate with an average half-life of 151 days, ranging from 76 to 240 depending on the soil and environmental conditions (Duke, 2020; Maggi et al., 2020; University of Hertfordshire, 2013). Due to its ubiquitous and massive application, glyphosate is always at the centre of controversial discussions (Meftaul et al., 2020; The Swiss Federal Council, 2021) and a consequence of the continuous application over the past decades, glyphosate-resistant weeds have emerged worldwide (Heap, 2011; Powles & Yu, 2010). The main concern, however, is about possible consumer health and environmental impacts of glyphosate and AMPA. Contaminations in soil, water and some food products have been found at concentrations that may pose toxicological and ecological risks (Bai & Ogbourne, 2016). Residues were found in different plant and animal materials, suggesting a possible accumulation through the food chain (Druart et al., 2011; Reddy et al., 2004). However, there is a general agreement that dietary exposure of glyphosate is well below the critical amounts of causing harm (Vicini et al., 2021). Still the International Agency for Research on Cancer classifies glyphosate as ‘probably carcinogenic to humans’, based on limited research on humans but sufficient evidence from animal research (Guyton et al., 2015).

Terbuthylazine (2-tert-butylamino-4-chlor-6-ethylamino-1,3,5-triazin) is also a broad-spectrum herbicide that is directly applied onto soil and is then mainly taken up by roots. Its mode of action is to inhibit the Hill reaction and CO_2 sorption in the photosynthesis (Linan, 2009). Chemically, it belongs to the chlorotriazine group of herbicides, of which atrazine and simazine have been banned for use due to environmental concerns by now in most countries. While terbuthylazine also works after the emergence of weeds, it is often used for pre-emergence treatments as for instance in maize cultivation. Concerns about terbuthylazine safety are growing because of its toxicity at low doses (Brumovský et al., 2017). Terbuthylazine half-life is of ~70 days, but can vary from 5 to 115 days depending on soil characteristics, including soil moisture, and temperature (Chiaia-Hernandez et al., 2017; Curran, 2001; Dolaptsoglou et al., 2007; Navarro et al., 2004) and its long-term risk on non-target organisms such as mammals, plants, invertebrates and aquatic organisms (Bottoni et al., 2013; López-Roldán et al., 2013; Palma et al., 2014; Tsaboula et al., 2016). It has been proposed that terbuthylazine should be classified in the carcinogen category 3 by the European Food Safety Authority (Bottoni et al., 2013).

Environmental impact assessments of herbicides are often based on their chemical properties (degradation and persistence in soil) and in vitro toxicological studies, whereas much less is known about their effects on soil microorganisms and functioning. The use of herbicides increased concerns regarding their non-target effects on soil microorganisms (Allegrini et al., 2017; Borowik et al., 2017; Lancaster et al., 2010; Stark & Banks, 2003; Wyszkowska et al., 2016). It is critical to understand the effects of herbicides on the soil microbiome because soils provide a wide range of key ecosystem functions. Applications of herbicides can affect the structure of soil microbiomes. Taxonomically, Lancaster et al. (2010) and Ramirez-Villacis et al. (2020) showed a higher abundance of Burkholderia and both Burkholderia and Firmicutes, respectively, following glyphosate application. Other soil microorganisms, such as arbuscular mycorrhizal fungi (AMF), were negatively impacted by glyphosate application on both target and non-target plants (Helander et al., 2018). The same studies reported an overall negative impact of terbuthylazine on the soil microbiome members such as Azotobacter, actinomycetes and fungi. Comparing the effects of glyphosate and terbuthylazine on rhizosphere microbes, it was shown that both herbicides affected the bacterial community, but that glyphosate was considered to have a lower effect size (Barriuso et al., 2010).

Alterations in microbiome composition can lead to changes in the soil functioning such as changes in activities of soil enzymes. Soil enzymes play an important role in maintaining the agricultural quality of soils and nutrient cycling (Kandeler et al., 1999; Sinsabaugh et al., 2002). The response of soil enzymes to anthropogenic factors, such as herbicide application, is more rapid compared to other soil variables. Hence, the activities of soil enzymes have been considered as one of the earliest and most sensitive indicators to evaluate soil quality (Ladd, 1985; Miller & Dick, 1995; Theriot et al., 2013). Enzymes in soil are responsible for nearly any biochemical cycles, including important transformation in the carbon (C), nitrogen (N) and phosphorus (P) cycles (Aon et al., 2001; Wallenstein & Burns, 2015). For that reason, it is important to assess the effects of

environmental chemicals on soil enzymatic activity to infer the potential hazard of the substances in agricultural systems. For instance, repeated applications of glyphosate resulted in a shift in the composition of bacterial groups involved in key processes for C and N cycling in soil (Allegrini et al., 2017). Another study working directly on soil enzyme activities, found out that in presence of Gly both β-glucosidase and phosphatase activities were reduced with time compared with the control soil (Tejada, 2009). However, it has also been shown that glyphosate can enhance glucosidase and N-β-acetylglucosaminidase activities, but this depended on the soil type and herbicide doses (Nguyen et al., 2018). Similar to glyphosate, also terbuthylazine (combined with other herbicides) was found to negatively affect soil functioning being an inhibitor of soil enzymes such as dehydrogenases, catalase, urease, β-glucosidase, arylsulfatase and phosphatases (Borowik et al., 2017; Wyszkowska et al., 2016). In another study, it was shown that atrazine was the only one, among the selected herbicides, that induced a reduction in the activity of N-β-acetylglucosaminidase in the field that was statistically relevant (Rose et al., 2018). Our study will compared Gly and Tb in their effects on soil enzyme activities taking into consideration the mode of application (pre- or post-emergence) and soil moisture as possible factor influencing their bioavailability.

Besides the desired weed-killing properties of herbicides, some products also have an impact on crop performance. Glyphosate residues were detected both in crops and weeds throughout the season following herbicide application (Helander et al., 2018). Glyphosate was found to interfere with plant resistance by enhancing susceptibility to below- and aboveground pathogens (Johal & Huber, 2009) and to negatively impact the attraction of beneficial insects (Fuchs et al., 2021), both representing essential elements for the management of healthy agricultural systems. Finally, it has also been proven that increased glyphosate rates resulted in decreased nutrient accumulation, nodulation, roots and shoots biomass (Velini et al., 2008; Zobiole et al., 2011; Zobiole et al., 2010). Opposite to negative effects on plant growth, plants may also benefit directly from the application of herbicides. Hormesis, a dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition, is known for glyphosate applications (Brito et al., 2018; Ramirez-Villacis, 2020; Velini et al., 2008). In contrast with the known hormesis effect of Gly, several studies showed growth inhibition of the non-target crop by terbuthylazine application (Borowik et al., 2017; Wyszkowska et al., 2016), as well as reduced efficiency of the photosynthesis (Cañero et al., 2011). Other negative effects than on plant growth include reduced chlorophyll content, and lower concentration of iron in maize's roots (Bartucca et al., 2017, 2018). Other studies showed no phytotoxicity on maize plants after terbuthylazine treatment (Skrzypczak & Waniorek, 2007), in fact Tb is known to be rapidly metabolized in maize (Anastassiadou et al., 2020). Today, we still have a limited understanding whether such herbicide feedbacks on crop performance are direct effects or mediated through the soil microbiome.

Therefore, in this study we addressed the aforementioned research gaps by investigating the effects of herbicides - glyphosate and terbuthylazine - on soil functioning and feedbacks on crop performance. We tested

the hypothesis that herbicide application will change the soil microbiome composition and reduces soil functioning. We further asked whether such changes provoke feedbacks on the growth of non-target crops. We also addressed two technical questions related to the application of herbicides: first, we compared two modes of application, by applying the herbicides directly onto bare soil or by spraying it on a weed, corresponding to the usage of pre- and post-emergence herbicides. We expected the application on bare soil to have a bigger impact compared to when it reaches the soil only after spraying a weed. Second, we were interested to understand the role of soil moisture content on herbicide bioavailability and its consequences on the soil microbiome. We performed two experiments differentiated by a flush of water after herbicide treatment (i.e., simulating a heavy rainfall after application) otherwise maintained at the same soil moisture level (Experiment 1: no flush; Experiment 2: flush). We suspected greater herbicide effects close to water saturation. Time after herbicide application was also taken into consideration for analysis.

We conceived a study-system using controlled conditions in the glasshouse, whereby we filled pots with agricultural soil and planted them with the weed *Chenopodium album* or left them empty (Fig. 1). We performed two large experiments (one with, one without water flush) where we sprayed either onto bare soil or *C. album* the herbicides alongside a water control. Two weeks after the herbicide application, we planted maize in these pots to test for feedbacks on crop performance. Herbicide impacts on soil functioning were assessed based on soil enzyme activities and soil microbiome composition (profiling the bacteria and fungi with 16S rRNA gene and internal transcribed spacer (ITS) sequencing, respectively). Plant performance was documented with height, chlorophyll content and aboveground dry biomass. Our results reveal that a single application of both herbicides can change soil functioning and soil microbial composition but no effect on the subsequent crop; in addition, most of the observed changes were temporal- and context-dependent.

Material and methods

Greenhouse experiments

Soil: We performed these experiments with the Ap horizon of a soil under agricultural use from 'Q-Matte' in Frauenkappelen (46°57'20.5''N, 7°19'58.3''E), Switzerland. Main soil types in this area are Haplic Cambisols and Haplic Luvisols (IUSS Working Group WRB, 2022). The soil had a silty loam texture and was chosen, because our pre-tests revealed it to be free from glyphosate and terbuthylazine and their residues. Characteristics of the Q-matte soil are the following: texture (sand 37±3%, silt 53±2% and clay 10.2±0.8%) and micronutrients (plant available phosphorus 2.41±0.02 mg/kg, total carbon 26.49±0.07 g/kg, total organic carbon 25.41±0.09 g/kg, nitrogen 2.91±0.01 g/kg, sulphur 0.35±0.03 g/kg) and pH 6.6. The texture of the soils was analyzed by laser diffraction (Mastersizer 2000, Malvern Instruments GmbH, Herrenberg, Germany) after destruction of organic matter by boiling in H_2O_2 (35%) and dispersion of the sample ($(NaPO_3)12$-13.Na_2O and Na_2CO_3, shaking overnight). Soil was passed to 1 cm sieve to remove large stones and mixed thoroughly with 20% of autoclaved sand (Sand "CAPITO" 1-4 mm, 25 kg, LANDI Switzerland AG) before filling it into pots ("Rosentopf Soparco 2.0 L"; Hortima AG, Switzerland). Each pot had an underpot ("Untersetzer mit flachem Rand 16 cm"; Hortima AG, Switzerland) to avoid cross-contamination during watering. After filling the pots, the soil was left to rest one week under greenhouse conditions (light/dark 14h:10h, day 14°C-22°C (temperature limits), night 10°C-14°C, humidity 50%-70% (limits)). Soil moisture was kept at 60% water holding capacity during the entire duration of both experiments, by weighing the pots and adding the missing water. Both experiments were set up and analysed with the same protocols with the only difference of higher water holding capacity. In Experiment 1, we kept the water holding capacity constant at 60%. On the contrary, in Experiment 2 a water flush transiently increased the soil water content to 100% after herbicide application and we let it go back to pre-treatment levels of 60% (around 3 weeks). After its return to 60%, the Experiment 2 was continued like Experiment 1 except that it was stopped two weeks later because the maize plants were growing less in the higher soil moisture conditions.

Experimental approach: We developed an experimental approach to compare effects of herbicides and their modes of application, either as pre-emergence herbicide applied directly on soil or as post-emergence herbicide applied on the pre-planted weed *Chenopodium album,* which is one of the more robust and competitive weeds, widely distributed, capable of producing remarkable crop losses (CABI, 2021)(**Fig. 1, Fig. S1**). We performed two experiments: one with and one without water flush after the herbicide treatments, which should simulate a heavy rainfall event after herbicide application (respectively 7 and 4 replicates). Identical setups were used for the two experiments, and they were maintained at the same water holding capacity of 60%, except that soil moisture transiently increased to saturation (100% WHC) and was left to go back to 60% in Experiment 2 (Experiment 1: constant 60% soil moisture, no water flush). Sampling at 2 and 5

weeks after herbicide treatments, we assessed soil enzyme activities and microbiome composition and we tested for feedbacks on maize performance with the same analyses.

Plant growth: After 1 week of pre-incubation of the soil, two *Chenopodium album* (Herbiseed, United Kingdom) seedlings were transplanted into half of the experimental pots, i.e., the 'Weed' pots for the later post-emergence herbicide application (**Fig. 1**). For this, *C. album* seeds were germinated and pre-grown for two weeks in the greenhouse using the same conditions as detailed above. The pots with transplanted *C. album* were randomly positioned in the greenhouse alternating with the pots that were already filled with soil, i.e., the 'Soil' pots intended for the later pre-emergence treatment (herbicide applied directly on soil, **Fig. 1**). Planted and non-planted pots were maintained at constant soil moisture of 60% in the greenhouse for two more weeks (allowing *C. album* to grow) using the same conditions as detailed above. The timepoint of herbicide application included treatments with glyphosate, terbuthylazine or water for the controls (described below, **Fig. S1**). One week after the herbicide application, all *C. album* plants in all treatments were cut with scissors and their plant material was left on the pots. This step was necessary for an even carbon-input balance in all pots, in particular for the water-control treatment where *C. album* plants would have continued to grow. Another week later, we planted seeds of *Zea mays L.* (inbred line W22) (Tzin et al., 2015) into the pots. Seeds were surface sterilized by soaking them in commercial bleach containing 5 % active hypochlorite (Potz Javel-Wasser Natur, Migros, Switzerland) for 6 minutes, mixing from time to time. Afterwards, bleach was removed, and seeds were washed 5-6 times with autoclaved MilliQ water. For pre-germination, seeds were soaked in autoclaved MilliQ water in the dark for 8h. After 8h, the seeds were placed on a moist filter paper (Rundfilter Sorte 1 Whatman, 90mm, Huberlab, Switzerland) in plastic Petri dishes (Petri dish 94x16mm, without vents, sterile, Greiner Bio-One, Switzerland) overnight, before sowing them. Pots were manually randomized every week. Maize plants were fertilized weekly from week 2 onwards with a commercial fertilizer 0.2% (NPK, w/v; Plantaktiv Typ K, Hauert, Switzerland). At week 2 and 3, plants were fertilized with 100 ml and from week 4 onwards with 200 ml. The soil moisture content was measured in every pot every second day and water loss was compensated with tap water to maintain the pots constantly at 60% water holding capacity. Maize plants were phenotyped weekly (see below) and left to grow for 8 and 6 weeks for Experiment 1 and Experiment 2, respectively.

Application of herbicides: We worked in this study with agronomically relevant formulations and dosages of the two herbicides in accordance with the recommendations, which are within the range of the doses applied in agricultural fields in Switzerland. We utilized the commercial products Aspect® (Bayer, 29 % (333 g L^{-1}) terbuthylazine and 17.4 % (200 g L^{-1}) flufenacet) and Glyfos Best (Bayer, 40-50% (360 g L^{-1}) glyphosate present

as isopropylamine salt and 2,2,2-butoxyethosx-ethyxyethanol (1-2%) and alcohol, C8-C10 ethoxylate). For simplicity, we refer to the main herbicide ingredient of these formulations throughout this study. The terbuthylazine and glyphosate formulations were applied at a dose rate of 10 and 390 L ha^{-1} respectively. Both products were diluted with tap water to reach their 'spray' concentrations (terbuthylazine = 1.5 L ha^{-1} and glyphosate = 10 L ha^{-1}) and a volume of 0.6 mL was sprayed onto pots (surface area 153.9 cm^2) under a chemical hood. The herbicides were manually sprayed using amber glass bottle dispenser (Pumpzerstäuberfläschchen 50 mL braun, Gerber Instruments AG, Effretikon, Switzerland). Control pots were sprayed with 0.6 mL of tap water. We applied the herbicides on one half of the pots directly on soil (i.e., as the 'pre-emergence' treatment) and in the other half of pots (pots with *C. album* plants growing), we sprayed the herbicides on the weed (i.e., as the 'post-emergence' treatment).

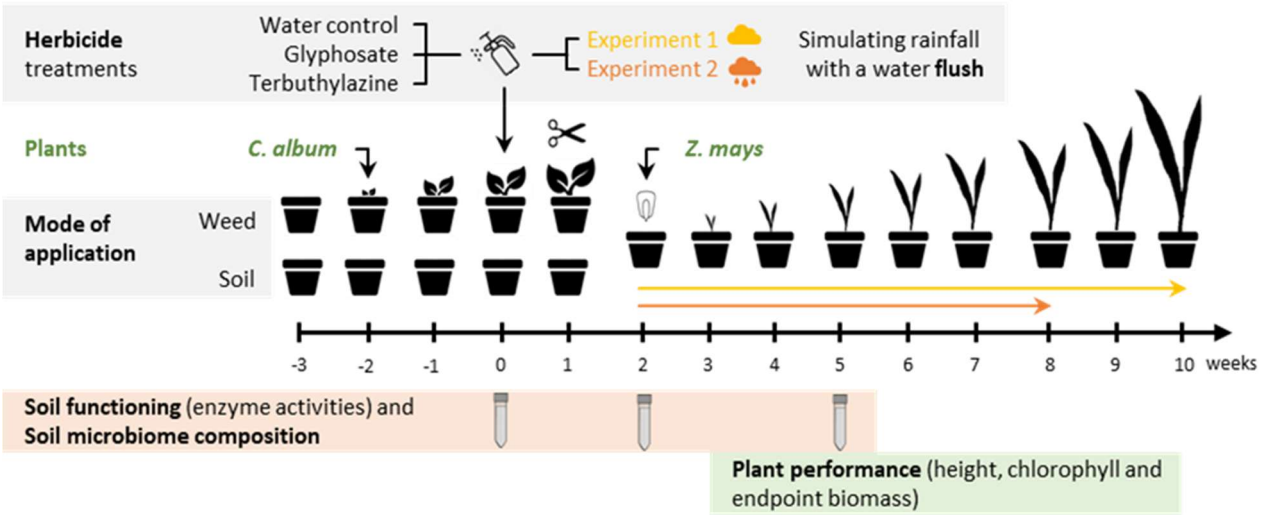


Figure 1. Experimental overview. Experimental setup consisted of three herbicide treatments (control, glyphosate and terbuthylazine) sprayed on both *C. album* and bare soil (n = 7). One week after *C. album* was treated with herbicides or water, the weed was cut and left in the pot. Experiments started with filling soil to pots three weeks before treatments. Soil was left to rest one week in the pot to let the microbiome get used to the new environment. *Chenopodium album* was transplanted in the pots and left to grow for two weeks. After that time, glyphosate and terbuthylazine were sprayed on *C. album* plants or on bare soil (week 0). Water was sprayed on the control pots. Sprayed and control plants were cut and left in the pots for another week. At week 2, maize seeds were sown in the pots and left to grow for 8 weeks in Experiment 1, and 6 weeks in Experiment 2. The two experiments differ in soil water content, where Experiment 1 is constant in water content through time (no flush) and in Experiment 2 it was increased with a flush of water, and later restored as in Experiment 1.

Analyses and measurements

Plant phenotyping and soil sampling: From the second week after sowing maize onwards, plant height and chlorophyll content were measured weekly. Height was determined by stretching the leaves and measuring the highest point relative to the soil surface of the pot using a foldable rule. Chlorophyll content was measured with a SPAD meter (Chlorophyll meter SPAD-502, Minolta Camera CO., LTD., Japan). The average of nine measurements (three at the base, three in the middle and three on the tip of the leaf) was taken as chlorophyll content value. At the end of the experiment, the shoot biomass was cut directly above the soil, dried at 70°C for 72h in an oven (UF 1060 Plus, Memmert Experts in Thermostatics, Hettich Laborapparate, Switzerland) and weighed. Soil was sampled before herbicide treatments (timepoint 0, **Fig. 1**), before maize sowing (timepoint 2 weeks) and during maize growth (timepoint 5 weeks) with a custom-made soil sampler (diameter: 0.8 cm) and corresponding to the soil depth between -5 to -15 cm from the soil surface. Each soil sample was thoroughly mixed after collection and then split for the different analyses (microbiome, enzymes activity, herbicide residues). Samples were stored as follows until analysis: microbiome samples at -80°C, enzymes activity samples at 4°C (less than 24h) and samples for herbicides residues at -20°C.

Activities of soil enzymes: We measured the activities of the extracellular soil enzymes using fluorogenic methylumbelliferone (MUF) based substrates (Marx et al., 2001). We employed the MUF-derivates 4-MUF N-acetyl-β-D-glucosaminide, 4-MUF β-D-glucopyranoside and 4-MUF phosphate disodium salt as substrates for the enzymes N-β-acetylglucosaminidase, β-glucosidase and acid phosphatase, respectively. The 10 mM stock solutions were prepared by dissolving the substrates in 300 µL dimethyl-sulfoxide and sterile water to make a final volume of 10 mL. The assays were based on 500 mg of soil, weighed into autoclaved 100 mL glass jars, to which 50 mL sterile water was added and then blended with a polytron (PT 1200 CL, Kinematica AG, Littau, Switzerland, 30 sec at 4.5x 1000 rpm) (De Cesare et al., 2000). Aliquots of 50 µL were taken from the soil suspensions (constantly mixing with a magnetic stirrer in a glass petri dish) and loaded to 96-well microtiter plates (pureGrade^{TM} sterile plates, BRAND, Germany). Three technical replicates for each biological sample were used for the assay. To each reaction, 50 µL of 0.1 M 2-(N-Morpholino)ethanesulforic acid (MES hemisodium salt, pH 6.1) were added as buffer (German et al., 2011). MUF-substrate stocks were diluted with MES to 1 mM working solutions. Finally, 100 µL serial dilutions of MUF-substrate solutions (0, 10, 20, 30, 40, 50, 100, 200 µmol g^{-1}) were added to the 96-well plates containing 50 µl of sample and 50 mL of MES buffer and well mixed by shaking for 1 minute directly in the fluorescence plate reader. Fluorescence measures of the samples were taken with a plate reader (Infinite M200 Pro, Tecan GmbH, Austria; excitation 355 nm, emission 460 nm) after substrate addition (t_0) and after 2 hours (t_1). For calibration and accounting for fluorescence quenching, standard plates were prepared with 50 µL of a composite soil solution, with 150, 145, 140, 130, 100, 70 and 30 µL of MES buffer and 0, 5, 10, 20, 50, 80 and 120 µL of MUF standards. The regression

slopes served to calculate the enzymatic activities relative to these standard measurements [µmol substrate g^{-1} soil h^{-1}]. The enzyme activities were fitted to a Michaelis-Menten model, which describes nonlinear saturation curves with v = (Vmax × [S])/(Km + [S]), with Vmax as the maximal rate of enzymatic activity under optimum substrate conditions, [S] as the added substrate concentrations and Km is the substrate concentration at which half of the maximum velocity is achieved (Loeppmann et al., 2016; Marx et al., 2001; Nannipieri et al., 2012; Sinsabaugh, 2010).

Microbiome profiling: Genomic DNA was extracted from 250 mg of soil samples with the DNeasy PowerSoil HTP 96 Kit (Marotz et al., 2017) following the manufacturer's instructions and eluted in 100 µL buffer. DNA concentrations were quantified by fluorescence (AccuClear® Ultra High Sensitivity dsDNA Quantification kit, Biotium, Fremont, CA, USA) mixing 10 µL of sample (diluted 1:4) to 198 µL of DNA Quantitation buffer and 2 µL of dye per reaction. Alongside the samples, a standard curve consisting of 10 µL of each of the eight standards (0, 0.03, 0.1, 0.3, 1, 3, 10, 25 ng/µl) were added three times for reference. Fluorescence was measured with the same plate reader as above after vortexing the plate, spin down and incubating it 5 min at room temperature in the dark (excitation wavelength 468 nm; emission wavelength 507 nm). After quantification, each sample was standardized to 1 ng µL-1 by dilution in autoclaved MilliQ water.

Library preparation consisted of a two-step PCR protocol of the Earth Microbiome Project (Wasimuddin et al., 2020) starting with PCR1 (reaction volume of 20 µL) containing Platinum Hot Start polymerase (0.8x; Invitrogen, Fisher Scientific, Switzerland), 200 nM of each primer (CS1-515f, ACACTGACGACATGGTTCTACAGTGYCAGCMGCCGCGGTAA and CS2-806r, TACGGTAGCAGAGACTTGGTCTGGACTACNVGGGTWTCTAAT; or CS1-ITS1f-F, ACACTGACGACATGGTTCTACACTTGGTCATTTAGAGGAAGTAA and CS2-ITS-2R, TACGGTAGCAGAGACTTGGTCTGCTGCGTTCTTCATCGATGC), 3% BSA and H_2O (PCR-grade water and UV-treated), plus 3 µl of DNA template (3 ng total amount). The PCR cycling started with a denaturation at 94°C for 3 min, followed by 25 cycles of denaturation at 94°C for 45'', annealing at 50°C for bacteria and 55°C for fungi for 60'', elongation at 72°C for 90'' and a final elongation at 72°C for 10 min. The PCR products were cleaned-up with magnetic beads (SPRIselect, Beckman Coulter, Brea, U.S.A.) at a 1:1 ratio of beads to DNA samples. Beads were thoroughly mixed with the samples by pipetting up and down, incubated at room temperature for 1 min and the plates were placed on a magnetic stand until the supernatant was cleared (MicroMag Separator, Kisner Biotech GmbH & Co., Germany). The supernatant was carefully removed, and the beads were washed with 180 µl freshly prepared 85% ethanol (keeping the plate on the magnetic stand).

The excess of ethanol was carefully removed with a small pipette. After removing the plate from the magnetic stand, 20 µl of Tris (pH 8.0) was used to resuspend the beads by pipetting up and down. The plate was

incubated 1 min at room temperature and then placed on the magnetic stand until the supernatant was cleared. Finally, the supernatant was transferred, avoiding contamination by beads, to a new plate for the second PCR.

The reaction volume of PCR2 was 25 µl and contained 5 µl of template of the bead-purified DNA, 5 µl of PCR-grade water, 10 µl of PCR master mix (2x) and 5 µl of primers 400 nM (Access Array™ Barcode Library, Fluidigm, San Francisco, U.S.A.). We utilized the unidirectional Access Array Barcode system with the PCR primers PE1-CS1-F and PE2-[BC]-CS2-R to prepare a ready-to-load library. These primers contain the paired-end (PE) adapters required for Illumina sequencing, the linker sequences CS1 and CS2 to bind to the amplicons of PCR1 and one of 384 10-mer barcodes (BC). Cycling of PCR2 consisted of an initial denaturation at 94°C for 3 min, followed by 10 cycles of denaturation at 94°C for 45'', annealing at 60°C for bacteria and 55°C for fungi for 60'', elongation at 72°C for 90'' and a final elongation at 72°C for 10 min. Negative control samples were include in each PCR assay and both PCR1 and PCR2 reactions were verified on a 1.5% agarose gel to lack contamination. After PCR2, the clean-up with SPRIselect beads was repeated. Finally, an equimolar pool of all samples was done, purified again with SPRIselect beads and fluorometrically quantified (Qubit™, Thermo Fisher Scientific, Switzerland). All 188 bacteria and 188 fungi were combined at a ratio of 19:1 (to obtain more bacterial reads) in one library. The library was then sequenced (v3 chemistry, 300 bp paired end) using custom primers following the instructions of Access Array Barcode system on an Illumina MiSeq instrument at the next generation sequencing NGS platform of the University of Bern.

The sequencing centre provided us demultiplexed sequences. In a first step we used cutadapt (V2.5) (Marcel Martin, 2011) to remove primers. Then we followed the methods we had used in Gfeller et al. (unpublished) by using the package dada2 (Callahan et al., 2016) in R 3.5.1 (R Core Team, 2018). We changed the truncation lengths to the minimal lengths of 240 bp for forward read and 160 bp for reverse reads.

Analytics of terbuthylazine: Soil samples were freeze dried for 72h, homogenized with mortar and pestle, and extracted by pressurized liquid extraction using in-cell clean-up technique as previously described (Chiaia-Hernández et al., 2020). In short, 2 g of soil was weighted and transferred to 10 ml stainless steel extraction cells filled with 1 g of activated florisil (60/100 mesh, Supelco, Bellefonte, USA). In addition, 0.5 g of diatomaceous earth (Hydromatrix ISOLUTE HM-N, Biotage, Sweden) was added to increase solvent channelling and the remaining empty cell space was filled with glass beads (2 mm, Dr Grogg Chemie AG, Switzerland).

Soils were extracted with a mixture of ethylacetate and acetone (70:30%, v/v) in two static extraction cycles of 5 min at 80°C and a rinsing volume of 60%, each followed by 100 s of purging with N2 using a pressurized liquid extraction (PLE) using an ASE 350 system (Thermo, Sunnyvale, U.S.A). Subsequently, soil extracts were

spiked with 25 µL of a mixed solution containing 4 internal standards (triadimenazole-D4, propiconazole-D3, terbuthylazine-D5 and difeconazole-D6; each at 2.5 ng μL^{-1}) with an absolute amount of 62.5 ng of each compound. Soil extracts were narrowed to 0.5 mL with N2 using a solvent evaporator (TurboVap, Biotage, Sweden) with a bath temperature of 40°C and inlet pressure of 3 bars. The concentrated extract was filtered (0.22 µm) into HLPC glass vials and stored at -20°C until analysis.

Separation of analytes was accomplished at 35°C on a 3.0 × 2.0 mm × 3.5 µm particle size C18 security guard cartridge (Waters Corp., Milford, MA) connected to a 2.1 × 50 mm × 3.5 µm particle size XBridge C18 column (Waters Corp., Milford, MA) by injecting 10 µL of extract using 100% Milli-Q water (mobile phase A) and methanol (mobile phase B) with formic acid (0.1% v/v), 4.5 mM ammonium formate and 0.5 mM ammonium fluoride added to the eluents on an high performance liquid chromatography HPLC system (Agilent 1260 Infinity II, Santa Clara, CA). Detection and quantification of the analytes, which included terbuthylazine, 2 main transformation products (terbuthylazine-2-hydroxy and desethylterbuthylazine) and flufenacet, were carried out using a mass spectrometer (6460 Triple Quad MS system, Agilent, Santa Clara, CA) with positive electrospray ionization (JetStream technology) using dynamic multiple reaction monitoring. Limits of detection (LOD, ≥ 0.1 $\mu g/kg_{dw}$) and additional details on the separation and detection are described in detail by Chiaia-Hernández et al. 2020.

Analytics of glyphosate and AMPA: The extraction of glyphosate and AMPA was performed according to Todorovic et al. (2013) with the following modifications: First, the air-dried soil samples were mixed with 40 mM Na-tetraborat decahydrat in a ratio of 1:4 (w/v) in 50 ml polypropylene centrifuge tubes and shaken for 4 h at 250 rpm on a horizontal shaker. The amounts of soil samples varied between 2 and 9 g (≥ 2 g needed for analysis), and the buffer was adjusted accordingly.

After shaking, the mixture was centrifuged at 2'012 RCF (relative centrifugal force) (= 19.7 g) for 20 min (Allegra 6KR Centrifuge, Beckman Coulter, Germany) and 6 mL of supernatant was transferred to new 3 mL polypropylene centrifuge tubes. Therefore, the sample from the prior step had to be split. The split extracts were further centrifuged at 20'817 RCF (=204 g) for 20 min using an ultra-centrifuge (Centrifuge 5417 C, Eppendorf, Germany) to pellet smallest soil particles. After extraction, the samples from the individual replicates of the 3 mL centrifuge tubes were bulked and a subsample of 2 mL was stored at -18°C in polypropylene Eppendorf tubes until analysis.

The samples needed to be derivatized using 9-Fluorenylmethyl chloroformate (FMOC) for fluorescence detection using HPLC. We utilized the method described by Macherey-Nagel (Macherey & Nagel GmbH & Co. KG, 2017) with the following modifications: first, 1'000 µL of 2.5 mM FMOC (Alfa Aesar by Thermo Fischer Scientific, Heysham, UK; dissolved in acetonitrile) and 100 µL of a water-dissolved derivatization buffer (50 mM $Na_2B_4O_7$ x $10H_2O$, 50 mM NaCl, and 200 mM H_3BO_3) were added to 2 ml of soil extracts, after, the mixture

was adjusted to pH of 9.5 by adding 20 % NaOH. After intensive shaking for 4 hours at 250 rounds on a horizontal shaker (IKA KS260 basic, IKA-Werke, Staufen, Germany), the samples were incubated at room temperature for 30 min. Subsequently, 3 mL of ethyl acetate was added (liquid-liquid extraction), the samples were manually agitated for 1 minute and incubate at room temperature for 10 min until an aliquot of 1 mL of the water phase was collected and transferred to a 5 mL brown glass vial. Finally, the FMOC derivatization process was terminated by adding 30 µL of 37 % HCl, shaking the extracts for 30 min again at 250 rpm on a horizontal shaker and ultimately stored in the dark at +4 °C until measurement.

The efficiency of derivatization was determined relative to a five-step series dilution of labelled phosphonomethylen ^{14}C-labelled glyphosate (ANAWA, Wangen, Switzerland) provided by American Radiolabeled Chemicals, Inc. (St. Louis, MO. USA)), ranging from 1.2 to 120 µg mL^{-1}. We determined a derivatization efficiency of 81.0 ± 2.3 % of our extracts to FMOC-derivatives, which we then used to calculate total glyphosate and AMPA concentrations in the samples. Of note, also the analytical standards of glyphosate and AMPA were FMOC derivatizes using the same protocol for consistency and eliminating systematic errors. The FMOC-derivatised extracts were separated on a HPLC system (1260 Infinity II, Agilent Technologies, Germany) with following column setting: 5 µm guard column (EC 4/3 NUCLEODUR C18 Gravity, Macherey-Nagel, Germany) and a 5 µm analytical column (EC 250/3 NUCLEODUR® C18 Gravity, Macherey-Nagel, Germany). The injection volume was 50 µL and separation was achieved using 0.1 % phosphoric acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.5 mL min^{-1} with the following gradient listed in Table S1 (total length 46 min). Glyphosate, with a retention time of ca. 19.5 min, was measured with a diode array detector (part of the Infinity II HPLC system; wavelength 208 nm) and AMPA (retention time 26.5 min, wavelength 266 nm) measured. Peak integration was performed manually using the Agilent OpenLAB software Version 2.2 (Agilent Technologies, Germany). Calibration was performed externally using four different AMPA and glyphosate standards (99 % purity) obtained from Sigma-Aldrich Chemie GmbH (Munich, Germany). Calibration points for AMPA were at 0.009, 0.018, 0.18, and 1.8 µg mL^{-1} and for glyphosate at 0.0107, 0.0215, 0.215, and 2.15 µg mL^{-1}, respectively. The calibration showed a linear regression between concentration and peak area (R^2 = 1). Limit of quantification (LOQ) was set to a signal to noise ratio of 1:10 yielding a LOQ of 0.0107 µg mL^{-1} and the limit of detection (LOD) was set to a signal to noise ratio of 1:3 yielding a LOD of 0.003 µg mL^{-1}.

Statistical analyses

General: All statistical analyses were performed in R (version 4.1.2). Due to scale, we did not manipulate all the factors – *herbicides*, *modes of application, time* and *soil moisture* – in the same experiment. We prioritized to keep the factors *herbicide, modes of application* and *time* combined in the same experiments, while we studied the factor *soil moisture* over two experiments (Experiment 1 with constant soil moisture; Experiment 2 with transient soil moisture after the water flush). Therefore, we always display both experiments side-by-

side in separate graphs. For the statistics we inspected with the Shapiro-Wilk test each type of data whether it satisfied normality assumptions and we applied a data transformation (square-root, log, or rank) if necessary. Eventual transformations are indicated in the statistic tables of each data type. Following this general approach permitted to assess all data types with the same statistical test, i.e., ANOVA. Each experiment was analysed separately with the generic full-factorial model 'data ~ *herbicide* (Hc) * *mode of application* (Ap) * *time* (Ti)'.

Enzyme activities: The fluorescence data was transformed to enzymatic activity rates (pM substrate g^{-1} soil h^{-1}) first, by translating the fluorescence data to substrate amounts based on the slope of the MUF-substrate standard curves and second, by subtracting the background values (t_0, substrate addition) from the actual activity values (t_1 after 2h of assay time; $t_1 - t_0$ fluorescence). In addition, enzyme activities were adjusted to the exact sample weights, volumes of the soil suspension (50 ml) and pipetted soil suspension volumes (50 µl). The obtained enzymatic activity data was then screened for outliers within technical, biological replicates and substrate concentrations using the InterQuartile Range (IQR) approach (R-bloggers, 2021), considering values exceeding ±1.5*IQR as outliers. Each sample group (biological and technical replicates) of this filtered enzymatic activity data was then fitted to a non-linear regression model nls() following Michaelis-Menten kinetics (Marx et al., 2001) to extract enzyme parameters such as Vmax (maximal velocity). Subsequently, Vmax values of the technical replicates were averaged, and we subtracted the background soil enzymatic activities before the herbicide application (t_0) for the two time points at 2 (t_2) and 5 (t_5) weeks (t_2-t_0, t_5-t_0 enzymatic activities). Vmax values were inspected for normality and transformed as needed (transformations in **Table S3**). The only exception to the general statistic approach was the dataset for the N-β-acetylglucosaminidase of Experiment 2. Differences between sample groups were tested with ANOVA for the factors *herbicide, mode of application* and *time* (~Hc*Ap*Ti).and for pairwise differences with the TukeyHSD test.

Microbiota comparisons: Sequence yield was too low for eight bacteria profiles, and they were excluded from the analysis. We found significant differences in sequencing depths between sample groups for the fungi. Therefore, we normalized the data by rarefication because this technique efficiently removes the confounding factor of differences in sequencing depth (Weiss et al., 2017). For consistency we also rarefied the bacteria data (bacteria: 20'000; fungi: 1'000 sequences per sample). The many samples (188 bacterial profiles; 188 fungal) required us to perform the PCR reactions on two separate plates. We found a discernible plate effect in the microbiota profiles, particularly for the fungi. Therefore, we included plate-to-plate variance as a co-factor in all statistical models to account for this technical variance. Differences in beta diversity were assessed with a permutational multivariate analysis of variance (PERMANOVA, ~Hc*Ap*Ti + Plate; 999 permutations) using Bray-Curtis (BC) distances. The comparative alpha diversity analysis was performed on mean values after

rarefying the dataset 100 times and each time calculating Shannon diversity. Differences in Shannon diversity between sample groups were tested with ANOVA for the factors *herbicide, mode of application* and *time* (~Hc*Ap*Ti + Plate) and we used Tukey's post-hoc test to further test for pair-wise differences. Beta diversity was analysed based on the BC index performing (PERMANOVA, ~Hc*Ap*Ti + Plate; 999 permutations), ordination and differential abundance analyses. For differential abundance testing, in brief, edgeR fits a negative binomial generalized log-linear model to the number of reads for each ASV, conducts a likelihood ratio test for a given coefficient contrast and adjusts the family-wise error rate with a Benjamini-Hochberg correction. We report cumulative relative abundance (cRA) of certain ASV groups, this refers to summing up all ASVs of a sample group.

Plant performance: Weekly plant height and chlorophyll content measurements and shoot biomass (dry weight) at harvest were inspected for normality and transformed if needed (Table S4). Chlorophyll (even after rank transformation) was the only data without 'Shapiro-Wilk support'. Nevertheless, we utilized ANOVA for reasons of consistency in the statistical analysis throughout the entire study. Differences between *herbicide, mode of application* and *time* were tested with ANOVA (~Hc*Ap*Ti +error(SampleID)) and subsequently with TukeyHSD for pairwise tests using the R package *emmeans*. SampleID was included in the model to account for the repeated measurements of the same plant over time.

Results

Mode of application has no effect on soil herbicide contents

We verified the herbicide contents in soil just before, 2 and 5 weeks after application by measuring glyphosate, terbuthylazine and their degradation products (**Fig. 2**). The utilized soil did not have a legacy of glyphosate or terbuthylazine as we neither detected the two herbicides or their degradation products in the soil before our treatments (timepoint 0), nor in the non-treated control samples at 2 and 5 weeks of the experiment.

The glyphosate levels in soil of the first experiment were ~400 µg kg^{-1} and generally tended to be higher when applied directly to soil than onto the weed and they increased with time (**Fig. 2A**). In Experiment 2, higher amounts (~1000 µg kg^{-1}) were measured after two weeks, but they disappeared completely by 5 weeks and no major difference between the two modes of application was found. Glyphosate was the main compound measured in both experiments, while its degradation product AMPA was detected in relatively small amounts but following the same patterns as glyphosate.

The terbuthylazine measurements revealed that we primarily detected the main compound in soil alongside small amounts of Flufenacet (co-formulated ingredient, see methods) and traces of the degradation products terbuthylazine-desethyl and 2-hydroxy-terbuthylazine (**Fig. 2B**). Terbuthylazine was generally higher in the first experiment reaching maximum levels of ~65 µg kg^{-1} soil compared to the second experiment (>25 µg kg^{-1}). In the first experiment, the levels of terbuthylazine increased from 2 to 5 weeks, and we did not find a difference whether the herbicide was applied to soil or onto the weed. Compared to the first experiment, the low levels of terbuthylazine were constant when spraying the weed while a small peak of herbicide was detected at two weeks when applied directly to soil.

Taken together, these analyses confirm the presence of herbicides in the treated soils and allow us to conclude that the soil herbicide levels were largely unaffected by their modes of application. The water flush resulted in higher but transient amounts of glyphosate and lower levels of terbuthylazine (Experiment 2) while soil herbicide levels increased under constant soil moisture with time with relatively higher terbuthylazine compared to glyphosate concentrations (Experiment 1).

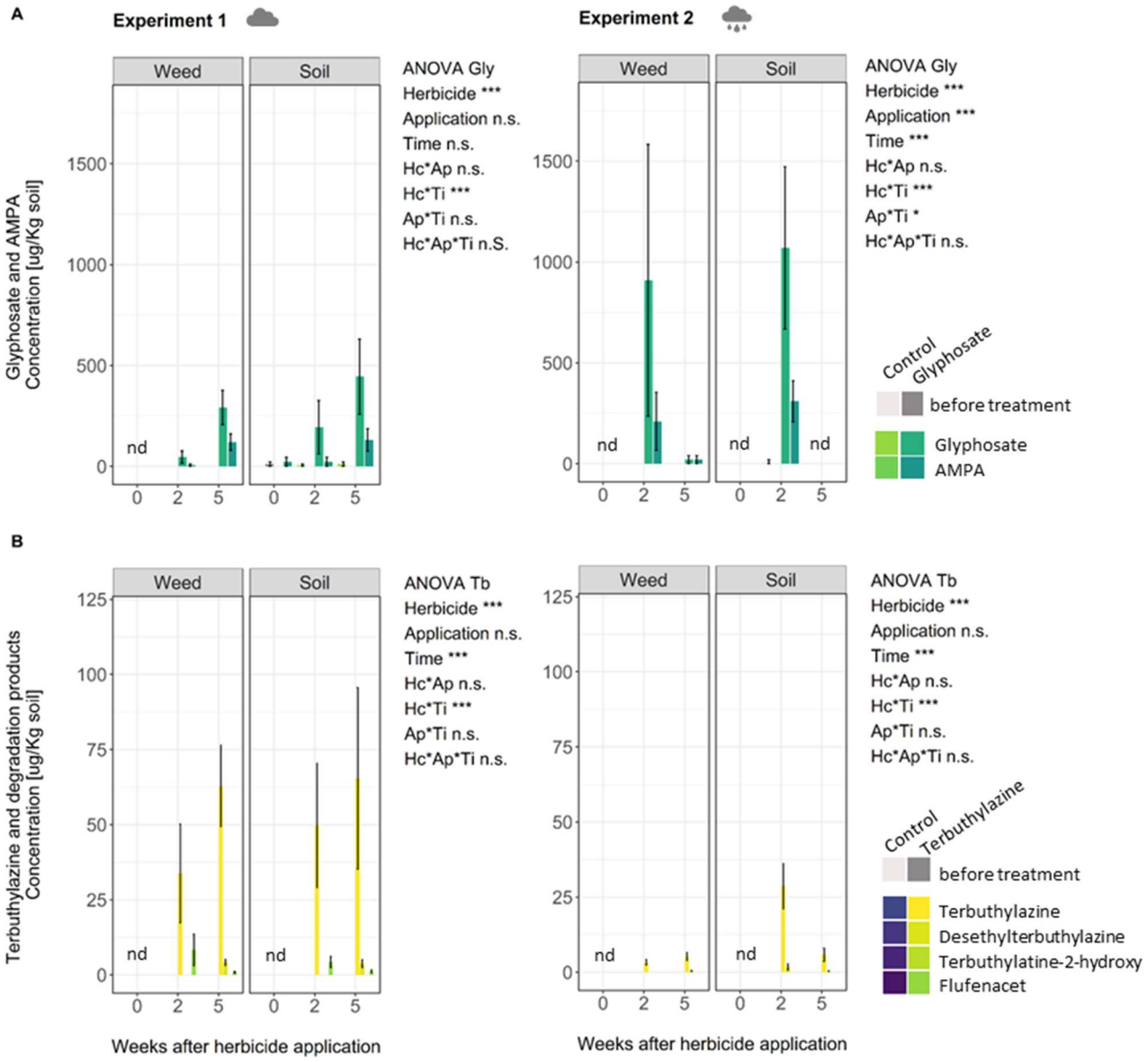


Figure 2. Herbicide levels after applying them onto bare soil or onto the weed (n = 4). (A) Quantification (in µg/Kg of soil) of glyphosate and its degradation product AMPA in soil samples from pots sprayed with glyphosate and water as control. At timepoint 0, we sampled soil before spraying to confirm that there were no herbicides residues in the soil before the experiment. Datasets were rank transformed to perform statistics. (B) Quantification of terbuthylazine (in µg/Kg of soil) and several of its degradation products in soil samples from pots sprayed with terbuthylazine and water as control. Left panels represent Experiment 1 with 60% constant water holding capacity (WHC); right panels represent Experiment 2 with 60% WHC and the flush. Nd = non-detected. P-values: "n.s." non-significant, "." marginally significant, *p <0.05, **p <0.01, ***p <0.001.

Soil microbial communities composition were not affected by herbicides, modes of application and soil moisture

To investigate the soil microbiome, we sequenced 16s rRNA gene amplicons for bacterial profiles and amplicons of the first internal transcribed spacer region for fungal profiles of all treatments of both experiments at two (i.e., at maize planting) and five weeks after applying the herbicides (**Fig. 1**). Bacterial and fungal profiles consisted of 19'462 to 97'860 (median: 41'264 sequences) and 1'048 to 6'108 (median: 1'989 sequences) high-quality sequences per sample, respectively (**Fig. S2**). Rarefaction analysis revealed that with 20'000 bacterial and 1'000 fungal sequences samples we largely captured the diversity in the microbiome samples (**Fig. S3**). Many samples were required to perform the PCR reactions on two separate plates, which resulted in a discernible plate effect in the microbiota profiles (**Fig. S4**). To account for this plate-to-plate variance, we included this factor as a co-variable in all statistical models so that effects of the experimental factors were quantified independent of technical variance. The taxonomic display at class level, indicative for the spectrum of detected taxa, revealed that the experimental soil was mainly hosting Alphaproteobacteria, Gammaproteobacteria, Verrucomicrobia and Acidobacteria (**Fig. S4A**). They were all similarly abundant and accounted together for ca. 75% of the bacterial community. The remaining 25% consisted of Bacteroidetes, Actinobacteria, Firmicutes, Deltaproteobacteria, Gemmatimonadetes, Chloroflexi, Planctomycetes, Rokubacteria and Thaumarchaeota. The fungal community was predominantly composed of Ascomycota, Mortierellomycota and Basidomycota (**Fig. S4B**). The statistical comparison did not reveal compositional differences at phylum level between the experiments, the modes of application or herbicide treatments, but the compositions changed with time (**Table S5**).

Different modes of application do not differentially affect soil microbiomes

We performed an in-depth microbiome analysis examining the impacts of the herbicides and their modes of application on alpha and beta diversity as well as on the abundance of individual taxa. We first asked whether the mode of application – spraying the herbicides on soil or onto weeds – would affect the microbiome differently. ANOVA of Shannon diversity revealed for both experiments a significant effect for the mode of application for fungal, but not bacterial communities. In Experiment 1, fungal alpha-diversity was higher when spraying the herbicides onto the weeds compared to direct application to soil while the opposite was found in Experiment 2 (**Fig. 3A, Table S6**). Mode of application had only a minor and insignificant impact on microbiome composition (beta diversity) with 1.1 to 1.9% effect size (as approximated by R2 values of PERMANOVA; **Table S7**). Consistently, only few bacterial and fungal community members varied in their abundance whether the herbicides were sprayed on soil or onto weeds (**Fig. 3B, Annexes Chapter I**). They were all very low in abundance; cumulatively they constituted only between 1 and 2% of the microbial communities and with little overlap between the two experiments. In summary, there was no consistent reaction of the microbial

communities to spraying herbicides either onto bare soil or onto the weeds. We concluded that mode of application had no effect on soil bacterial and fungal communities.

Bacterial communities are more sensitive to herbicides than fungal communities

Secondly, we investigated the effects of the herbicides on the microbiome asking how the effects of glyphosate and terbuthylazine compare to each other. In both experiments, neither the bacterial nor the fungal alpha diversity were detectably affected by the herbicides (Table S6). However, herbicides had a small, yet significant impact on microbiome composition (Table S7).

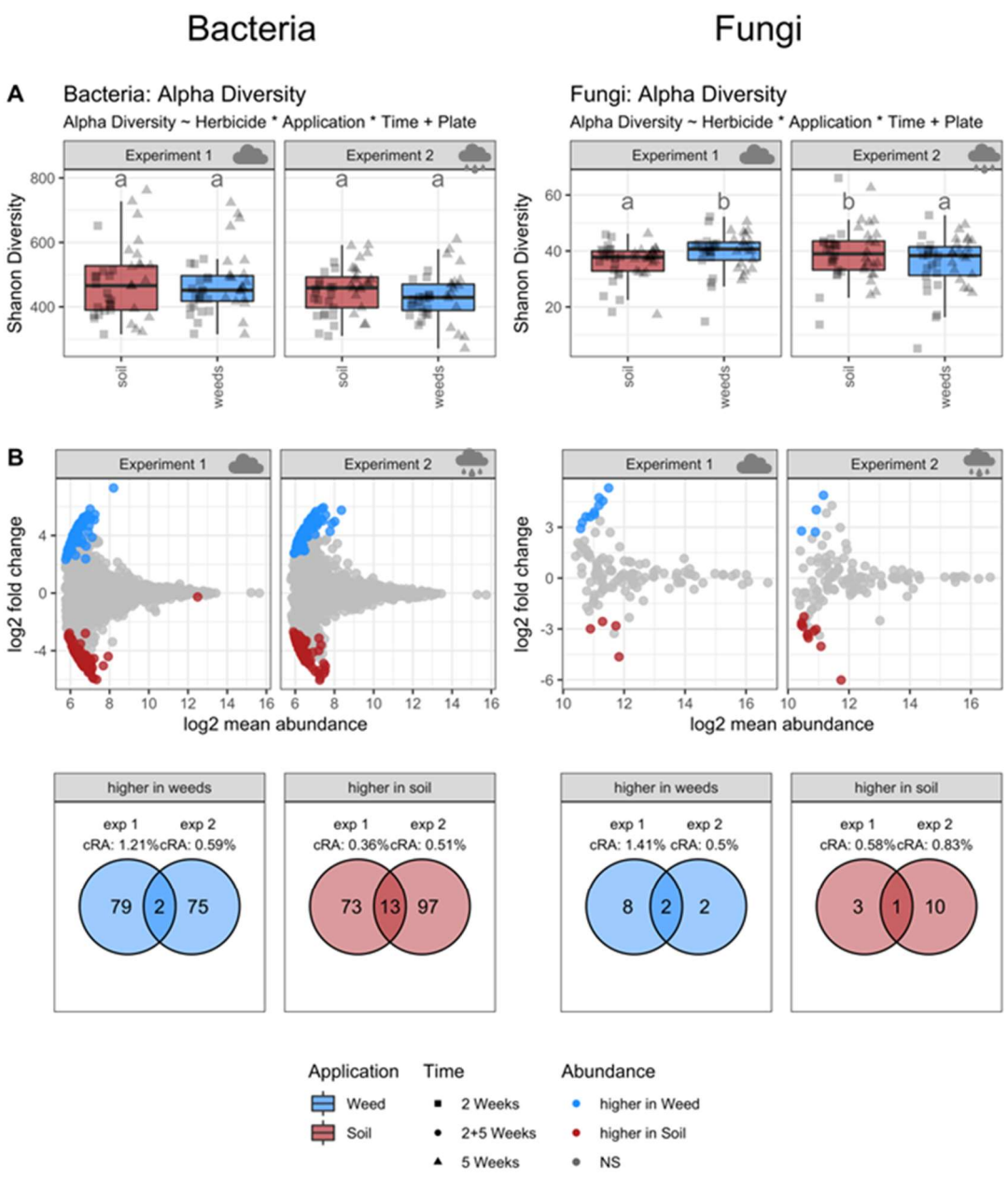


Figure 3. Alpha diversity and sensitive ASVs upon different mode of application. (A) Alpha diversity represented with the Shannon index for bacteria (left) and fungi (right) respectively. Alpha diversity is shown in two different panels for the two experiments and in different colours for the different modes of application. In red, bacterial and fungal alpha diversity when herbicides are sprayed directly on bare soil; in blue, when herbicides were applied on the weed. Different letters symbolize statistically significant differences in the alpha diversity between the treatments ($p<0.05$). (B) Sensitivity of ASVs to the different modes of application for bacteria and fungi. The panels separate bacteria (left) from fungi (right). The upper panels contain grey coloured ASVs, which are not significantly different in relative abundance between modes of application, blue coloured ASVs, which are more abundant in samples sprayed on soil and red coloured ASVs, which are more abundant in samples sprayed on weeds. The lower panels show the number of sensitive ASVs for bacteria (top) and fungi (bottom) which could either be found in Experiment 1, Experiment 2 or in both. For each experiment there is the cumulative relative abundance (cRA) of sensitive bacteria or fungi indicated in the plots.

Regardless of whether spraying glyphosate or terbuthylazine, both led to significant shifts in the bacterial beta diversity in both experiments accounting for around 3% of effect size (Fig. 4A). Besides numerous low abundant bacteria, also multiple high abundant community members reacted sensitive to the herbicides (Fig. 4B, Annexes Chapter I). With constant soil humidity (Experiment 1), 108 out of 1713 ASVs were glyphosate-sensitive, cumulatively accounting for only up to 6% of the bacterial community, while 141 out of 1633 ASVs terbuthylazine-sensitive taxa only made 1.5% (Fig. 4C). Out of these herbicide sensitive ASVs, we found 36 rather low abundant bacterial ASVs which were sensitive to both herbicides. In contrast, after a water flush (Experiment 2) we found more terbuthylazine-sensitive bacteria (108/1638; 4% cumulative abundance) compared to glyphosate-sensitive taxa (91/1575; 1.7% cumulative abundance), out of which 32 bacterial ASVs were sensitive to both herbicides. These contrasting patterns between both experiments were consistent with very little overlap of herbicide sensitive ASVs.

In contrast to the bacteria, applying glyphosate or terbuthylazine hardly provoked any shifts in fungal community in both experiments (Fig. 4A). We detected effect sizes of 1.6 to 3%, where only terbuthylazine showed a significant shift in the fungal beta diversity in Experiment 2. In both experiments, only a handful of low abundant fungi reacted sensitive to herbicide application (Fig. 5B, Annexes Chapter I) and together they constituted less than 1.6% of cumulative abundance in the fungal community without overlap between herbicides or experiments (Fig. 4C). Recapitulating the microbiome data, the application of herbicides provoked changes to the soil microbial community compositions with the bacterial communities reacting more sensitive to glyphosate and terbuthylazine than fungal communities.

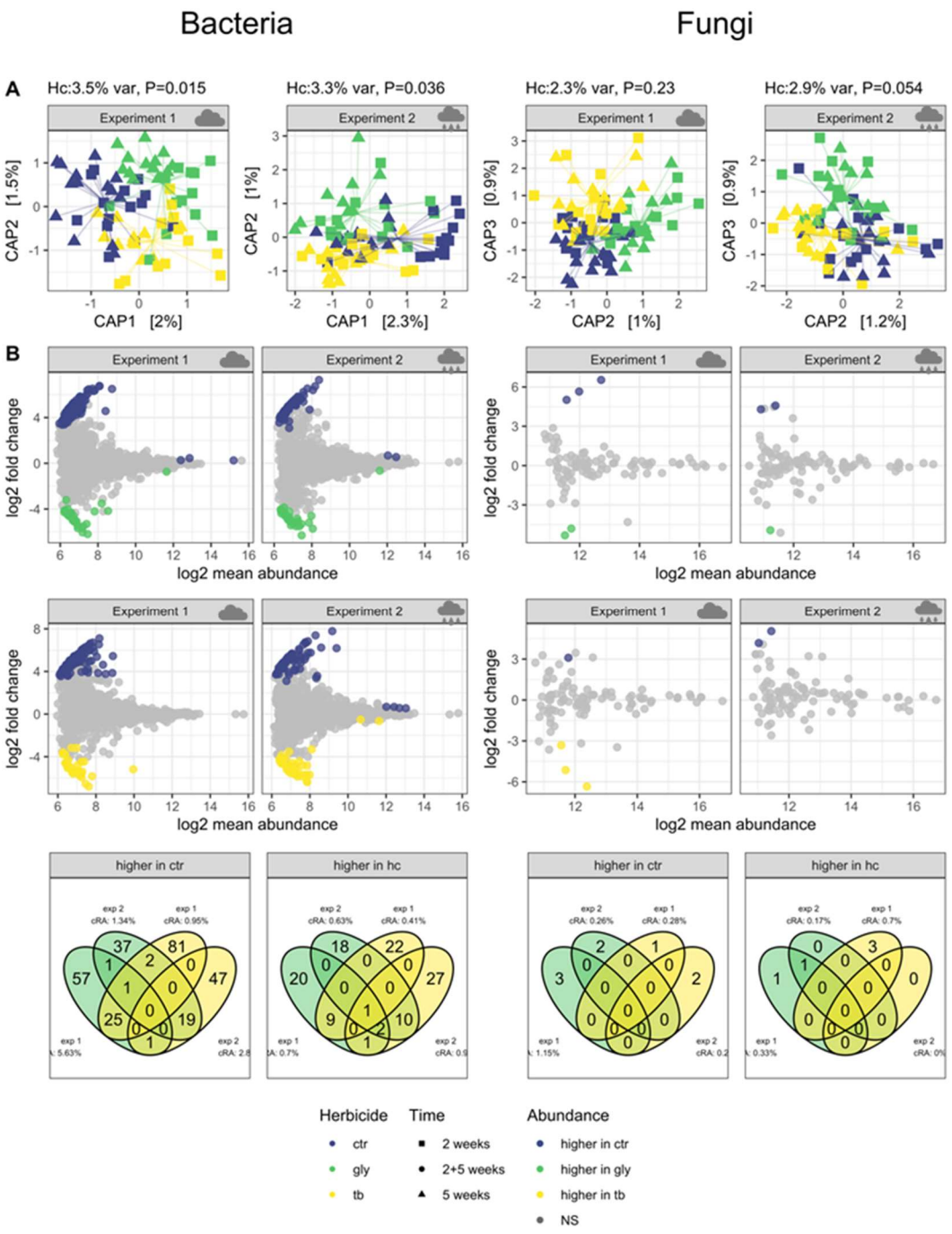


Figure 4. Constrained Analysis of Principal Coordinates and sensitive ASVs upon herbicide application. (A) CAP for bacteria (left) and fungi (right). Panels are separated by experiment (1 or 2). Soil microbiomes dissimilarities upon treatments were tested based on the following model: *Bray-Curtis-distance ~ Hc*Ap*Ti + Plate*. P-values: *p<0.05, **p<0.01, ***p<0.001. (B) Sensitive ASVs to glyphosate (top) or terbuthylazine (middle) for bacteria (left) and fungi (right). Within bacteria or fungi, the panels represent Experiment 1 (left) or Experiment 2 (right). ASV in grey do not show a response in their relative abundance between treating them with water or the herbicide. The blue coloured ASVs are more abundant in samples treated with water, green coloured AVs are more abundant in glyphosate-treated samples and yellow coloured ASVs are more abundant after treating with terbuthylazine. The lower panels show the number of sensitive ASVs between the different experiments and treatments with the corresponding cRA.

Soil enzyme activities change as a function of herbicide, mode of application and time after application

We investigated the effects of herbicides on extracellular enzyme activities for three key enzymes of soil N, P and C cycles. For these measurements we focused on substrate turnover rates, represented by the enzymes' maximal velocities (Vmax), and we quantified herbicide-triggered changes 2 and 5 weeks after application. To represent the soil N cycle, we measured the enzyme N-β-acetylglucosaminidase, also known as chitinase. In Experiment 1 we found a trend of decreasing chitinase activity with time and a lower chitinase activity when spraying the herbicides on soil (Fig. 5A). However, soil chitinase activity remained largely unaffected when applying the herbicides onto the weed, whereas it consistently decreased after direct application to soil. In the Experiment 2 with the transiently higher water content, there were no changes in chitinase activity.

Secondly, we quantified the activity of the enzyme acid phosphatase involved in P cycling in soil. In Experiment 1, acid phosphatase activity consistently decreased with time, which was unaffected by the herbicides and unaffected by the mode of application (Fig. 5B). In contrast to Experiment 1, changes in acid phosphatase activity were much less pronounced in Experiment 2 and the decreasing activities due to the herbicides were lost with time. The significant effect of mode of application is most likely due the strong decrease in enzymatic activity in the soil of weeds sprayed with the control treatment. One interpretation is that the application of the herbicides on the weeds prevents this decrease in soil acid phosphatase activity.

Finally, we determined the activity of β–glucosidase, an enzyme of the C cycle. Changes of β–glucosidase activities were in the same range in both experiments but generally decreased with time without a water flush (Experiment 1) while increasing with time with a water flush (Experiment 2; Fig. 5C). Mode of application was significant in Experiment 1 because the direct spraying of the herbicides on soil caused a transient increase of β–glucosidase activity, which was not seen when spraying both herbicides onto the weed. In Experiment 2 we noticed that the β–glucosidase activity increased with time in all treatments except when weeds were sprayed with the control treatment. This observation suggests an enhanced C-availability after herbicide application on weeds under high soil moisture levels.

In summary, we found that the changes in soil extracellular enzymatic activities mostly varied with time. Effects of glyphosate and terbuthylazine were often similar. We demonstrated that the herbicides alone and the modes of application alone did not have overall consistent effects on the activities of the three soil enzymes studied. However, in some cases we detected an herbicide effect depending on the mode of application: only when applying the herbicides on the weeds, some enzymatic activities were different compared to water-treated control plants including the reduced decreases of chitinase (Experiment 1) and acid phosphatase (Experiment 2) or enhanced increase of β–glucosidase (Experiment 2). Finally, as a tendency, the enhanced soil moisture due to the water flush resulted in fewer or opposite changes in soil enzymatic activities. Overall, these findings allow to conclude that the herbicide treatments only marginally affected soil nutrient cycling.

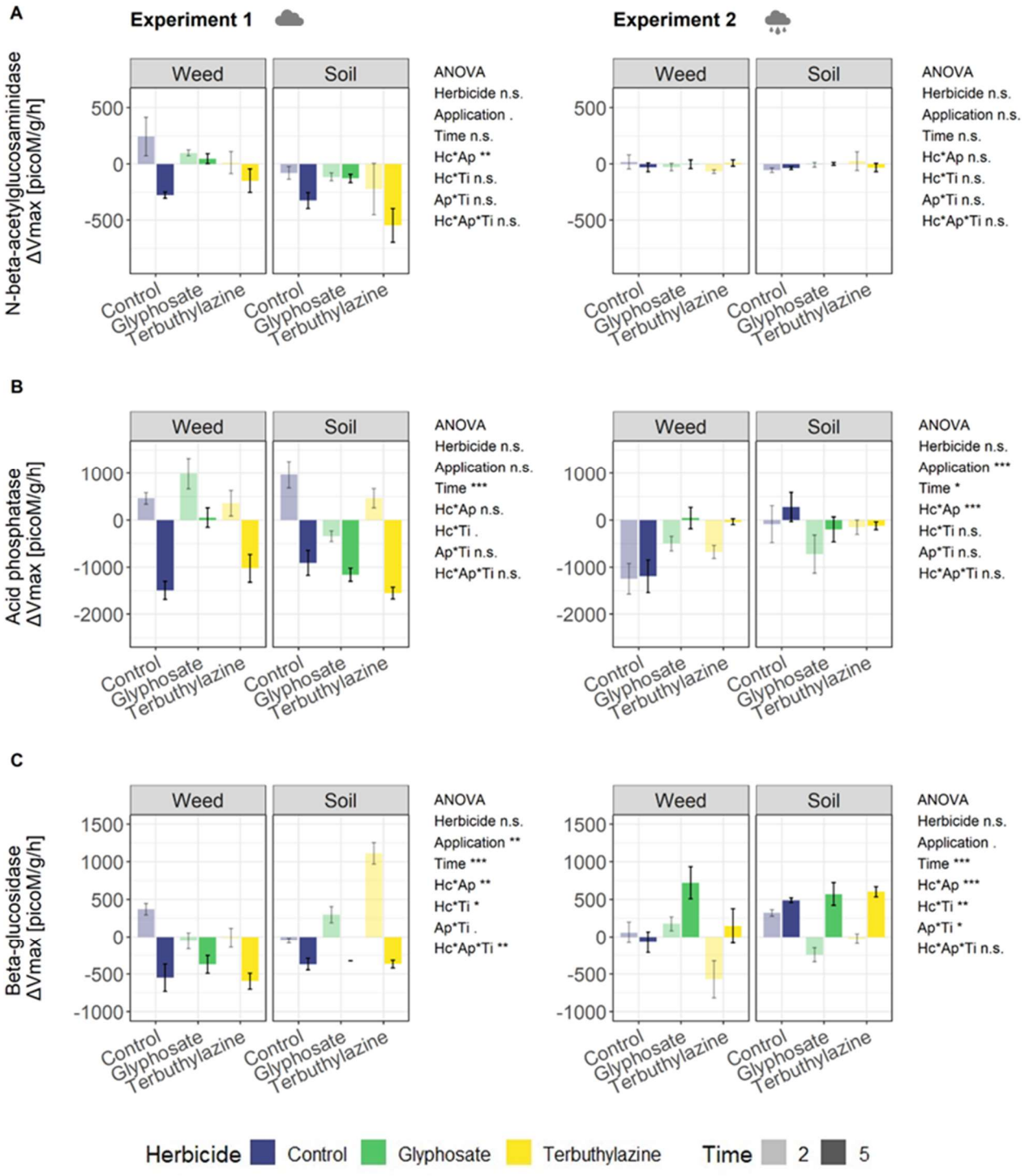


Figure 5. Activity of three selected soil enzymes in response to herbicide application (n = 4). (A) The delta of the activity between week 0 (before herbicide application) and two and five weeks after spraying (week 2 and week 5) is represented for N-β-acetylglucosaminidase (N cycle). (B) The acid phosphatase for P cycle. (C) The β-glucosidase for the C cycle. In the clear color, there is the delta between week 2 and week 0, in dark color the delta between week 5 and week 0. Left-side hand panels represent Experiment 1 and right-side panels Experiment 2. P-values: "n.s." non-significant, "." marginally significant, *p<0.05, **p<0.01, ***p<0.001. Left panels represent Experiment 1; right panels represent Experiment 2.

Herbicides and mode of application does not alter plant growth and health of the subsequent crop

The final goal of this study was to test whether herbicide applications would feedback on crop performance. To this end, we grew maize plants in pots in which we had earlier sprayed the herbicides glyphosate and terbuthylazine (**Fig. 1**). On a weekly basis we measured plant height and chlorophyll content and shoot biomass at the end of the experiments. Maize plants grew shorter after a water flush (Experiment 2) compared to Experiment 1 with constant soil moisture. In Experiments 1 and 2, plants grew significantly taller with time, but their height did not differ consistently due to herbicide treatments or their mode of application (**Fig. 6A**). In Experiment 2 the significant interaction of herbicide and their mode of application (Hc*Ap: $p = 0.002$) pointed to the reduced growth of maize plants in pots where the weed was previously sprayed with terbuthylazine. The post-hoc analysis identified a significant difference between glyphosate and terbuthylazine when sprayed on the weed. Secondly, we approximated shoot chlorophyll content with a SPAD meter and found the SPAD values to be in a similar range in both experiments, decreasing over time and with a greater decrease in the second experiment (**Fig. 6B**). We did not find differences in SPAD values due to herbicide or modes of application. Finally, we recorded maize shoot biomass (as dry weight), which was clearly lower for plants growing - even two weeks longer compared to Experiment 1 - in the moist soil after the water flush (Experiment 2; **Fig. 6C**). In both experiments, yield was unaffected by glyphosate and terbuthylazine and their modes of application. Altogether these results show that earlier herbicide applications on soil or on weeds only had minor or no effects on the performance of the subsequent crop.

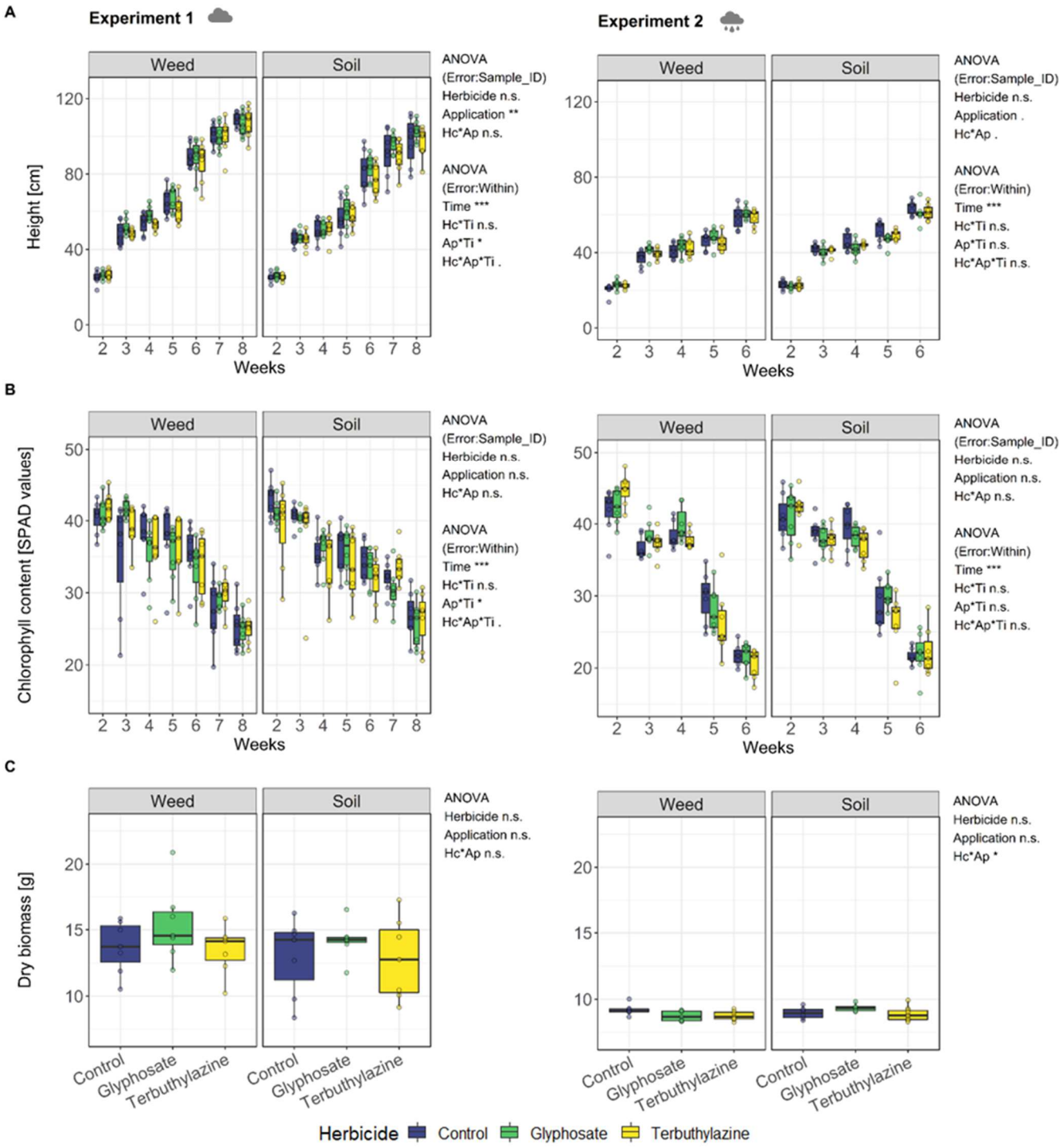


Figure 6. Plant height and chlorophyll content in response to herbicide application (n = 7). (A) Plant height and (B) chlorophyll content, expressed by SPAD values, are displayed weekly. (C) Leaves dry biomass was taken after 8 and 6 weeks of growth, respectively for Experiment 1 and 2. Applications onto bare soil and onto the weed are shown with side-by-side graphs. All left-hand side panels represent Experiment 1 and the right-hand side panels Experiment 2. P-values: n.s. non-significant, *p<0.05, **p<0.01, ***p<0.001.

Discussion

We investigated the effects of herbicides and their modes of application on soil extracellular enzyme activity, soil microbiome composition and plant performance. We performed two experiments, where we applied the two herbicides glyphosate and terbuthylazine both either onto bare soil and onto a weed, one experiment with and one without a water flush after herbicide application. We then measured the activities of three soil enzymes as a proxy for soil functioning and sequenced the soil microbiome to determine the diversity and composition. Finally, we scored plant performance, recording plant height and chlorophyll content weekly, and the shoot biomass at the end of the experiments. With this setup we tested the hypothesis that herbicide application reduces soil functioning, changes soil microbiome composition, and provokes feedbacks on growth of subsequent crop plants. We also studied the questions related to modes of application and soil moisture.

All our measurements on extracellular enzyme activities and plant performance must be interpreted relative to the amounts of herbicides detected in the soils. Therefore, we first validated the presence of the herbicides in the soils and answered the technical questions of this study. We found very similar amounts of glyphosate and terbuthylazine in soil irrespective of their mode of application – whether being sprayed directly on bare soil or onto the weed *Chenopodium album* (**Fig. 1**). In contrast, we found that soil moisture – manipulated by flushing (or not) the soil with water after herbicide application – had pronounced effects on the soil herbicide contents. We measured transiently much higher amounts of glyphosate that then disappeared with time after the flush treatment. This peak of glyphosate in soil is reminiscent of enhanced glyphosate leaching after rainfall (Kepler et al., 2020; Vereecken, 2005). The subsequent disappearance of glyphosate in the later timepoint indicates its leaching or chemical or microbial degradation. At high soil moisture conditions we measured also much lower levels of terbuthylazine, also suggesting a faster metabolization under these conditions (Sahid & Teoh, 1994). Without a water flush however, so in low soil moisture conditions, we generally found the opposite with higher amounts of herbicides that still increased with time As we were expecting higher herbicide effects under higher soil moisture, these findings of comparing soil moisture conditions point to important temporal dynamics of herbicide bioavailability in soil. However, our findings are tainted with the limitation that we compared soil moisture in two experiments due to priority and scale issues (see methods). Future studies should measure the concentration of herbicides not only in the soil matrix but also in the leachate and in the soil solution. Furthermore, setups that manipulate soil moisture within the same experiment are needed to corroborate the temporal dynamics of herbicide bioavailability in soil and the resulting consequences on soil functioning, microbiome composition and feedbacks on plant growth.

The soil microbiota profiling provided support for the hypothesis that herbicide applications change soil microbiome diversity and composition. Although we did not find major differences in microbiome diversity and composition due to the modes of application, we did find significant shifts in microbiome composition after herbicide applications, which were stronger for bacteria than for fungi (**Fig. 6**).

Under constant soil moisture (Experiment 1), we found more ASVs sensitive to glyphosate than ASVs sensitive to terbuthylazine (6% vs. 1.5%). However, under higher soil moisture (Experiment 2) we found more terbuthylazine-sensitive ASVs compared to glyphosate-sensitive ASVs (4% vs. 1.7%). Hereby, multiple highly abundant ASVs reacted sensitive to herbicide application. In previous work, glyphosate was shown to trigger a shift in soil microbial communities after repeated applications (Allegrini et al., 2017; Lancaster et al., 2010), and only subtle changes in a one-time spraying experiment (Zabaloy et al., 2012). Glyphosate is being considered as an herbicide that is environmentally only marginally aggressive (Barriuso et al., 2010) despite the fact that also in our study, a single application was sufficient to provoke discernible shifts in community composition. Environmental toxicity, however, is not only an issue of quantities and the number of applications but also requires the assessment of the temporal dynamics of herbicide bioavailability in soil. Our chemical analysis indicated a fast herbicide turnover, particularly under high soil moisture conditions, suggesting that shifts in microbiome composition are probably also transient.

Although terbuthylazine was reported earlier to have negative effects on soil microbial communities (bacteria and fungi) in formulation with other compounds (Borowik et al., 2017; Wyszkowska et al., 2016), we did not observe significant community shifts for fungi from one application. It is not uncommon that fungi were observed to be insensitive to herbicide applications (Pasaribu et al., 2011; Powell et al., 2009; Sheng et al., 2012). A possible explanation could be that fungi, which vastly expand into soil thanks to their hyphae, and therefore might be less affected by localized applications of a toxic compound. At the same time, this might explain why we found the bacteria to be more sensitive to herbicide application, possibly because they are directly exposed to the localized application of the herbicide. However, it has also been shown that glyphosate application can reduce AMF colonization in both target and non-target plants (Helander et al., 2018; Zaller et al., 2014). Other studies showed as well that agricultural management can have a greater effect on the soil microbiota than herbicide toxicity (Kepler et al., 2020).

We measured soil extracellular enzymatic activities to approximate microbial nutrient cycling in soil. Glyphosate was already known to affect soil enzymatic activity acting as C, P and N source (Mijangos et al., 2009), and terbuthylazine was shown to inhibit several enzymes, including β-glucosidase and phosphatases (Borowik et al., 2017; Wyszkowska et al., 2016). The enzyme activities varied in our experiments mostly with time and the effects of glyphosate and terbuthylazine were often similar to each other and are comparable to those found in the control (**Fig. 2**). We found the herbicide effects being mainly dependent on soil moisture. For instance, we found an herbicide-increased β–glucosidase activity under high soil moisture and only when spraying the herbicides on the weed. Possible explanations are that the roots would exudate more low-molecular compounds when sprayed, or when the fine roots die-back first, releasing easily available C-sources as substrate for the β-glucosidase. Or we found a reduced decrease of chitinase activity compared to water-treated control in lower soil moisture conditions.

Based on our results we rejected the hypothesis that herbicide applications generally reduce soil extracellular enzyme activities, and we concluded that soil enzymatic activities are subjected to a high context-dependency including moisture levels and presence/absence of herbicides in soil. This points out the importance of considering herbicide applications along with other conditional parameters, including time and weather. We found a tendency of fewer changes in soil enzymatic activities under high soil moisture (**Fig. 2**) which coincided with low herbicide levels at the late measurement (**Fig. 1**). This suggests a rather temporary effect than consistently negative effects on soil functioning. Probably a more precise time series of combined measurements of herbicide levels, soil enzyme activities or isotope approaches are needed to decipher the enviro-temporal conditions of herbicide effects on nutrient cycling in soil. Future experiments are also needed to connect soil microbiome community changes and soil enzymes activity measurements.

Thirdly, we tested the hypothesis that herbicide applications provoke negative feedbacks on the growth of the subsequent crop plant. One underlying assumption for this hypothesis was that the altered soil microbiome might negatively affect the growth of a next crop generation. Although we found changes in soil microbiome composition after herbicide exposure (**Fig. 6**), we disproved this hypothesis: herbicide application did not affect maize height, chlorophyll content or yield. There was one small exception to this finding; the reduced height of plants growing in soil of previously terbuthylazine treated weeds in high moisture conditions. We believe that this finding is not relevant to the agricultural context, first because the final biomass was then unaffected by the herbicide treatment (**Fig. 8C**) and secondly, because herbicides are not typically applied in high soil moisture conditions, e.g., when a heavy rainfall is forecasted. The alternative underlying assumption to test the aforementioned hypothesis was that herbicides like glyphosate have positive physiological effects on crop performance at low doses (Fuchs et al., 2021; Helander et al., 2018; Zobiole et al., 2011). This so called 'hormesis' effect was only reported for low doses of glyphosate (Brito et al., 2018; Ramirez-Villacis, 2020) while also having disappearing in presence of a functional microbiome (Ramirez-Villacis, 2020). In our experiments we applied moderate doses of the herbicides (10 L ha^{-1} glyphosate and 1.5 L ha^{-1} terbuthylazine) as recommended by Agroscope, the Swiss Confederation's centre of excellence for agricultural research. Despite the moderate dosage, we did not observe hormesis effects due to herbicides as previously reported in the literature.

Overall, our study revealed little impact from a single application of herbicides on soil extracellular enzyme activities, discernible shifts in soil bacterial communities but without affecting the performance of the subsequent crop plant. Our work highlights the important premise of herbicide bioavailability in soil, which is strongly context-dependent, e.g., the soil moisture levels affected the temporal dynamics of herbicide metabolization. In the general context of agriculture, our study confirms that herbicides and their timing of application (absence of rain) are generally very well designed. Furthermore, herbicides are intended to act only on their targets and to sorb to the soil particles and thereby becoming less bioavailable.

A limitation of our study is that these conclusions are restricted to single applications of herbicide and their short-term effects. Although our results reveal that a single application of herbicide can change soil functioning and soil microbiome composition, it reveals a high temporal and context-dependency of these transient effects. Therefore, and maybe more importantly than worrying about off-target effects of an applied herbicide, it is probably of notable importance to specifically understand the conditions when an herbicide compound is chemically degraded or metabolized by microbes in soil so that it vanishes without long-term negative effects on soil health.

Author statement

Experiments were planned and conducted by VC. Co-authors contributed to the microbiome analysis (Fig. 3, 4) and to the analysis and measurements of terbuthylazine and glyphosate in the soil.

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Supplementary information

Figure S1. Experimental setup greenhouse. (A) Two *Chenopodium album* plants in each pot with "weed" treatment. (B) Chenopodium album after herbicides spraying. (C) Maize seedlings planted after herbicides application. Wood sticks were inserted where the soil was sampled (several sampling in one pot), to keep the soil structure. (D) Herbicides application on weed or bare soil under the chemical hood (E) Experiments in the greenhouse.

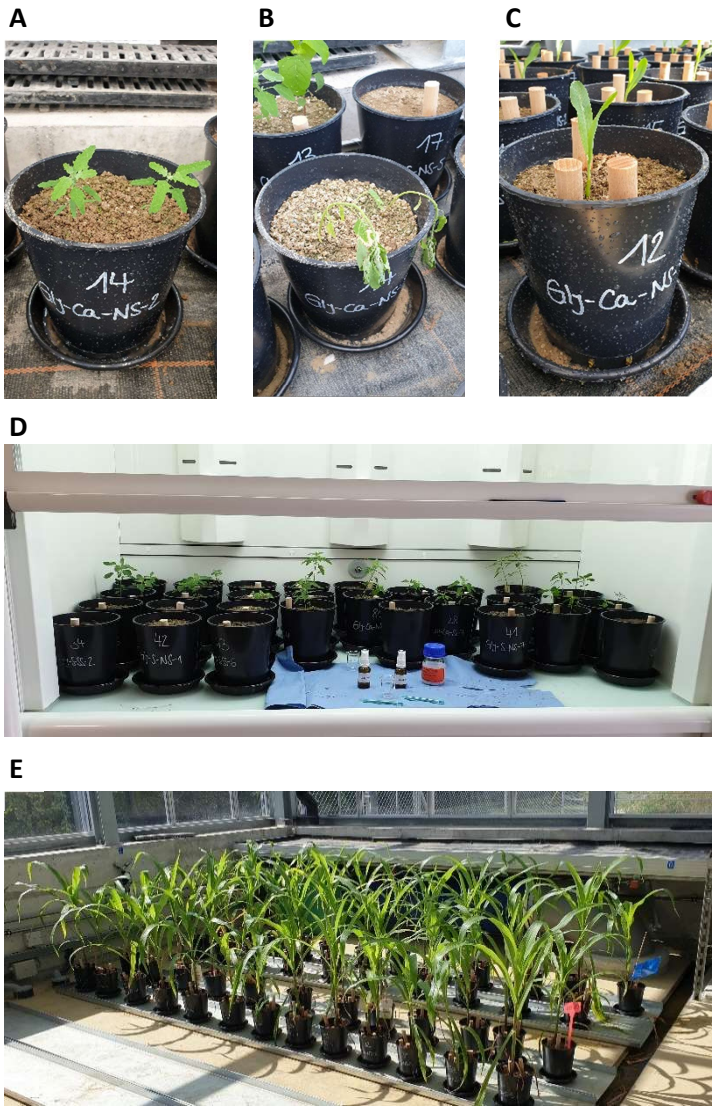


Table S1. Gradient timetable of the solvents A (H2O+H3PO4 at pH 3) and B (acetonitrile) for glyphosate and AMPA extraction from soil.

Time (min)	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-46
% A	75	70	65	50	10	10	50	75	75
% B	25	30	35	50	90	90	50	25	25

Table S2. Glyphosate and terbuthylazine and their degradation products were measured in soil to determine plant exposure to herbicides in the two experiments. Variables were untransformed (§), log transformed (¥), sqrt transformed (∞) or ranked transformed (†) to meet the requirements to perform the analysis. F-values and significance levels for a three-way analysis of variance with herbicide (Hc), application (Ap) and time (Ti) as separate factors and their interaction term are shown. P-values: ns not significant, . 0.1 < P < 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0 < P < 0.001.

	Herbicide	Application	Time	Hc*Ap	Hc*Ti	Ap*Ti	Hc*Ap*Ti
Variable	F	F	F	F	F	F	F
Glyphosate-exp1†	0.000129	2.58E-13	0.002205	4.80E-12	0.060306	0.349343	0.98654
AMPA-exp1†	2.00E-16	0.424078	0.00000143	0.000232	0.597756	0.958316	0.957457
Terbuthylazine-exp1†	1.13E-08	0.244	2.32E-07	0.728	1.84E-09	0.163	0.435
Desethylterbuyhalzine-exp1†	2.00E-16	0.0797	0.6601	0.1956	3.06E-06	0.915	0.6898
Terbuthylazine-2-hydroxy-exp1†	2.00E-16	1.46E-05	2.00E-16	0.06706	0.00126	0.32023	0.02019
Flufenact-exp1†	2.00E-16	0.000879	0.068287	0.117476	3.73E-07	0.700641	0.606476
Glyphosate-exp2†	2.00E-16	3.40E-11	2.00E-16	2.00E-16	3.61E-06	2.00E-16	0.041
AMPA-exp2†	2.00E-16	2.00E-16	2.00E-16	4.90E-15	0.00000176	2.00E-16	2.00E-16
Terbuthylazine-exp2†	2.00E-16	5.96E-05	0.014843	0.886696	0.000132	0.039978	0.039978
Deserthylterbuyhalzine-exp2†	5.44E-16	0.38499	0.25991	7.34E-05	2.02E-08	0.00889	0.00889
Terbuthylazine-2-hydroxy-exp2†	2.00E-16	0.078	1.70E-11	0.448	0.274	0.274	0.274
Flufenact-exp2†	3.08E-06	0.8006	4.63E-06	0.0532	0.1186	0.7279	0.7279

Table S3. N-β-acetylglucosaminidase, acid phosphatase and β-glucosidase were measured to determine soil functioning in the two experiments. Variables were untransformed (§), log transformed (¥), sqrt transformed (∞) or ranked transformed (†) to meet the requirements to perform the analysis. F-values and significance levels for a three-way analysis of variance with herbicide (Hc), application (Ap) and time (Ti) as separate factors and their interaction term are shown. P-values: ns not significant, . 0.1 < P < 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0 < P < 0.001.

	Herbicide	Application	Time	Hc*Ap	Hc*Ti	Ap*Ti	Hc*Ap*Ti
Variable	F	F	F	F	F	F	F
N-beta-acetylglucosaminidase Exp1¥	0.71359	0.11864	0.3182	0.00333	0.51606		
Acid phosphatase Exp1†	0.13556	0.02056	1.09E-13	7.50E-06	0.00571	0.57797	0.41453
Beta-glucosidase Exp1§	0.378	0.324	0.324	0.378	0.378	0.324	0.378
N-beta-acetylglucosaminidase Exp2§	0.664	0.782	0.58	0.308	0.617	0.812	0.213
Acid phosphatase Exp2§	0.236995	0.007352	0.024448	0.000631	0.685881	0.729835	0.469879
Beta-glucosidase Exp2†	0.118253	0.006096	2.38E-07	0.000271	0.000693	0.035427	0.906534

Table S4. Plant height, chlorophyll content and leaves dry biomass were measured to determine plan performance in the two experiments. Variables were untransformed (§), log transformed (¥), sqrt transformed (∞) or ranked transformed (†) to meet the requirements to perform the analysis. F-values and significance levels for a three-way analysis of variance with herbicide (Hc), application (Ap) and time (Ti) as separate factors and their interaction term are shown. P-values: ns not significant, . 0.1 < P < 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0 < P < 0.001.

	Error:Sample_ID			Error:Within			
	Herbicide	Application	Hc*Ap	Time	Hc*Ti	Ap*Ti	Hc*Ap*Ti
Variable	F	F	F	F	F	F	F
Plant height Exp1†	0.32919	0.00135	0.9621	<2e-16	0.9732	0.0282	0.058
Chlorophyll Exp1†	0.6516	0.8587	0.5653	3.31E-62	0.1682	0.02159	0.06679
Plant height Exp2†	0.9798	0.07319	0.06392	1.16E-86	0.911	0.2872	0.4128
Chlorophyll Exp2†	0.6064	0.7799	0.206	2.04E-60	0.1902	0.117	0.4279

Figure S2. Final sequencing depth after demultiplexing and quality filtering for bacteria (left) and fungi (right). The upper panels show the sequences per treatment group. In the lower panels are the sequences sorted by depth.

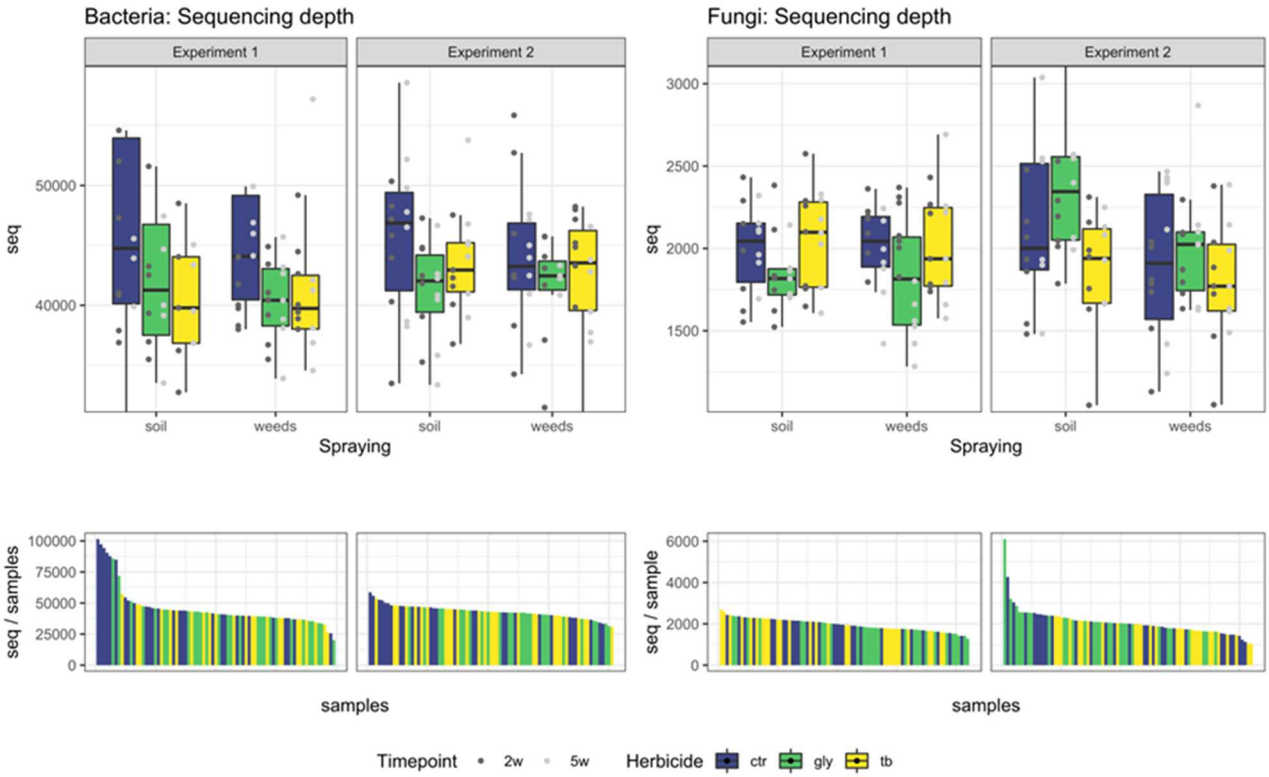


Figure S3. Rarefaction plot for bacteria (left) and fungi (right). The dashed line represents the rarefaction threshold.

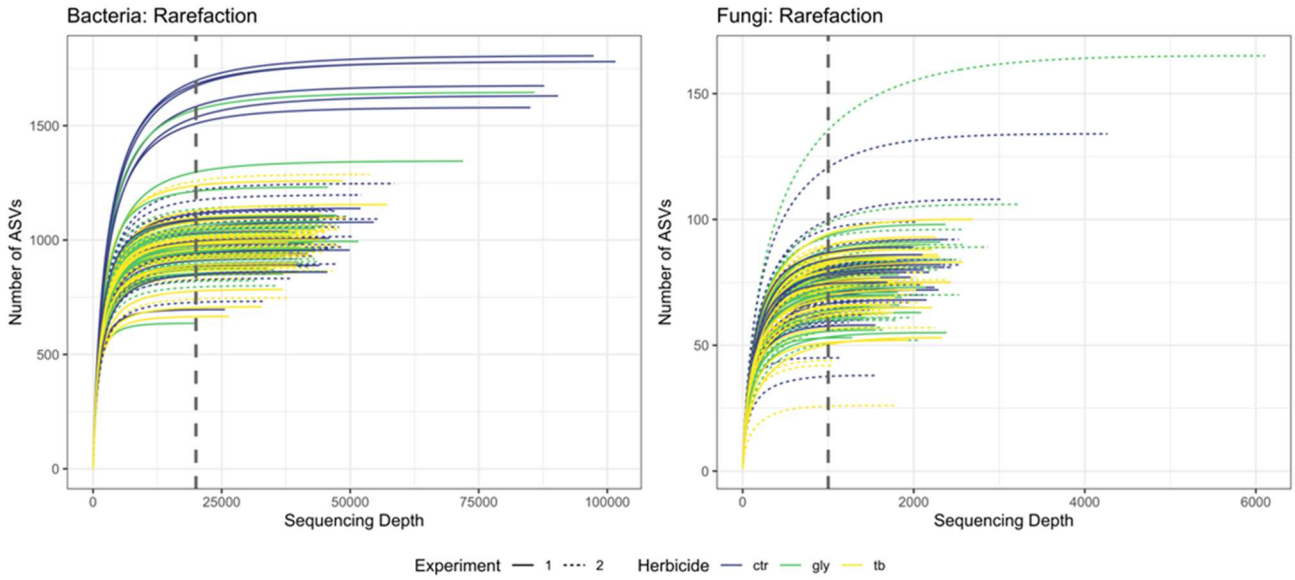


Figure S4. Phyla abundance for bacteria (top) and fungi (bottom). The abundance has been measured after 2 weeks (upper panels) and after 5 weeks (lower panels).

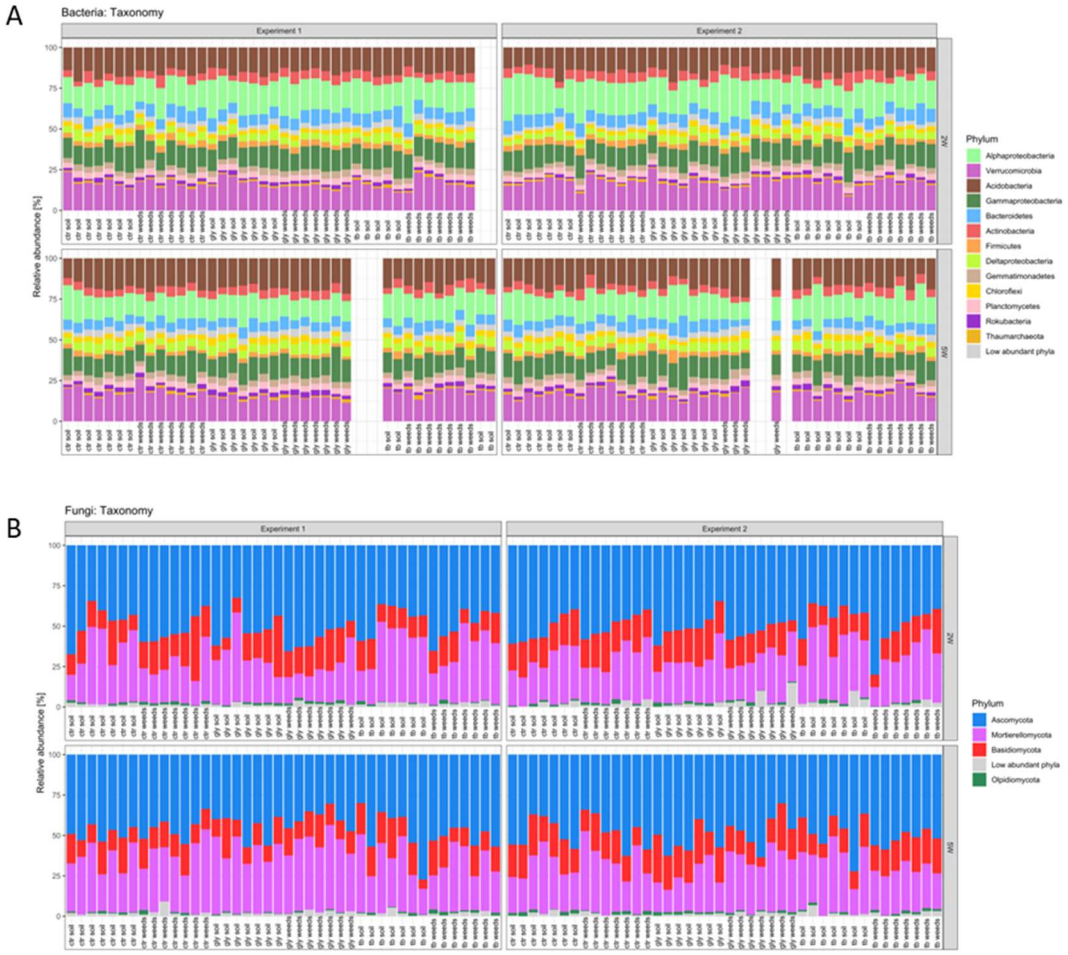


Table S5. PERMANOVA on the relative phyla abundances (bacteria and fungi).

Taxa	Experiment	Factor	Df	SumOfSqs	R2	F	Pr(>F)
Bacteria	Experiment1	Herbicide	2	0.022719026	0.044326	2.10369	0.039
Bacteria	Experiment1	Application	1	0.002410082	0.004702	0.446328	0.796
Bacteria	Experiment1	Time	1	0.076350136	0.148962	14.13943	0.001
Bacteria	Experiment1	Plate	1	0.011662762	0.022754	2.159849	0.078
Bacteria	Experiment1	Herbicide:Application	2	0.008359844	0.01631	0.774088	0.583
Bacteria	Experiment1	Herbicide:Time	2	0.020970133	0.040913	1.941749	0.082
Bacteria	Experiment1	Application:Time	1	0.00865977	0.016895	1.603719	0.158
Bacteria	Experiment1	Herbicide:Application:Time	2	0.005030222	0.009814	0.465778	0.878
Bacteria	Experiment1	Residual	66	0.356387099	0.695323	NA	NA
Bacteria	Experiment1	Total	78	0.512549076	1	NA	NA
Bacteria	Experiment2	Herbicide	2	0.009392883	0.013271	0.629487	0.683
Bacteria	Experiment2	Application	1	0.003505995	0.004953	0.469926	0.706
Bacteria	Experiment2	Time	1	0.074763281	0.105628	10.0209	0.002
Bacteria	Experiment2	Plate	1	0.01548491	0.021878	2.07552	0.101
Bacteria	Experiment2	Herbicide:Application	2	0.015747942	0.022249	1.055388	0.375
Bacteria	Experiment2	Herbicide:Time	2	0.027498435	0.038851	1.842876	0.12
Bacteria	Experiment2	Application:Time	1	0.021136929	0.029863	2.833088	0.048
Bacteria	Experiment2	Herbicide:Application:Time	2	0.032937914	0.046536	2.207416	0.054
Bacteria	Experiment2	Residual	68	0.50733024	0.716772	NA	NA
Bacteria	Experiment2	Total	80	0.707798529	1	NA	NA
Fungi	Experiment1	Herbicide	2	0.004059071	0.002904	0.192097	0.951
Fungi	Experiment1	Application	1	0.025432619	0.018194	2.407215	0.108
Fungi	Experiment1	Time	1	0.074003262	0.05294	7.004461	0.003
Fungi	Experiment1	Plate	1	0.448733821	0.321014	42.47297	0.001
Fungi	Experiment1	Herbicide:Application	2	0.016586092	0.011865	0.784943	0.503
Fungi	Experiment1	Herbicide:Time	2	0.031435483	0.022488	1.487695	0.21
Fungi	Experiment1	Application:Time	1	0.048297802	0.034551	4.571421	0.017
Fungi	Experiment1	Herbicide:Application:Time	2	-0.00080989	-0.00058	-0.03833	1
Fungi	Experiment1	Residual	71	0.750126451	0.536623	NA	NA
Fungi	Experiment1	Total	83	1.397864714	1	NA	NA
Fungi	Experiment2	Herbicide	2	0.036275667	0.029714	1.65611	0.146
Fungi	Experiment2	Application	1	0.007011821	0.005744	0.640228	0.569
Fungi	Experiment2	Time	1	0.01040294	0.008521	0.94986	0.39
Fungi	Experiment2	Plate	1	0.251600573	0.206093	22.97287	0.001
Fungi	Experiment2	Herbicide:Application	2	0.051612876	0.042277	2.356306	0.046
Fungi	Experiment2	Herbicide:Time	2	0.029511982	0.024174	1.347324	0.218
Fungi	Experiment2	Application:Time	1	0.01950652	0.015978	1.78108	0.124
Fungi	Experiment2	Herbicide:Application:Time	2	0.037293703	0.030548	1.702587	0.134
Fungi	Experiment2	Residual	71	0.77759712	0.63695	NA	NA
Fungi	Experiment2	Total	83	1.220813202	1	NA	NA

Table S6. Alpha diversity of bacteria and fungi.

Taxa	Experiment	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Bacteria	Experiment1	Herbicide	2	28546.04	14273.02	1.778962	0.176812
Bacteria	Experiment1	Application	1	167.6438	167.6438	0.020895	0.885506
Bacteria	Experiment1	Time	1	82941.03	82941.03	10.33761	0.002019
Bacteria	Experiment1	Plate	1	92269.43	92269.43	11.50029	0.001179
Bacteria	Experiment1	Herbicide:Application	2	5079.772	2539.886	0.316567	0.729747
Bacteria	Experiment1	Herbicide:Time	2	22518.45	11259.23	1.403329	0.253014
Bacteria	Experiment1	Application:Time	1	7.180655	7.180655	0.000895	0.976224
Bacteria	Experiment1	Herbicide:Application:Time	2	30952.45	15476.22	1.928927	0.153408
Bacteria	Experiment1	Residuals	66	529533.1	8023.228	NA	NA
Bacteria	Experiment2	Herbicide	2	24.55294	12.27647	0.002736	0.997268
Bacteria	Experiment2	Application	1	8164.981	8164.981	1.819369	0.181863
Bacteria	Experiment2	Time	1	36300.61	36300.61	8.088712	0.005878
Bacteria	Experiment2	Plate	1	8068.822	8068.822	1.797942	0.184424
Bacteria	Experiment2	Herbicide:Application	2	2063.106	1031.553	0.229857	0.795262
Bacteria	Experiment2	Herbicide:Time	2	38442.51	19221.26	4.282992	0.017704
Bacteria	Experiment2	Application:Time	1	1827.175	1827.175	0.407142	0.525567
Bacteria	Experiment2	Herbicide:Application:Time	2	19276.34	9638.169	2.147633	0.124614
Bacteria	Experiment2	Residuals	68	305171.1	4487.81	NA	NA
Fungi	Experiment1	Herbicide	2	39.26436	19.63218	0.486217	0.616981
Fungi	Experiment1	Application	1	245.8339	245.8339	6.088401	0.01602
Fungi	Experiment1	Time	1	38.67409	38.67409	0.957815	0.331061
Fungi	Experiment1	Plate	1	398.2342	398.2342	9.862797	0.002459
Fungi	Experiment1	Herbicide:Application	2	11.2607	5.630351	0.139443	0.87008
Fungi	Experiment1	Herbicide:Time	2	146.3265	73.16323	1.811984	0.170801
Fungi	Experiment1	Application:Time	1	0.001223	0.001223	3.03E-05	0.995624
Fungi	Experiment1	Herbicide:Application:Time	2	243.2287	121.6144	3.011941	0.055523
Fungi	Experiment1	Residuals	71	2866.796	40.37741	NA	NA
Fungi	Experiment2	Herbicide	2	257.7705	128.8853	1.927211	0.153092
Fungi	Experiment2	Application	1	220.3692	220.3692	3.295163	0.073707
Fungi	Experiment2	Time	1	171.4856	171.4856	2.564209	0.113748
Fungi	Experiment2	Plate	1	1430.508	1430.508	21.39027	1.64E-05
Fungi	Experiment2	Herbicide:Application	2	31.90178	15.95089	0.238512	0.788428
Fungi	Experiment2	Herbicide:Time	2	173.974	86.987	1.300709	0.278748
Fungi	Experiment2	Application:Time	1	19.80511	19.80511	0.296144	0.588015
Fungi	Experiment2	Herbicide:Application:Time	2	590.3277	295.1638	4.41356	0.015607
Fungi	Experiment2	Residuals	71	4748.238	66.87659	NA	NA

Table S7. Beta diversity of bacteria and fungi.

Taxa	Experiment	Factor	Df	SumOfSqs	R2	F	Pr(>F)
Bacteria	Experiment1	Herbicide	2	0.234707	0.034848	1.479239	0.005
Bacteria	Experiment1	Application	1	0.100062	0.014857	1.261276	0.11
Bacteria	Experiment1	Time	1	0.406396	0.06034	5.122606	0.001
Bacteria	Experiment1	Plate	1	0.107756	0.015999	1.358258	0.065
Bacteria	Experiment1	Herbicide:Application	2	0.160305	0.023801	1.010318	0.402
Bacteria	Experiment1	Herbicide:Time	2	0.21963	0.03261	1.384218	0.026
Bacteria	Experiment1	Application:Time	1	0.105574	0.015675	1.330754	0.059
Bacteria	Experiment1	Herbicide:Application:Time	2	0.164661	0.024448	1.037771	0.338
Bacteria	Experiment1	Residual	66	5.236029	0.777422	NA	NA
Bacteria	Experiment1	Total	78	6.735118	1	NA	NA
Bacteria	Experiment2	Herbicide	2	0.239731	0.033349	1.505315	0.034
Bacteria	Experiment2	Application	1	0.092576	0.012878	1.1626	0.188
Bacteria	Experiment2	Time	1	0.608648	0.08467	7.643608	0.001
Bacteria	Experiment2	Plate	1	0.085309	0.011868	1.071344	0.291
Bacteria	Experiment2	Herbicide:Application	2	0.179836	0.025017	1.129219	0.218
Bacteria	Experiment2	Herbicide:Time	2	0.271303	0.037741	1.703556	0.013
Bacteria	Experiment2	Application:Time	1	0.097443	0.013556	1.223728	0.142
Bacteria	Experiment2	Herbicide:Application:Time	2	0.198901	0.027669	1.248936	0.121
Bacteria	Experiment2	Residual	68	5.414726	0.753251	NA	NA
Bacteria	Experiment2	Total	80	7.188473	1	NA	NA
Fungi	Experiment1	Herbicide	2	0.307869	0.022576	1.151189	0.207
Fungi	Experiment1	Application	1	0.256061	0.018777	1.914933	0.023
Fungi	Experiment1	Time	1	0.508188	0.037265	3.800445	0.001
Fungi	Experiment1	Plate	1	1.994018	0.146219	14.91212	0.001
Fungi	Experiment1	Herbicide:Application	2	0.25102	0.018407	0.938617	0.562
Fungi	Experiment1	Herbicide:Time	2	0.295031	0.021634	1.103183	0.263
Fungi	Experiment1	Application:Time	1	0.223866	0.016416	1.674166	0.041
Fungi	Experiment1	Herbicide:Application:Time	2	0.307139	0.022522	1.148457	0.222
Fungi	Experiment1	Residual	71	9.493978	0.696184	NA	NA
Fungi	Experiment1	Total	83	13.63717	1	NA	NA
Fungi	Experiment2	Herbicide	2	0.403149	0.029268	1.414353	0.056
Fungi	Experiment2	Application	1	0.159251	0.011561	1.117389	0.273
Fungi	Experiment2	Time	1	0.213983	0.015535	1.501419	0.062
Fungi	Experiment2	Plate	1	1.646235	0.119514	11.55086	0.001
Fungi	Experiment2	Herbicide:Application	2	0.375691	0.027275	1.318025	0.093
Fungi	Experiment2	Herbicide:Time	2	0.326681	0.023716	1.146085	0.221
Fungi	Experiment2	Application:Time	1	0.170214	0.012357	1.19431	0.185
Fungi	Experiment2	Herbicide:Application:Time	2	0.360274	0.026155	1.263939	0.109
Fungi	Experiment2	Residual	71	10.11896	0.734619	NA	NA
Fungi	Experiment2	Total	83	13.77444	1	NA	NA

Figure S5. The PCR has been done on two different plates. This effect is shown in a Constrained Analysis of Principal Coordinates (CAP) for bacteria (left) and fungi (right) using the following model: *Bray-Curtis-distance ~ Plate.*

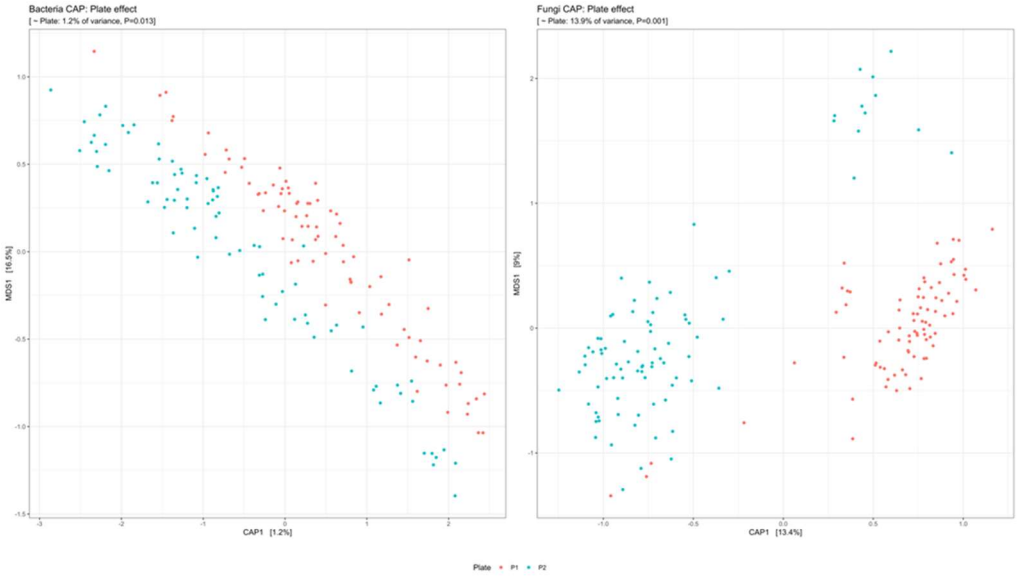


Table S8. Pair-wise comparison between treatments of plant performance (plant height and chlorophyll content). In Figure S6, a visual representation of the differences. P-values: ns not significant, . 0.1 < P < 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0 < P < 0.001.

Variables	p-value	
Height Exp1:		
Glyphosate-Control	0.07508	.
Terbuthylazine-Control	0.7476	ns
Terbuthylazine-Glyphosate	0.01076	*
Soil-Weed	1.80E-11	***
Chlorophyll Exp1:		
Glyphosate-Control	0.8444	ns
Terbuthylazine-Control	0.3574	ns
Terbuthylazine-Glyphosate	0.6926	ns
Soil-Weed	0.7906	ns
Height Exp2:		
Glyphosate-Control	0.9927	ns
Terbuthylazine-Control	0.9376	ns
Terbuthylazine-Glyphosate	0.9721	ns
Soil-Weed	0.001743	**
Chlorophyll Exp2:		
Glyphosate-Control	0.9952	ns
Terbuthylazine-Control	0.6029	ns
Terbuthylazine-Glyphosate	0.5439	ns
Soil-Weed	0.7447	ns

Chapter II

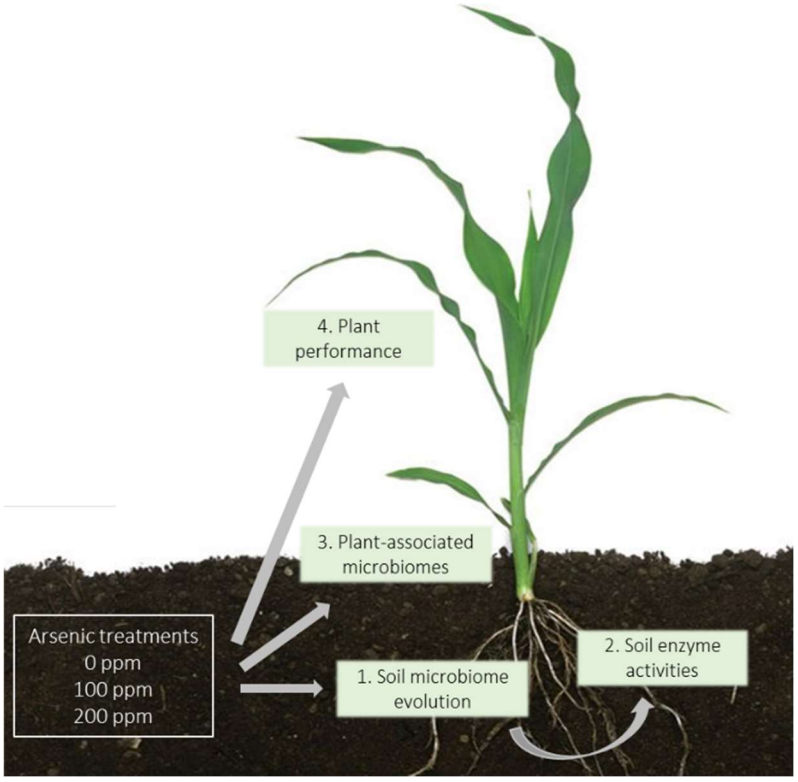
Impact of arsenic on soil and plant microbial communities, soil enzyme activities and maize growth

Veronica Caggìa[1], Hang Guan[2], Jan Waelchli[3], Gabriel Deslandes[1], Sandra Spielvogel[4,5], Adrien Mestrot[2], Moritz Bigalke[2,6] and Klaus Schlaeppi[1,3]

[1] Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland
[2] Institute of Geography, University of Bern, Hallerstrasse 12, CH-3012 Bern, Switzerland
[3] Department of Environmental Sciences, University of Basel, Bernoullistrasse 32, 4056 Basel, Switzerland
[4] Institute of Plant Nutrition and Soil Science, Christian-Albrechts-Universität, Hermann-Rodewald-Strasse 2, D-24118 Kiel, Germany
[5] Institute of Bio- and Geosciences, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany
[6] Institute of Applied Geoscience, Technical University Darmstadt, Schnittspahnstrasse 9, D-64287 Darmstadt, Germany

Abstract

Arsenic is a toxic contaminant present in soils and the evaluation of its environmental and ecological risks under aerobic conditions are still relatively unknown. In this study, we tested the hypothesis that increasing arsenic concentrations in soil triggers a shift in soil microbial communities and consequently negatively impacts the activities of N-β-acetylglucosaminidase, acid phosphatase, β-glucosidase, β-xylosidase and cellobiohydrolase in soil. We further expected the plant-associated microbiomes (roots, leaves, and kernels) to be influenced by soil pollution by arsenic albeit to a smaller extent than the soil microbiome. Furthermore, we hypothesized that this would result in decreasing maize growth and yield. We grew maize plants in increasing arsenic concentrations (0, 100 and 200 ppm) for 5 months, sampling soil every month and plant's parts after three months. Our results showed no effect of arsenic on neither soil microbial communities over time nor soil enzyme activities. Likewise, root, leaf and kernel microbiomes were not impacted by increasing arsenic concentrations in soil. Only soil microbial diversity at month three was increased in presence of arsenic. We further found that yield rather than growth was affected with the dry shoot biomass being significantly lower in the highest arsenic concentration. Overall, our study suggested that soil bacterial and fungal communities were highly resilient to arsenic pollution, as neither the community composition nor the enzymatic activities were influenced. On the plant level, specific microbiomes did not change because of the contamination in soil, but the much lower dry aboveground biomass accumulation showed toxic effects of 200 ppm of arsenic. In conclusion, our study revealed that neither soil microbial communities nor enzymes activities mediated arsenic negative effects on plant growth.



Key words: arsenic, soil enzyme activity, soil microbes, plant-associated microorganisms, *Zea mays* yield

Introduction

Arsenic (As) is a toxic metalloid naturally occurring in nature and belonging to the group A of carcinogens (Cubadda et al., 2017; IARC, 2004). In some regions around the world, arsenic levels in groundwater are higher than the maximum permissible level of 10 µg/L, recommended by the World Health Organization (WHO, 2018). Naturally, arsenic in water originates from the bedrock and soils (Hamidian et al., 2019; Keshavarzi et al., 2011). Usually arsenic is found in natural soils in concentrations of less than 10 mg/kg (Adriano, 2001; Wenzel, 2013), however naturally or anthropogenically contaminated sites ranging from 100 till 10'000 mg/kg also exist (Wenzel, 2013). Therefore, elevated concentrations of arsenic are present in drinking and groundwater but also in agricultural soils and crops growing on them (Amaibi et al., 2019; Mensah et al., 2020), representing potential direct and indirect threat to human health. The general understanding about the impact of arsenic on the soil ecosystem is of high importance for agricultural management and to ensure food safety and security.

Soil microorganisms represent the first organisms that get in touch with the presence of toxic arsenic. In fact, nearly all soil microbes show resistance to inorganic forms of arsenic, such as arsenite (AsIII) and arsenate (AsV; (Paez-Espino et al., 2009)). They have the potential, depending on soil properties (Mestrot et al., 2011), of transforming As into volatile forms (Paez-Espino et al., 2009). Several fungi, including *Fusarium* and *Trichoderma*, can methylate inorganic As species to organic ones (Bentley & Chasteen, 2002). Plant growth promoting microbes are known to assist plants in nutrient mining and consequently in better growth, as well as to minimize the effects of biotic and abiotic stresses including heavy metals (Ma et al., 2016). In fact, arsenic-related genes of microbes are associated to both resistance mechanisms and metabolism, by encoding for proteins involved in efflux (Rosen, 2002) or methylation and oxidation (Andres & Bertin, 2016). A lot of studies have investigated arsenic pollution in rice paddy fields, which present anaerobic conditions where arsenite (AsIII) is predominant (Akinbil & Haque, 2012; Srivastava et al., 2012; Munish Kumar Upadhyay et al., 2020; Wu et al., 2022). However. The inorganic species arsenate (AsV) is mainly found under aerobic soil conditions where maize is grown (Punshon et al., 2018). Understanding the consequences of arsenic contamination of soil microbial communities in well-aerated soils is required to understand the selection of microbes by the plant and assess their potential in sustainable agriculture.

Soil microbial communities drive most of the soil biogeochemical cycles, therefore, changes in soil enzyme activities present good proxies for changes in important elemental cycles in soil (e.g., the C-, N- and P-cycle). The use of soil enzyme activities as quality indicators is commonly used to assess environmental and ecological risk of arsenic pollution (Majumder et al., 2022). In fact, the presence of arsenic in soil can have a negative impact on sensitive bacteria, which can cause a decrease in their cellular and metabolic functions (Lorenz et al., 2006). In particular, activities of soil arylsulfatase and alkaline phosphatase decreased because of arsenic, being the phosphatase activity was particularly sensitive due to the chemical similarity between phosphate

and arsenate (Speir et al., 1999). Other soil enzymes such as urease, invertase and xylanase were shown to be unaffected by As pollution in soil or even increased as in the case of invertase and xylanase (Lorenz et al., 2006). In contrast, other studies have shown the vulnerability of β-glucosidase, phosphatase, urease and arylsulfatase to heavy metal contamination (Cang et al., 2009). The limitation of many of these studies have been performed on anaerobic rice cultivation (Choudhury et al., 2011; Jiang et al., 2022; Majumder et al., 2022). To date, comparatively little is known about N-β-acetylglucosaminidase, acid phosphatase, β-glucosidase, β-xylosidase and cellobiohydrolase activities in aerobic soil conditions, such as for instance in maize cultivation.

Zea mays is the most widely and intensively cultivated crop in the world, according to the Food and Agriculture Organization of the United Nations (FAOSTAT, 2004). It represents a staple food for both animals and humans. In most of the rural areas, maize is responsible for 50% of the human? protein intake and 70% of the caloric consumption (Serna-Saldivar et al., 2008). Thus, especially in contaminated areas, arsenic transfer through the soil-plant system constitutes an important risk for human exposure (Rosas-Castor et al., 2014). Crop plants exposed to arsenic can show several adverse characteristics such as chlorosis, growth inhibition, and oxidative stress (Zemanová et al., 2021). Arsenic is transported into the plant through a variety of transporters, for example P-transporters for AsV (Cao et al., 2019), aquaporins (Kamiya et al., 2009) and silicon transporters (Yamaji et al., 2015) for AsIII, and transporters of organic compounds for organic arsenic species (Tang et al., 2017). Plants possess a variety of arsenic tolerance and detoxification approaches. One of them consists of several transporters that restrict or reduce the uptake in roots, and/or control the translocation to upper plant parts (Bali & Sidhu, 2021). Furthermore, plants can enhance their tolerance to arsenic by its detoxification and sequestration through phytochelatins (Tang et al., 2019). Further chemicals, produced by the plant (salicylic acid) or not (silicone) also mitigate arsenic toxicity in the plant system, by respectively interacting with hormones and activating antioxidants alerting the plant, and outcompeting arsenic uptake through silicone transporters (Sharma et al., 2020; Zama et al., 2018). Whether and to which degree plant-associated microbes (roots, leaves, and kernels) may help with As tolerance and enrichment of arsenic-specific microbes following arsenic contamination in soil remain largely unknown.

In this study, we addressed the previously mentioned knowledge gaps by studying the impact of increasing concentrations of inorganic arsenic (0, 100 and 200 ppm) on soil enzymes activities, on both soil and plant microbiomes and on plant growth and yield. We quantified plant growth, by measuring both plant height and final aboveground biomass to assess the impact of arsenic on plant performance. With our setup we tested our hypotheses that increasing arsenic concentration in soil causes shifts in soil microbial communities and therefore negatively affects the activities of soil enzymes. We also expect that soil As pollution results in changes in the plant-associated microbiomes, by having greater impact on roots, and much smaller impact on

leaves and kernels microorganisms. Finally, we anticipated a negative relationship between plant growth and arsenic concentration.

We performed an experiment under controlled greenhouse conditions using a soil from an agricultural field site in Switzerland with silty loam texture to test the effects of soil pollution by inorganic arsenic and subsequent plant performance. We specifically investigated soil functioning, soil microbial communities, plant-related microbiomes and plant growth and yield. We found that soil bacterial and fungal communities were not impacted by increasing arsenic concentrations. Similarly, also soil enzyme activities did not show an increase or decrease in presence of arsenic compared to the control. Concerning the plant-associated microbiomes, we could not find any differences due to arsenic contamination in neither of the compartments. Roots, leaves, and kernels showed very distinct microbiomes among each other but no-arsenic dependent signature. Our results suggest that soil microbial communities and soil functioning are not affected under the experimental conditions with AsV up to 200 mg/kg soil. On the other hand, we could observe the negative of these concentrations on plant yield, as plants grown in the 200 ppm variant had a significantly lower biomass.

Methods

Greenhouse experiment

Soil: We performed these experiments with a silty loam soil (Ap horizon) under agricultural use from 'Q-Matte' in Frauenkappelen (46°57'20.5''N, 7°19'58.3''E), Switzerland, because our pre-tests revealed it to be very low in arsenic content (2.9 ± 0.5 mg/kg, **Table S1**). Main soil types in this area are Haplic Cambisols and Haplic Luvisols (IUSS Working Group WRB, 2022). Characteristics of the Q-matte soil are described in **Table S1**. The soil was stored in a pile outside the greenhouse facility of the Institute of Plant Sciences at the University of Bern (Ostermundigen, Switzerland). The pile was sown to ensure a natural outside storage. The soil was taken from both sides of the pile for homogeneity and sieved to 1 cm. To test arsenic effects, we applied three different arsenic concentrations to the soil (0, 100, 200 ppm As): As-0ppm (naturally containing 2.91 ± 0.54 mg kg^{-1}), As-100ppm and As-200ppm. Sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$, ≥ 98.0%; Sigma-Aldrich®, Switzerland) was spiked to the soil that was incubated at room temperature for 8 weeks at 50% water holding capacity (WHC), to allow the stabilization of As in the matrix and to simulate soil aging (Jing Song et al., 2006). Arsenate was dissolved in MilliQ water, according to the arsenic concentration and WHC, and the soil was watered in four steps with the arsenic solution and mixed thoroughly in between the waterings to allow homogeneity.

Plants growth: We filled the pots (Rosentopf Soparco 7.0 L, Hortima AG, Switzerland) with 6.5 kg of soil and placed them with an underpot (Untersetzer Similcotto anthrazit Arca, Ø29 cm Hortima AG, Switzerland), to avoid cross-contamination in water leaching out during watering, in the greenhouse (n = 10). To sterilize seeds of *Zea mays L.* (inbred line W22) (Tzin et al., 2015), we soaked them in commercial bleach containing 5% active hypochlorite (Potz Javel-Wasser Natur, Migros, Switzerland) for 6 minutes, shaking the flask from time to time. We removed the bleach, and the seeds were rinsed 5-6 times with autoclaved MilliQ water. For pre-germination, we soaked the seeds in autoclaved MilliQ water for 8h and afterwards they were placed overnight on a moist filter paper (Rundfilter Sorte 1 Whatman, 90mm, Huberlab, Switzerland) in plastic Petri dishes (Petri dish 94x16mm, without vents, sterile, Greiner Bio-One, Switzerland). Three seeds were planted in each pot to ensure germination, and after germination the extra seedlings were removed. Maize plant grew in the greenhouse for 5 months (light/dark 14h:10h, daily temperature ranges 14°C-22°C, night 10°C-14°C, humidity range 50%-70%). We placed the pots in a randomized manner in the greenhouse and they were watered to keep WHC at 50%, by weighing the pots every 3 days and adding when needed the missing weight in water. Plants were fertilized weekly from week 2 onwards with a commercial fertilizer 0.2% (NPK, w/v; Plantaktiv Typ K, Hauert, Switzerland). At week 2 and 3, plants were fertilized with 100 ml and from week 4 onwards with 200 ml of solution.

Analyses and measurements

Soil and plant sampling: We sampled the soil for analysis with a custom-made soil sampler (diameter: 0.8 cm) and corresponding to the soil depth between -5 to -15 cm from the soil surface. For enzyme activities assay we sampled at month 0 (when maize was sewn) and at month 3 and stored the soil samples at 4°C until analysis (less than 24h). For the soil microbiome analysis, soil was sampled monthly (0-5) and for plant microbiome, roots and leaves were sampled at month 3 and kernels at month 5; everything was stored at -80°C. Roots were also sampled between -5 and -15 cm (same depth increment as the soil) and for the leaves we selected the 3rd youngest leaf to be sampled. A visual summary of the experimental timeline and sampling can be found in **Fig. 1**.

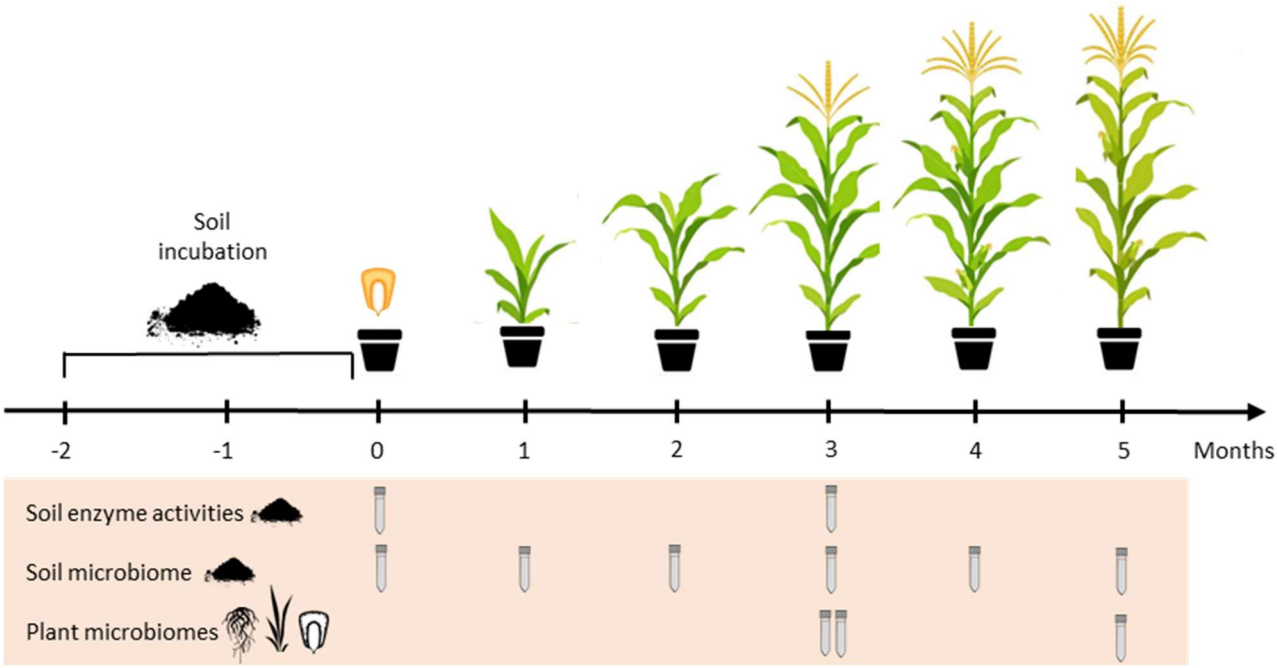


Figure 1. Experimental design and timeline. The experiment started with 2 months of soil incubation after arsenic spiking. At month 0, maize seeds were sown, and they grew for 5 months until they produced cobs and dried out. Soil for enzyme activities and microbiome analysis were sampled as shown. Roots and leaves were sampled at month 3 and kernels at month 5.

Soil microbiota profiling: Genomic DNA was extracted from 250 mg of soil sample (n = 5) with the DNeasy PowerSoil HTP 96 Kit (Berg-Lyons et al., 2018; Marotz et al., 2017) following the manufacturer's instructions and eluted in 100 µL buffer. DNA concentrations were quantified by fluorescence (AccuClear® Ultra High Sensitivity dsDNA Quantification kit, Biotium, Fremont, CA, USA) mixing 10 µL of sample (diluted 1:4) to 198 µL of DNA Quantitation buffer and 2 µL of dye per reaction. Alongside the samples, a standard curve consisting of 10 µL of each of the eight standards (0, 0.03, 0.1, 0.3, 1, 3, 10, 25 ng/µl) were added three times for

reference. Fluorescence was measured with the same plate reader as above after vortexing the plate, spin down and incubating it 5 min at room temperature in the dark (excitation wavelength 468 nm; emission wavelength 507 nm). After quantification, each sample was standardized to 1 ng µL-1 by dilution in autoclaved MilliQ water.

Library preparation consisted of a two-step PCR protocol of the Earth Microbiome Project (Wasimuddin et al., 2020) starting with PCR1 (reaction volume of 20 µL) containing Platinum Hot Start polymerase (0.8x; Invitrogen, Fisher Scientific, Switzerland), 200 nM of each primer (CS1-515f, ACACTGACGACATGGTTCTACAGTGYCAGCMGCCGCGGTAA and CS2-806r, TACGGTAGCAGAGACTTGGTCTGGACTACNVGGGTWTCTAAT; or CS1-ITS1f-F, ACACTGACGACATGGTTCTACACTTGGTCATTTAGAGGAAGTAA and CS2-ITS-2R, TACGGTAGCAGAGACTTGGTCTGCTGCGTTCTTCATCGATGC), 3% BSA and H_2O (PCR-grade water and UV-treated), plus 3 µl of DNA template (3 ng total amount). The PCR cycling started with a denaturation at 94°C for 3 min, followed by 25 cycles of denaturation at 94°C for 45'', annealing at 50°C for bacteria and 55°C for fungi for 60'', elongation at 72°C for 90'' and a final elongation at 72°C for 10 min. The PCR products were cleaned-up with magnetic beads (SPRIselect, Beckman Coulter, Brea, U.S.A.) at a 1:1 ratio of beads to DNA samples. Beads were thoroughly mixed with the samples by pipetting up and down, incubated at room temperature for 1 min and the plates were placed on a magnetic stand until the supernatant was cleared (MicroMag Separator, Kisner Biotech GmbH & Co., Germany). The supernatant was carefully removed, and the beads were washed with 180 µl freshly prepared 85% ethanol (keeping the plate on the magnetic stand). The excess of ethanol was carefully removed with a small pipette. After removing the plate from the magnetic stand, 20 µl of Tris (pH 8.0) was used to resuspend the beads by pipetting up and down. The plate was incubated 1 min at room temperature and then placed on the magnetic stand until the supernatant was cleared. Finally, the supernatant was transferred, avoiding contamination by beads, to a new plate for the second PCR.

The reaction volume of PCR2 was 25 µl and contained 5 µl of template of the bead-purified DNA, 5 µl of PCR-grade water, 10 µl of PCR master mix (2x) and 5 µl of primers 400 nM (Access Array™ Barcode Library, Fluidigm, San Francisco, U.S.A.). We utilized the unidirectional Access Array Barcode system with the PCR primers PE1-CS1-F and PE2-[BC]-CS2-R to prepare a ready-to-load library. These primers contain the paired-end (PE) adapters required for Illumina sequencing, the linker sequences CS1 and CS2 to bind to the amplicons of PCR1 and one of 384 10-mer barcodes (BC). Cycling of PCR2 consisted of an initial denaturation at 94°C for 3 min, followed by 10 cycles of denaturation at 94°C for 45'', annealing at 60°C for bacteria and 55°C for fungi for 60'', elongation at 72°C for 90'' and a final elongation at 72°C for 10 min. Negative control samples were include in each PCR assay and both PCR1 and PCR2 reactions were verified on a 1.5% agarose gel to lack contamination. After PCR2, the clean-up with SPRIselect beads was repeated. Finally, an equimolar pool of all

samples was done, purified again with SPRIselect beads and fluorometrically quantified (Qubit™, Thermo Fisher Scientific, Switzerland). The library was then sequenced (v3 chemistry, 300 bp paired end) using custom primers following the instructions of Access Array Barcode system on an Illumina MiSeq instrument at the next generation sequencing NGS platform of the University of Bern.

Plant microbiota profiling: Genomic DNA was extracted from roots, leaves and kernels' samples with the NucleoSpin 96 Tissue (96-wells kit, Macherey Nagel, Switzerland) following the manufacturer's instructions and eluted in 50 µL. Replicates were n = 10 for roots and leaves, while for kernels we have n = 6 from the control soil and n = 4 from the 100 ppm contaminated soil (no kernels from the 200 ppm treatment). DNA concentrations were quantified by fluorescence (AccuClear® Ultra High Sensitivity dsDNA Quantification kit, Biotium, Fremont, CA, USA) mixing 10 µL of sample (diluted 1:4) to 198 µL of DNA Quantitation buffer and 2 µL of dye per reaction. Alongside the samples, a standard curve consisting of 10 µL of each of the eight standards (0, 0.03, 0.1, 0.3, 1, 3, 10, 25 ng/µl) were added three times for reference. Fluorescence was measured with the same plate reader as above after vortexing the plate, spin down and incubating it 5 min at room temperature in the dark (excitation wavelength 468 nm; emission wavelength 507 nm). After quantification, each sample was standardized to 2 ng µL-1 by dilution in autoclaved MilliQ water.

For the bacterial library, first PCR reaction was performed with the non-barcoded 16S rRNA gene primers 799-F (AACMGGATTAGATACCCKG, (Chelius & Triplett, 2001)) and 1193-R (ACGTCATCCCCACCTTCC, (Bodenhausen et al., 2013)). A second PCR tagged the PCR product with custom-made barcoding primers: a padding sequence (GGTAG) followed by a barcode (8 nucleotides) and finishing by the 799-F or 1193-R sequence. The first PCR program consisted of a touchdown approach after an initial denaturation step of 2 minutes at 94°C. The five first cycles consisted of denaturation at 94°C for 30 seconds, annealing at 58°C for 45 seconds and elongating at 72°C for 10 seconds. Five more cycles were done with 30 seconds of annealing at 57°C and 20 seconds of elongation. Five more cycles were done with 30 seconds of annealing at 56°C and 30 seconds of elongation. After that, 25 more cycles were done with 30 seconds of annealing at 55°C. In total, there was 40 cycles, which were followed by a final elongation at 72°C for 10 minutes. The second PCR program consisted of an initial denaturation step of 2 minutes at 94°C, 10 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 65°C for 30 seconds, and a final elongation at 65°C for 10 minutes.

For the fungal library, first PCR reaction was performed with the internal transcribed spacer (ITS) region primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA, (Gardes & Bruns, 1993)) and ITS2 (GCTGCGTTCTTCATCGATGC, (White et al., 1990)). A second PCR tagged the PCR product with custom-made barcoding primers: a padding sequence (GGTAG) followed by a barcode (8 nucleotides) and finishing by the ITS1-F or ITS2 sequence. The first PCR program consisted of an initial denaturation step of 2 minutes at 94°C, 23 cycles of denaturation at

94°C for 45 seconds, annealing at 50°C for 60 seconds, elongation at 72°C for 90 seconds, and a final elongation at 72°C for 10 minutes. The second PCR program was similar, with the difference that the number of cycles was reduced to 7.

All PCR reactions were performed with the 5-Prime Hot Master Mix (Quantabio, QIAGEN, Beverly, MA, U.S.A.). All PCR products and pooled libraries were purified with SPRIselect beads (Beckman Coulter, Brea, CA, U.S.A.) according to manufacturer protocol with a ratio of 1:1; and were quantified with the AccuClear® Ultra High Sensitivity dsDNA quantification kit (Biotium, Fremont, CA, USA). Bacterial and fungal subpools were assembled by library type by adding an equal mass of each PCR product. To keep the ≈ 450 bp long DNA fragment, the bacterial subpool was cleaned on agarose gel and purified with the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel GmbH, Düren, Germany). One run with soil-samples from different timepoints and one run with soil-, roots-, leaves- and kernels- samples were paired-end sequenced (v3 chemistry, 300 bp paired end) on an Illumina MiSeq instrument at the NGS platform of the University of Bern.

Bioinformatics: We received demultiplexed samples from the NGS platform. In all files, we removed primers with cutadapt (V3.4, (Martin, 2011)). Following the methods of Gfeller et al. (unpublished), we inferred exact sequences variants and assigned taxonomies with dada2 (V1.16.0, (B. J. Callahan et al., 2016)) in R (V4.0.0, (R Core Team, 2022)). We adapted the methods by allowing maximum 2 expected errors and using different truncation lengths for soil samples (forward: 240 bp, reverse: 160 bp).

Enzymes activity: We measured the activities of the extracellular soil enzymes using fluorogenic methylumbelliferone (MUF) based substrates (Marx et al., 2001). We employed the MUF-derivates 4-MUF N-acetyl-β-D-glucosaminide, 4-MUF-β-D-cellobioside, 4-MUF β-D-glucopyranoside, 4-MUF-β-D-xylopyranoside and 4-MUF phosphate disodium salt as substrates for the enzymes N-β-acetylglucosaminidase, cellobiohydrolase, β-glucosidase, β-xylosidase and acid phosphatase, respectively. The 10 mM stock solutions were prepared by dissolving the substrates in 300 µL dimethyl-sulfoxide and sterile water to make a final volume of 10 mL. The assays were based on 500 mg of soil, weighed into autoclaved 100 mL glass jars, to which 50 mL sterile water was added and then blended with a polytron (PT 1200 CL, Kinematica AG, Littau, Switzerland, 30 sec at 4.5x 1000 rpm) (De Cesare et al., 2000). Aliquots of 50 µL were taken from the soil suspensions (constantly mixing with a magnetic stirrer in a glass petri dish) and loaded to 96-well microtiter plates (pureGrade™ sterile plates, BRAND, Germany). Three technical replicates for each biological sample (n = 4) were used for the assay. To each reaction, 50 µL of 0.1 M 2-(N-Morpholino)ethanesulforic acid (MES hemisodium salt, pH 6.1) were added as buffer (German et al., 2011). MUF-substrate stocks were diluted with MES to 1 mM working solutions. Finally, 100 µL serial dilutions of MUF-substrate solutions (0, 10, 20, 30, 40,

50, 100, 200 µmol g^{-1}) were added to the 96-well plates containing 50 µl of sample and 50 mL of MES buffer and well mixed by shaking for 1 minute directly in the fluorescence plate reader. Fluorescence measures of the samples were taken with a plate reader (Infinite M200 Pro, Tecan GmbH, Austria; excitation 355 nm, emission 460 nm) after substrate addition (t_0) and after 2 hours (t_1). For calibration and accounting for fluorescence quenching, standard plates were prepared with 50 µL of a composite soil solution, with 150, 145, 140, 130, 100, 70 and 30 µL of MES buffer and 0, 5, 10, 20, 50, 80 and 120 µL of MUF standards. The regression slopes served to calculate the enzymatic activities relative to these standard measurements [µmol substrate g^{-1} soil h^{-1}]. The enzyme activities were fitted to a Michaelis-Menten model, which describes nonlinear saturation curves with v = (Vmax × [S])/(Km + [S]), with Vmax as the maximal rate of enzymatic activity under optimum substrate conditions, [S] as the added substrate concentrations and Km is the substrate concentration at which half of the maximum velocity is achieved (Loeppmann et al., 2016; Marx et al., 2001; Nannipieri et al., 2012; Robert L. Sinsabaugh, 2010).

Statistical analysis

Enzymes activities: The fluorescence data were transformed to enzymatic activity rates (pM substrate g^{-1} soil h^{-1}) first, by translating the fluorescence data to substrate amounts based on the slope of the MUF-substrate standard curves and second, by subtracting the background values (t_0, substrate addition) from the actual activity values (t_1 after 2h of assay time; $t_1 - t_0$ fluorescence). In addition, enzyme activities were adjusted to the exact sample weights, volumes of the soil suspension (50 ml) and pipetted soil suspension volumes (50 µl). The obtained enzymatic activity data was then screened for outliers within technical, biological replicates and substrate concentrations using the InterQuartile Range (IQR) approach (R-bloggers, 2021), considering values exceeding ±1.5*IQR as outliers. Each sample group (biological and technical replicates) of this filtered enzymatic activity data was then fitted to a non-linear regression model nls() following Michaelis-Menten kinetics (Marx et al., 2001) to extract enzyme parameters such as Vmax (maximal velocity). Subsequently, Vmax values of the technical replicates were averaged. Vmax values were inspected for normality and transformed as needed (transformations in Table S2). Differences between sample groups were tested with ANOVA for the factors *arsenic* and *time* (~As*Ti).and for pairwise differences with the TukeyHSD test.

Soil microbiota profiling: We discarded three fungal samples with low sequencing yield. Bacterial sample groups varied significantly in sequencing depth (Kruskal-Wallis test, bacteria: $p<0.05$, fungi: $p>0.05$). To remove different sequencing depths, we normalised by rarefication. For consistency, we rarefied bacterial and fungal samples (bacteria: 25'000; fungi: 9'000 sequences per sample). Four bacterial and five fungal samples were detected as outliers and removed (CLOUD test, $p<0.05$). Alpha diversity was calculated as mean values after rarefying the dataset 100 times and each time calculating Shannon diversity. The effect of arsenic over

time on alpha diversity was tested with ANOVA (~Arsenic * Timepoint). Beta diversity was analysed by PERMANOVA with BC-distances (~Arsenic * Timepoint; 999 permutations), unconstrained and constrained ordinations. We further investigated the data by PERMANOVA for each timepoint (BC, ~Arsenic; 999 permutations) and corrected for multiple testing with a BH-Correction.

Plant phenotyping: Plant height and dry biomass were inspected for normality and transformed as needed (transformations in Table S3). Differences between sample groups were tested with ANOVA for the factors *arsenic* and *time* (~As*Ti).and for pairwise differences with the TukeyHSD test.

Plant microbiota profile: We removed 29 bacterial and 11 fungal samples due to too low sequencing depth. Samples were normalised by total sum scaling since no significant differences in sequencing depth were found (Kruskal-Wallis test, bacteria: $p>0.05$, fungi: $p>0.05$). Two bacterial and one fungal sample were removed because they were detected as outliers (CLOUD test, $p<0.05$). Shannon diversity was used as index for alpha-diversity to test the effect of Arsenic on the microbes in different compartments (ANOVA, ~Arsenic * Plant-compartments). Further we used Tukey's post-hoc test to test for pair-wise differences. BC-distances were calculated, PERMANOVA was used to test for differences in beta diversity (~Arsenic * Plant-compartments; 999 permutations), and outcomes were visualised in unconstrained and constrained ordinations. We further tested the Arsenic effect on beta diversity by PERMANOVA for each plant compartment (BC, ~Arsenic; 999 permutations) and corrected for multiple testing with a BH-Correction.

Results

The compositions of soil bacterial and fungal communities were not affected by arsenic over time

During five months of maize growing, we profiled the soil microbiota monthly samples for bacterial 16s rRNA gene amplicons and fungal first internal transcribed spacer region amplicons. We received bacterial and fungal samples ranging from 26'737 to 89'934 (median: 43'855) and 9'468 to 120'053 (median: 20'395) high-quality sequences, respectively. A rarefaction analysis confirmed that rarefying at 25'000 bacterial sequences and 9'000 fungal sequences per sample captured the microbiota diversity present in these samples (Figure S1). Around 80% of the bacterial microbiota community consisted of Acidobacteria, Alphaproteobacteria, Gammaproteobacteria and Verruccomicrobia in approximately similar abundances (Fig. 2A). In lower abundances we found Actinobacteria, Bacteriodetes, Chloroflexi, Deltaproteobacteria, Firmicutes, Gemmatimonadetes, Latescibacteria, Planctomycetes, Rokubacteria and other low abundant bacteria. A major part of the fungal community belonged in similar amounts to Ascomycota and Mortierellomycota. Next to them we found Basidiomycota, Chytridiomycota and other low abundant phyla.

We investigated the effect of applying arsenic to the microbiome over time. The alpha diversity remained unaffected in bacterial samples (ANOVA: $p = 0.57$) and fungal samples (ANOVA: $p = 0.71$) (Fig. 2B). No constant effect on the community composition over time was found (PERMANOVA: all $p > 0.05$) as visualized in a CAP ordination (Fig. 2C). In conclusion, we can say that different concentrations of arsenic did not affect the soil microbial community over the five months of our experiment duration.

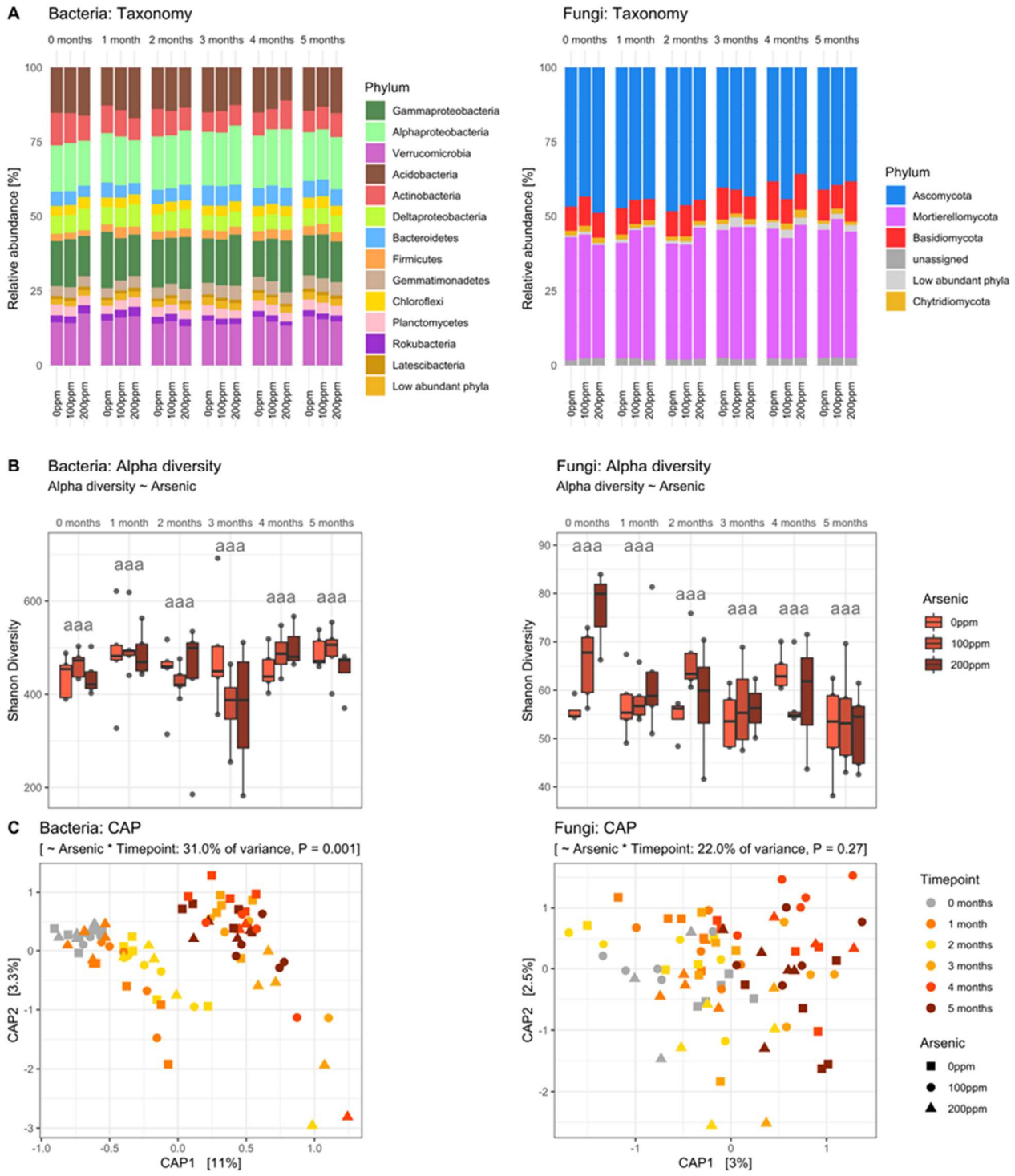


Figure 2. Soil microbiome over time in arsenic conditions. The mean abundances of bacterial (left) and fungal phyla (right) over five months under different arsenic concentrations are shown (A). Panels (B) represent alpha diversity measured by Shannon index and panels (C) beta diversity using Bray-Curtis distances and CAP analysis.

Soil enzyme activities were not impacted by arsenic in soil

To investigate the impact of the different arsenic concentrations on soil functioning, we measured the activities of five soil enzymes involved in the C-, N- and P-cycling. We selected the N-β-acetylglucosaminidase for the nitrogen cycle, the acid phosphatase for the phosphate cycle, as well as β-glucosidase, β-xylosidase and cellobiohydrolase for the degradation of increasingly complex substances of the carbon cycle. Enzyme activities were measured before sowing maize seeds (0 months) and three months after. For the N cycle, we did not observe any differences in N-β-acetylglucosaminidase activities due to the presence of arsenic, neither due to the presence of the maize plant (**Fig. 3A**). Acid phosphatase activities did not differ among different arsenic concentrations in soil, but it significantly increased at month 3 (**Fig. 3B**). The timepoint of 3 months corresponded to a BBCH stage of 60, when the phenological developmental is about at the stage of flowering (**Figure S2,** (Lancashire et al., 1991)). Similar to N-β-acetylglucosaminidase, also β-glucosidase, β-xylosidase and cellobiohydrolase activities did not show influences triggered by arsenic nor time (**Fig 3C-E**). Overall, we observed higher variation in the enzyme activities after three months. To summarize, we can say that we did not observe changes in the activity of these soil enzymes when exposed to increasing concentrations of arsenate.

Arsenic pollution impacts plant height and total shoot biomass

To evaluate the toxic impact of arsenic on plant growth, we measured weekly plant height, and we took the total shoot biomass at the end of the experiment (month 5). We observed that until week 6 (**Fig. 4A**), plant height differed significantly among the three arsenic treatments, showing a decreased growth with increasing arsenic concentrations. After week 6, this difference slowly disappeared between plants growing in 0 ppm and 100 ppm soils and from week 9 onwards, there was no difference any longer between the treatments. Likewise, also the maize plants growing in the soil with highest arsenic concentrations, had similar heights compared to the control and the 100 ppm treatments. However, this took much longer than the plants growing in 100 ppm, because only after 12 weeks (4 months) plant heights reached the same ranges as for 0 and 100 ppm. On the other hand, we noticed that the total shoot dry biomass (stem, leaves, cob) significantly decreased for plants growing in 200 ppm arsenic conditions compared to the control and the 100 ppm (**Fig. 4B**). Taken together these results show that there is a clear negative effect of arsenic on plant growth that is greater with increasing arsenic concentration. Nevertheless, arsenic mainly affected the shoot biomass of plants growing in 200 ppm soil while plant height did not differ among treatments by the end of the experiment. We concluded that arsenic in soil mostly influenced plant performance by having a negative impact on plant yield, while plant height was different among treatments at the beginning but equalized towards the end of the experiment.

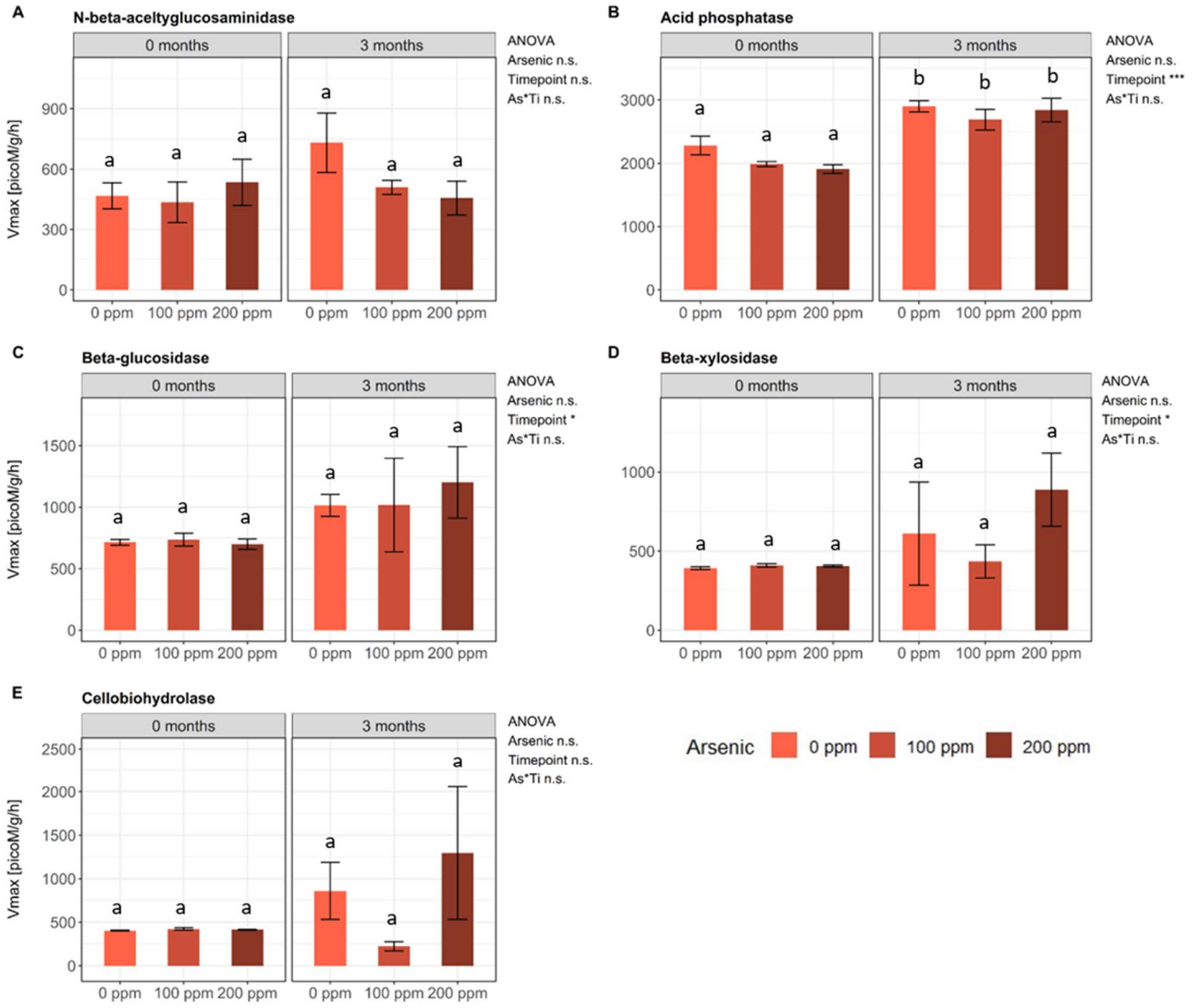


Figure 3. Enzyme activities in arsenic conditions. The panels represent respectively the following enzymes: (A) N-β-acetylglucosaminidase, (B) acid phosphatase, (C) β-glucosidase, (D) β-xylosidase, (E) Cellobiohydrolase. Each panel shows two facets that represent two timepoints (0 months - before sowing maize plants, 3 months). P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001. Different letters mean statistical significance among treatments.

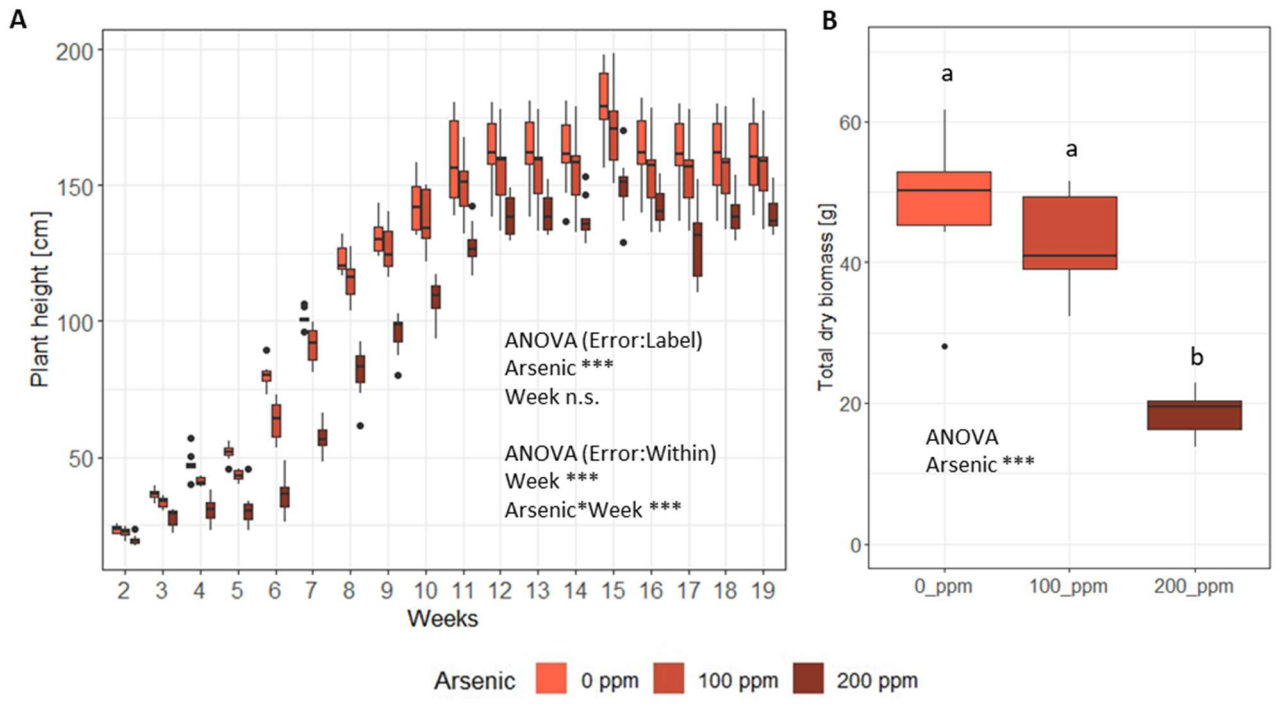


Figure 4. Plant response to arsenic treatment. The panels represent plant height (A) and the total aboveground dry biomass (including stem, leaves, and cob) (B) in the respective arsenic concentration in soil (0, 100, 200 ppm). P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001. Different letters mean statistical significance among treatments.

Soil microbial community changes because of the arsenic treatment but not the microbiomes of other plant compartments

16s rRNA gene amplicons and fungal first internal transcribed spacer region amplicons were sequenced from soil, roots, leaves, and kernels to investigate arsenic effects on microbes in different plant compartments after three months of maize growth. The sequencing of bacterial samples yielded 5'168 to 34'252 (median: 15'711) and 520 to 2'062 (median: 1'343) high-quality sequences for fungal samples. Leaf samples had a high sample-to-sample variation, but around 50% of the bacterial microbiome belonged to Gammaproteobacteria while the other 50% mainly belonged to Actinobacteria, Deltaproteobacteria, Firmicutes and Chloroflexi (**Fig. 5A**). Actinobacteria and Chloroflexi co-dominated the soil samples and made up to 75% of bacterial abundance. Abundant in the other 25% were Firmicutes and Gammaproteobacteria. 80% of the soil bacterial community had similar proportions of Actinobacteria, Alphaproteobacteria, Chloroflexi, Firmicutes and Gammaproteobacteria while the other 20% mainly belonged to Acidobacteria and Verrucomicrobia. Fungal communities were dominated by Ascomycota in root samples. Basidiomycota varied in abundance between ranging from absent to making up to 75% of the total community. Soil samples are co-dominated by Ascomycota and Mortierellomycota. Basidiomycota were similarly abundant in roots, varying between almost

absent and 75% of the total abundance. Fungal community members in kernel samples belong almost entirely to Ascomycota (Fig. 5A).

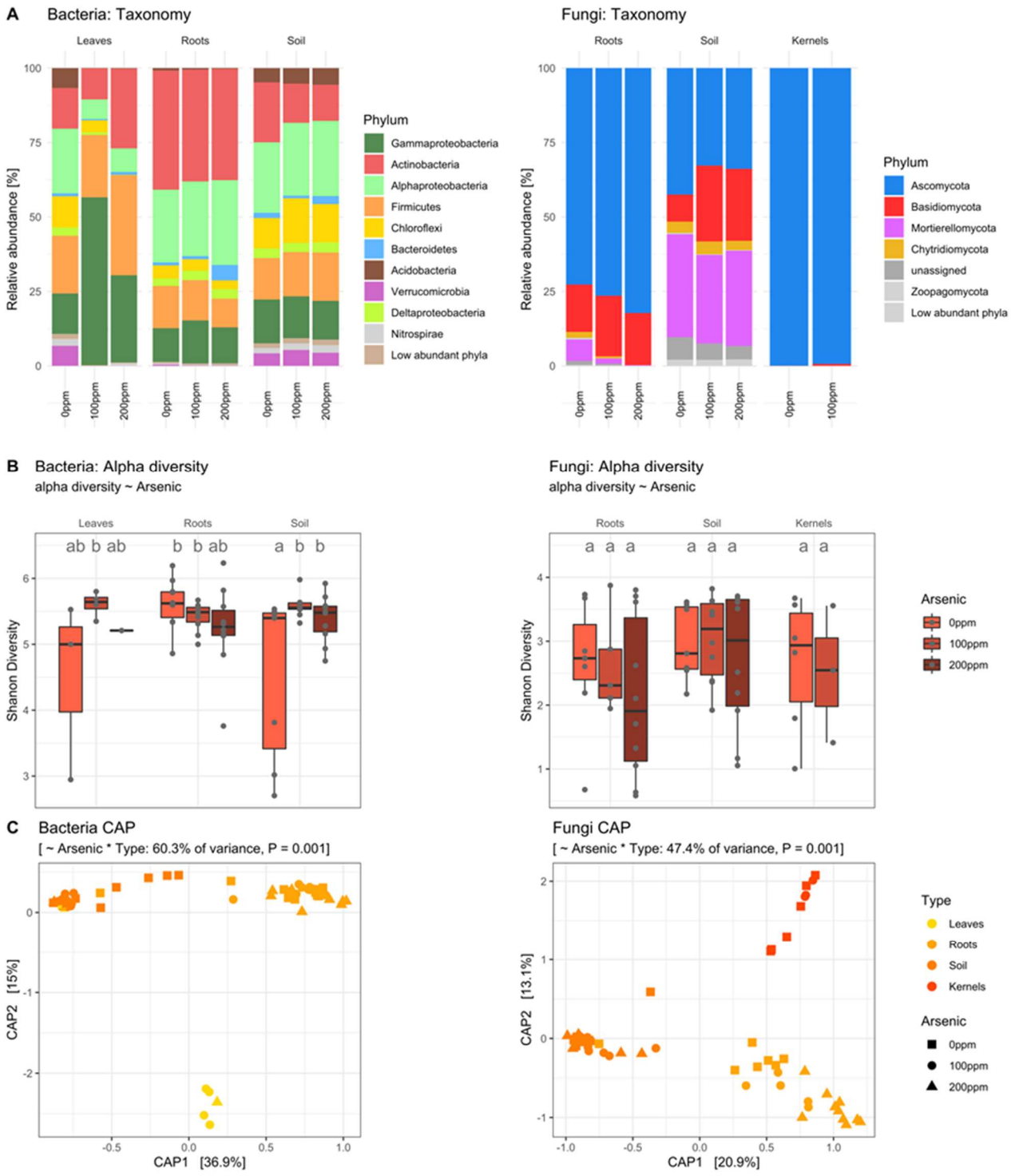


Figure 5. Microbiome in different plant compartments under arsenic conditions. The mean abundances of bacterial (left) and fungal phyla (right) in different plant compartments under arsenic concentrations are shown (A). Panels (B) represent alpha diversity measured by Shannon index and panels (C) beta diversity using Bray-Curtis distances and CAP analysis.

We analysed the effect of soil arsenic pollution on the microbial alpha and beta diversity in the different plant compartments (Fig. 5B). We found an increase on soil bacterial alpha diversity (ANOVA, $p = 0.04$) but not on fungal alpha diversity (ANOVA, $p = 0.35$). Further pairwise-comparisons between arsenic treatments showed higher bacterial alpha diversity in soil under arsenic conditions but not in other compartments. Bacterial and fungal communities in all compartments remained unaffected despite the arsenic treatment in soil (PERMANOVA: all $p > 0.05$) as shown in Fig. 5C. Take together these results showed an increase in soil bacterial diversity in presence of arsenic (and regardless of the concentration) while no changes in neither bacterial nor fungal communities in all other compartments.

Discussion

We investigated the effects of arsenic on soil enzymes activities, on soil and plant microbiomes as well as on plant performance. We performed a greenhouse experiment manipulating arsenic concentration in soil (0, 100 and 200 ppm) and measuring how soil and plant microbiomes, as well as soil enzymatic activity and plant growth were affected. We measured the activities of five enzymes as a proxy for soil functioning and sequenced both soil and plant microbiomes to determine their diversity and composition. We also scored plant growth, by measuring plant height and final biomass, to assess the impact of arsenic on plant performance. Using this set up we tested our hypothesis that increasing arsenic concentrations in soil cause shifts in soil microbial communities and negatively affect soil enzyme activities. We also expected changes in the plant-associated microorganisms, especially in the roots, and minor changes in the leaves and kernels microbiomes. In addition, we expect reduced growth of maize negatively correlated with the increasing arsenic concentration.

Arsenic is not the only factor that can influence soil microbes, in fact, it has been shown that bacterial communities' structure mainly by their soil type (Seulki et al., 2019; Sherlyn et al., 2018). An explanation that supports the fact that we did not observe any changes in soil microbial communities is that the soil we used, was incubated already for 2 months before use. This time could have given the time to the soil communities to adapt and activate arsenic resistance and metabolization mechanisms (Li et al., 2021; Yu et al., 2020) or that the concentration of arsenic was not high enough to impact the soil community. The fact that we did not observe changes in the soil microbial communities, is in line with the results on the enzyme activities where we also did not detect any influence by arsenic. Also, several studies have been working on the potential of fungi in arsenic bioremediation, and several saprophytic fungi are attractive candidates given their tolerance and accumulation of As in their biomass, but also their capability of producing siderophores (Ceci et al., 2020). This argument points to the fact that we do not necessarily need to see a shift in microbial communities, but soil microbes might still put in place strategies, such as production of siderophores, to counteract arsenic toxicity. Furthermore, arbuscular mycorrhizal fungi can also influence arsenic uptake in plant as they enhance and control the phosphate nutritional status in maize plant (Bai et al., 2008). To corroborate the DNA sequencing and enzymes activities in soil, expression of soil community functional genes could have been an additional analysis to verify the variations not only in genes responsible for As resistance but also for nutrient cycling (Xiong et al., 2012). Similarly, it would be worth to also introduce untargeted metabolites analysis to eventually identify the eventual enrichment of some metabolites because of arsenic presence.

Soil extracellular enzyme activities were measured to approximate the microbial element cycling in soil. Our results revealed that the activities of neither of the five measured enzymes were affected by the applied arsenic concentrations in soil. In fact, N-β-acetylglucosaminidase, acid phosphatase, β-glucosidase, β-xylosidase and cellobiohydrolase did not differ in their activities comparing control soil without arsenic and

the two arsenic contaminated soils (100 and 200 ppm). Controversial reports stem from previous studies that have shown an insensitivity of some enzyme activities to arsenic contamination (Lorenz et al., 2006), while others found that activity was adversely affected by arsenic (Cang et al., 2009; Speir et al., 1999). There are soil characteristics such as Fe/Al content, pH, soil organic matter, soil texture and cation exchange capacity that typically increase the mobilization of arsenic and thereby, increase the buffer capacity of the soil (Lake et al., 2021). In our case, iron content was relatively high and could explain the reduction in bio-accessibility of arsenic that would bind to it and be less accessible. Similarly, also the soil pH of 6.6 helps to have a reduced bio-accessibility, showed to be around 30%, compared to higher pH that sees it ramping until 60% with pH 8 (Lake et al., 2021). Furthermore, AsV that we used for the initial spiking of the soil is known to absorb faster to soil particles than other species (Pierce & Moore, 1980), it is also possible that the arsenic was not bioavailable and therefore did not disturb the biogeochemical cycles. In addition, environmental conditions and soil properties can influence the effectiveness and toxicity of arsenic in soil (Mclean & Bledsoe, 1992; Mestrot et al., 2011). We only observed an increase of acid phosphatase activity at the later timepoint compared to the early timepoint. This can be explained by the fact that at the earlier timepoint there was not a plant growing in the pot yet; however, at the later timepoint (3 months), maize was a fully grown and a mature plant. The increase in acid phosphatase can be therefore explained by the presence of the plant (van Wyk et al., 2017), which can trigger higher activity by mining for resources. This could be explained by the fact that at this developmental stage, maize plants were mining for phosphate to ensure the development of flowers and seeds (Naomi et al., 2021). Overall, the fact that we did not observe changes in enzyme activities can also be an unfortunate choice of the enzymes; in fact there is the possibility that there are other soil enzymes that would have been more sensitive to arsenic (Seulki et al., 2019). For example, sulphur-related enzymes could have been included as there is evidence that As biogeochemical cycling could interact with S cycling (Li et al., 2021). In conclusion, it could be useful to measure in future soil enzymes activities, by including also sulphur related enzymes, in soils with different characteristics to investigate to which extend we observe different impact of increasing arsenic concentrations.

To assess the impact of arsenic on plant growth and yield, we measured the maize height, and we dried the shoot biomass at the end of the experiment. Our results showed a negative effect of arsenic on plant height, mainly on young plants at the beginning of the experiment. However, after 7 weeks for the 100 mg/kg and 12 weeks for the 200 mg/kg of arsenic in soil, plants caught up with the control plants in term of height. Nevertheless, the big difference showed up in the shoot biomass, as the plants growing in the highly contaminated soil were much lighter than the two other treatments. This revealed a big impact of arsenic toxicity on maize yield. Similar results were obtained, where a significant decrease of dry matter weight in maize treated, however, with 100 mg/kg of arsenic already (Ci et al., 2012). This study suggested that maize adapted to arsenic concentration in soil, as there was no difference among plant heights by the end of the experiment probably because there were no other stresses (nutrient availability, light, …).

In addition, to investigate the possible effects of arsenic contamination in soil and plant-associated microbiomes, we sequenced the microbial communities of soil and several plant compartments (roots, leaves, and kernels). It has been shown that under heavy metal contaminated soils, several crops revealed selective rhizobiomes and indicated crop-specific heavy metal-microbe interactions (Sun et al., 2018), but in our study we did not observe difference in the roots-associated microbiome. Regarding the higher plant compartments, there are only few studies reporting the effect of heavy metals on plant leaves, which have shown an enrichment of some bacteria in the plant phyllosphere under contamination (Jia et al., 2018; Lui et al., 2017). We found an increase of species diversity in arsenic contaminated soil, compared to the control soil. This could eventually show an enrichment of taxa that can detoxify or cope better with arsenic. On the plant side, we did not find arsenic-specific changes in our bacterial and fungal communities in roots, leaves, and kernels; the community shifts that we observed were only compartment-dependent. It is surprising that we did not find any changes in the root microbiomes, as it is a compartment in direct contact with the arsenic contamination. It is less surprising that we did not find differences in leaf and kernel's microbiomes, it is in line with our hypothesis that compartments not directly in contact with the arsenic source would be only marginally or not at all affected. Altogether the results about plant phenotyping and microbiome under arsenic conditions suggest that arsenic contamination in soil have negative impact of plant yield and the diversity of the soil microbiome, but also that other trait such as plant height, roots, leaves, and kernels microbiomes stayed unaffected.

Overall, our study revealed negative impact of arsenic on plant performance in a soil-maize system with concentrations in soil of 200 mg/kg while no significant effects at 100 mg/kg. Our work highlights no impact of arsenic in soil microbial communities over time and enzyme activities in soil, and no influence as well on the plant-associated microbiomes. However, we noticed an increase of soil species diversity under arsenic conditions. We also observed the toxic effects of arsenic on crop yield, with a significant reduction in dry aboveground biomass accumulation for maize growing in the highly contaminated soil. This effect may suggest that the native soil microbial communities, which we worked with, are well-equipped to tolerate arsenic or they adapted to it during the soil incubation with arsenic (around 2 months). That could be the reason why we did not observe changes during our experiment. As far as the crop is concerned, maize mainly experienced an important reduction of biomass at the highest arsenic concentration, but no changes in the plant microbiomes. The next steps would be to further understand which potential changes in the soil microbial community can confer to the plant more resistance or susceptibility. This can help to unravel the potential in soil microbial communities to ensure crop yield in actively reducing arsenic toxicity in plant.

Author statement

VC performed the experiment together with a co-author. VC performed enzyme activities assays and collected plant phenotyping data. Co-authors performed microbiome analysis (Fig. 3, 5) and helped with the DNA library preparation.

Acknowledgements

Many thanks to Pia Bergamaschi, Andrea Weber and Xiaowen Lui for their help in setting up the experiment, taking all the samples and measurements and harvesting the experiment at the end; Bahar Razavi and Sebastian Loeppmann for helping in establishing the soil enzymes activities assay. Thank you also to Nichole Wetter for proofreading the English of this chapter. We acknowledge the University of Bern for supporting the Interfaculty Research Cooperation (IRC) 'One Health' (www.onehealth.unibe.ch).

Supplementary information

Table S1. Characteristics of the Q-Matte soil including the arsenic concentration.

pH	6.61 ± 0.04
Clay (%)	10.2 ± 0.8
Silt (%)	53 ± 2
Sand (%)	37 ± 3
Plant available P (mg/kg)	2.41 ± 0.02
Total carbon (g/kg)	26.49 ± 0.07
Nitrogen (g/kg)	2.91 ± 0.01
Sulfur (g/kg)	0.35 ± 0.03
Total organic carbon (g/kg)	25.41 ± 0.09
Arsenic (mg/kg)	2.9 ± 0.5
Magnesium (g/kg)	4.1 ± 0.5
Potassium (g/kg)	1.5 ± 0.1
Iron (g/kg)	17.7 ± 0.7
Manganese (g/kg)	0.74 ± 0.02

Table S2. N-β-acetylglucosaminidase, acid phosphatase, β-glucosidase, β-xylosidase and cellobiohydrolase were measured to determine soil functioning in different arsenic conditions. Variables were untransformed (§), log transformed (¥), sqrt transformed (∞) or ranked transformed (†) to meet the requirements to perform the analysis. F-values and significance levels for a two-way ANOVA with arsenic (As) and time point (Ti) as separate factors and their interaction term are shown. P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001. Different letters mean statistical significance among treatments.

	Arsenic	**Timepoint**	**As*Ti**
Variable	*F*	*F*	*F*
N-β-acetylglucosaminidase§	0.406	0.292	0.244
Acid phosphatase§	0.129	1.01e-06 ***	0.464
β-glucosidase†	0.7723	0.0341 *	0.2816
β-xylosidase†	0.1971	0.0369 *	0.2145
Cellobiohydrolase†	0.869	0.205	0.707

Table S3. Maize height and dry biomass were measured weekly and at the end respectively, to determine plant performance in different arsenic conditions. Variables were untransformed (§), log transformed (¥), sqrt transformed (∞) or ranked transformed (†) to meet the requirements to perform the analysis. F-values and significance levels for a two-way ANOVA with arsenic (As) and time point (Ti) with their interaction for plant height and only the factor arsenic for biomass. P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001. Different letters mean statistical significance among treatments.

	Error:Sample_ID		Error:Within	
	Arsenic	**Week**	**Week**	**As*W**
Variable	*F*	*F*	*F*	*F*
Plant height†	1.11e-08 ***	0.53	<2e-16 ***	<2e-16 ***

	Arsenic
Variable	*F*
Dry biomass†	0.0001 ***

Figure S1. Rarefaction of soil bacterial and fungal communities over time.

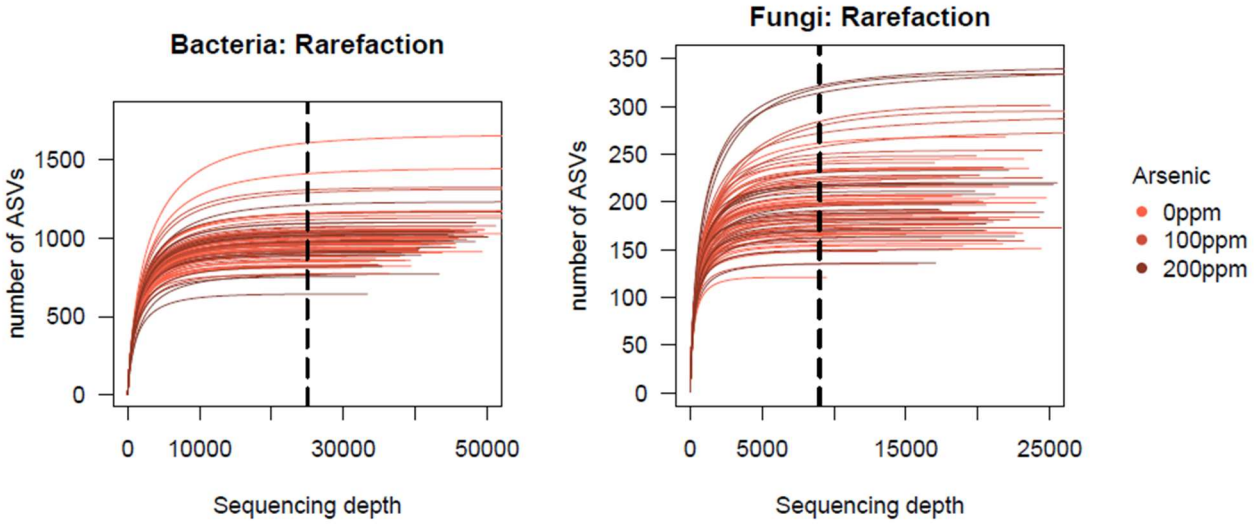


Figure S2. BBCH code corresponding to the development of maize over the entire experiment.

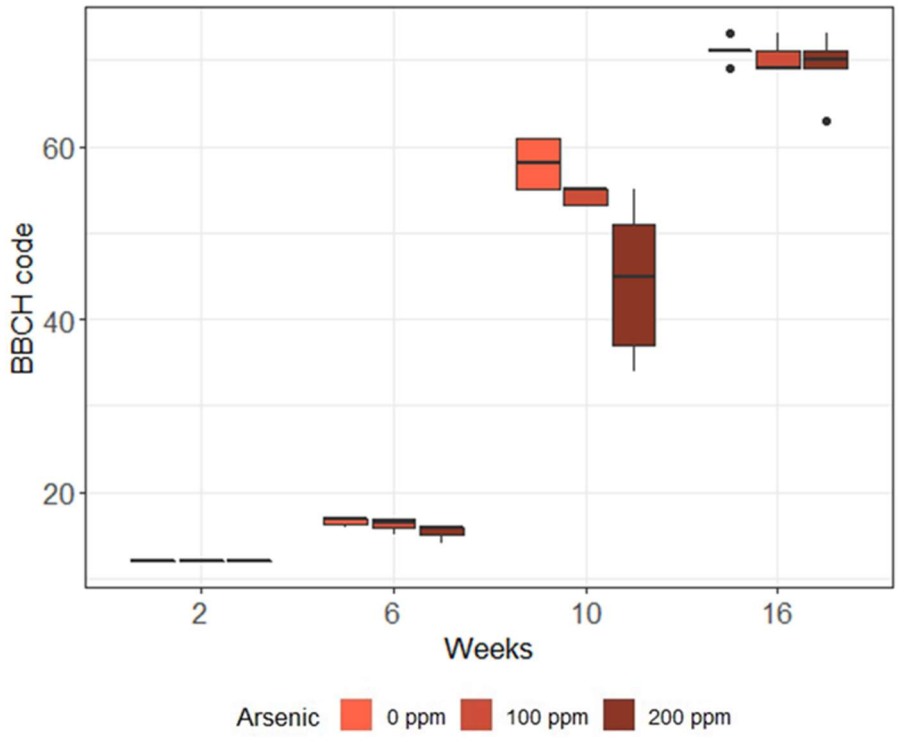


Table S4. Alpha diversity for soil microbial community.

factors	taxa	Sum.Sq	Mean.Sq	F.value	Pr..F.
Arsenic	bacteria	6614.706	3307.353	0.550386	0.579351
Timepoint	bacteria	60290.85	12058.17	2.006633	0.089106
Arsenic:Timepoint	bacteria	52115.7	5211.57	0.867272	0.567534
Residuals	bacteria	396604.2	6009.155	NA	NA
Arsenic	fungi	241.5426	120.7713	1.816901	0.171214
Timepoint	fungi	1170.764	234.1527	3.522627	0.007309
Arsenic:Timepoint	fungi	1063.634	106.3634	1.600146	0.128125
Residuals	fungi	4054.734	66.47105	NA	NA

Table S5. Beta diversity for soil microbial community.

taxa	Timepoint	Factors	SumOfSqs	R2	F	p	p_adj
bacteria	0 months	Arsenic	0.0547278	0.1193467	1.0841649	0.232	0.84
bacteria	0 months	Residual	0.4038335	0.8806533	NA	NA	NA
bacteria	0 months	Total	0.4585613	1	NA	NA	NA
bacteria	1 month	Arsenic	0.0470439	0.0783208	0.6798094	1	1
bacteria	1 month	Residual	0.5536132	0.9216792	NA	NA	NA
bacteria	1 month	Total	0.6006571	1	NA	NA	NA
bacteria	2 months	Arsenic	0.0460925	0.1107322	0.9961647	0.447	0.84
bacteria	2 months	Residual	0.3701599	0.8892678	NA	NA	NA
bacteria	2 months	Total	0.4162524	1	NA	NA	NA
bacteria	3 months	Arsenic	0.0573858	0.117863	0.9352758	0.578	0.84
bacteria	3 months	Residual	0.4295	0.882137	NA	NA	NA
bacteria	3 months	Total	0.4868858	1	NA	NA	NA
bacteria	4 months	Arsenic	0.0523843	0.1201853	0.9562206	0.6	0.84
bacteria	4 months	Residual	0.3834787	0.8798147	NA	NA	NA
bacteria	4 months	Total	0.435863	1	NA	NA	NA
bacteria	5 months	Arsenic	0.0454141	0.0986303	0.8753812	0.7	0.84
bacteria	5 months	Residual	0.415034	0.9013697	NA	NA	NA
bacteria	5 months	Total	0.4604481	1	NA	NA	NA
bacteria	0 months	Arsenic	0.0599364	0.1219047	1.1106282	0.257	0.4215
bacteria	0 months	Residual	0.4317299	0.8780953	NA	NA	NA
bacteria	0 months	Total	0.4916663	1	NA	NA	NA
bacteria	1 month	Arsenic	0.110138	0.1959982	1.950227	0.009	0.054
bacteria	1 month	Residual	0.4517955	0.8040018	NA	NA	NA
bacteria	1 month	Total	0.5619335	1	NA	NA	NA
bacteria	2 months	Arsenic	0.053746	0.0877976	0.7699833	0.888	0.888
bacteria	2 months	Residual	0.5584122	0.9122024	NA	NA	NA
bacteria	2 months	Total	0.6121582	1	NA	NA	NA
bacteria	3 months	Arsenic	0.0984018	0.1767168	1.5025419	0.037	0.111
bacteria	3 months	Residual	0.4584318	0.8232832	NA	NA	NA
bacteria	3 months	Total	0.5568336	1	NA	NA	NA
bacteria	4 months	Arsenic	0.0647678	0.1311381	0.9055856	0.574	0.6888
bacteria	4 months	Residual	0.4291224	0.8688619	NA	NA	NA
bacteria	4 months	Total	0.4938902	1	NA	NA	NA
bacteria	5 months	Arsenic	0.0524485	0.1303747	1.0494434	0.281	0.4215
bacteria	5 months	Residual	0.3498424	0.8696253	NA	NA	NA
bacteria	5 months	Total	0.4022909	1	NA	NA	NA
fungi	0 months	Arsenic	0.1024001	0.13041	1.0497704	0.329	0.579
fungi	0 months	Residual	0.682817	0.86959	NA	NA	NA
fungi	0 months	Total	0.7852171	1	NA	NA	NA
fungi	1 month	Arsenic	0.1133928	0.1235399	1.1276258	0.16	0.48
fungi	1 month	Residual	0.8044714	0.8764601	NA	NA	NA
fungi	1 month	Total	0.9178643	1	NA	NA	NA
fungi	2 months	Arsenic	0.1140591	0.1249106	0.9991826	0.386	0.579
fungi	2 months	Residual	0.7990665	0.8750894	NA	NA	NA
fungi	2 months	Total	0.9131256	1	NA	NA	NA
fungi	3 months	Arsenic	0.1173214	0.115816	0.9169043	0.627	0.7524
fungi	3 months	Residual	0.8956768	0.884184	NA	NA	NA
fungi	3 months	Total	1.0129982	1	NA	NA	NA
fungi	4 months	Arsenic	0.1134336	0.095414	0.8438244	0.942	0.942
fungi	4 months	Residual	1.0754233	0.904586	NA	NA	NA
fungi	4 months	Total	1.1888569	1	NA	NA	NA
fungi	5 months	Arsenic	0.1365193	0.1254367	1.1474228	0.146	0.48
fungi	5 months	Residual	0.9518326	0.8745633	NA	NA	NA
fungi	5 months	Total	1.0883518	1	NA	NA	NA
fungi	0 months	Arsenic	0.0850337	0.1355746	0.7841894	0.97	0.97
fungi	0 months	Residual	0.5421756	0.8644254	NA	NA	NA
fungi	0 months	Total	0.6272093	1	NA	NA	NA
fungi	1 month	Arsenic	0.1280611	0.1292008	1.1869628	0.049	0.147
fungi	1 month	Residual	0.8631176	0.8707992	NA	NA	NA
fungi	1 month	Total	0.9911786	1	NA	NA	NA
fungi	2 months	Arsenic	0.1329757	0.1662684	1.1965608	0.102	0.204
fungi	2 months	Residual	0.6667893	0.8337316	NA	NA	NA
fungi	2 months	Total	0.799765	1	NA	NA	NA
fungi	3 months	Arsenic	0.121421	0.1591377	0.9462766	0.643	0.7716
fungi	3 months	Residual	0.6415726	0.8408623	NA	NA	NA
fungi	3 months	Total	0.7629936	1	NA	NA	NA
fungi	4 months	Arsenic	0.1410994	0.1524642	1.0793466	0.258	0.387
fungi	4 months	Residual	0.7843599	0.8475358	NA	NA	NA
fungi	4 months	Total	0.9254593	1	NA	NA	NA
fungi	5 months	Arsenic	0.1817945	0.1528145	1.4430318	0.025	0.147
fungi	5 months	Residual	1.0078477	0.8471855	NA	NA	NA
fungi	5 months	Total	1.1896422	1	NA	NA	NA

Table S6. Alpha diversity and pairwise test for plant-associated microbiomes (soil, roots, leaves, kernels).

factors	taxa	Sum.Sq	Mean.Sq	F.value	Pr..F.
Arsenic	bacteria	2.377944	1.188972	3.267335	0.045486
Type	bacteria	1.005294	0.502647	1.38129	0.259681
Arsenic:Type	bacteria	5.599781	1.399945	3.847097	0.007852
Residuals	bacteria	20.37821	0.363897	NA	NA
Arsenic	fungi	1.992299	0.996149	1.053977	0.356164
Type	fungi	2.969442	1.484721	1.57091	0.217941
Arsenic:Type	fungi	0.184065	0.061355	0.064917	0.978185
Residuals	fungi	47.25672	0.945134	NA	NA

Taxa	Type	Arsenic	Shannon	.group
Bacteria	Leaves	0ppm	4.491096	ab
Bacteria	Leaves	100ppm	5.608205	b
Bacteria	Leaves	200ppm	5.208572	ab
Bacteria	Roots	0ppm	5.612284	b
Bacteria	Roots	100ppm	5.417915	b
Bacteria	Roots	200ppm	5.237831	ab
Bacteria	Soil	0ppm	4.487711	a
Bacteria	Soil	100ppm	5.580513	b
Bacteria	Soil	200ppm	5.386892	b
Bacteria	Roots	0ppm	2.637207	a
Bacteria	Roots	100ppm	2.623109	a
Bacteria	Roots	200ppm	2.115687	a
Bacteria	Soil	0ppm	2.971508	a
Bacteria	Soil	100ppm	3.034652	a
Bacteria	Soil	200ppm	2.70147	a
Bacteria	Kernel	0ppm	2.652599	a
Bacteria	Kernel	100ppm	2.504857	a

Table S7. Beta diversity for plant-associated microbiomes (soil, roots, leaves, kernels).

taxa	Type	Factors	SumOfSqs	R2	F	p	p_adj
bacteria	Leaves	Arsenic	0.26222687	0.1300637	0.7475475	0.539	0.539
bacteria	Leaves	Residual	1.75391441	0.8699363	NA	NA	NA
bacteria	Leaves	Total	2.01614128	1	NA	NA	NA
bacteria	Roots	Arsenic	0.13124326	0.0576722	1.1016323	0.313	0.539
bacteria	Roots	Residual	2.14443495	0.9423278	NA	NA	NA
bacteria	Roots	Total	2.27567821	1	NA	NA	NA
bacteria	Soil	Arsenic	0.08314095	0.0573663	0.9128617	0.497	0.539
bacteria	Soil	Residual	1.36615893	0.9426337	NA	NA	NA
bacteria	Soil	Total	1.44929988	1	NA	NA	NA
bacteria	Leaves	Arsenic	0.13674595	0.1746931	0.423341	0.5	0.9045
bacteria	Leaves	Residual	0.64603212	0.8253069	NA	NA	NA
bacteria	Leaves	Total	0.78277808	1	NA	NA	NA
bacteria	Roots	Arsenic	0.08702913	0.031394	0.5834066	0.982	0.982
bacteria	Roots	Residual	2.68513311	0.968606	NA	NA	NA
bacteria	Roots	Total	2.77216224	1	NA	NA	NA
bacteria	Soil	Arsenic	0.07890575	0.0529293	0.8383105	0.603	0.9045
bacteria	Soil	Residual	1.41187105	0.9470707	NA	NA	NA
bacteria	Soil	Total	1.4907768	1	NA	NA	NA
fungi	Roots	Arsenic	0.18466969	0.046543	0.4881495	0.952	0.991
fungi	Roots	Residual	3.78305583	0.953457	NA	NA	NA
fungi	Roots	Total	3.96772552	1	NA	NA	NA
fungi	Soil	Arsenic	0.09865544	0.0294988	0.4559318	0.991	0.991
fungi	Soil	Residual	3.24573001	0.9705012	NA	NA	NA
fungi	Soil	Total	3.34438545	1	NA	NA	NA
fungi	Kernels	Arsenic	0.23204462	0.1079043	0.8466917	0.502	0.991
fungi	Kernels	Residual	1.91842247	0.8920957	NA	NA	NA
fungi	Kernels	Total	2.15046709	1	NA	NA	NA
fungi	Roots	Arsenic	0.24446003	0.0449567	0.7060939	0.858	0.975
fungi	Roots	Residual	5.19321915	0.9550433	NA	NA	NA
fungi	Roots	Total	5.43767918	1	NA	NA	NA
fungi	Soil	Arsenic	0.10939953	0.0331444	0.5142093	0.975	0.975
fungi	Soil	Residual	3.19129365	0.9668556	NA	NA	NA
fungi	Soil	Total	3.30069318	1	NA	NA	NA

Chapter III

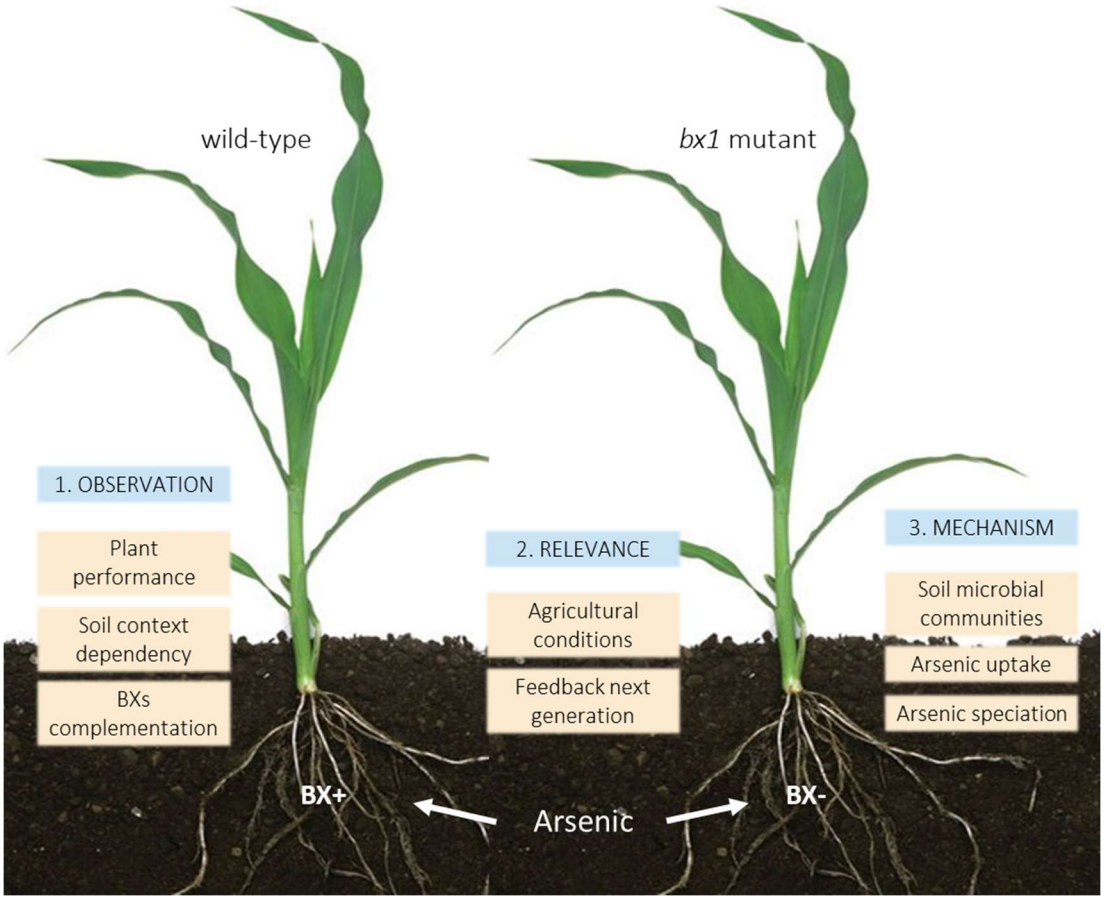
Root exuded benzoxazinoids increase arsenic tolerance of maize plants

Veronica Caggìa[1], Jan Waelchli[2], Gabriel Deslandes[1], Stephanie Pfister[3], Anika Sidler[3], Pierre Mateo[1], Christelle A. M. Robert[1], Sandra Spielvogel[4,5], Adrien Mestrot[3], Matthias Erb[1] and Klaus Schlaeppi[1,2]

[1] Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland
[2] Department of Environmental Sciences, University of Basel, Bernoullistrasse 32, 4056 Basel, Switzerland
[3] Institute of Geography, University of Bern, Hallerstrasse 12, CH-3012 Bern, Switzerland
[4] Institute of Plant Nutrition and Soil Science, Christian-Albrechts-Universität, Hermann-Rodewald-Strasse 2, D-24118 Kiel, Germany
[5] Institute of Bio- and Geosciences, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany

Abstract

By releasing secondary metabolites into the environment, plants can modify their environment to their advantage. So far, little is known about the potential of secondary metabolites to help plants to cope with heavy metals such as arsenic, whose toxicity is a widespread problem in agriculture. Here, we evaluated how benzoxazinoid, a major class of secondary compounds released by cereals such as wheat and maize, affects arsenic tolerance of maize plants. Benzoxazinoid producing maize plants were more tolerant to arsenic contamination than benzoxazinoid deficient mutant plants, in three different soils in the greenhouse and the field. Benzoxazinoid soil complementation of mutant plants largely restored the wild-type phenotype, suggesting that benzoxazinoids act directly in the soil. Arsenic levels were lower in the roots, but not the shoots of wild type plants. Microbiota analysis showed only very subtle changes between the two maize genotypes in arsenic contaminated conditions, suggesting that microbes were not responsible for the differential growth and root As accumulation phenotypes. In conclusion, we show that exuded benzoxazinoids can help maize plants to tolerate benzoxazinoids across different environments. This finding expands our knowledge on the role of plant secondary metabolites in counteracting arsenic toxicity in soil and how this phenomenon is agriculturally relevant for crop rotation systems.



Key words: arsenic, benzoxazinoids, arsenic tolerance, W22 wild-type, *bx1* mutant, uptake and speciation

Introduction

Arsenic is naturally found in soil and groundwater due to natural geogenic origins (Amini et al., 2008), but also due to contaminations which can also be generated from anthropogenic activities, such as agriculture (Han et al., 2003; Matschullat, 2000). When crops are grown in arsenic-contaminated soil or irrigated by contaminated groundwater, As is taken up by crops, thereby entering the food chain and may ultimately reach humans through livestock feed or direct consumption of plant-based products. It is therefore clear that arsenic in the environment causes harmful effects to micro- and macrofauna, crop plants, livestock and humans (Guan et al., 2021; Zhao et al., 2010). Plants suffer from many physiological damages due to arsenic exposure.

Soils represent a long-term sink for toxic elements such as arsenic (Abid et al., 2020). Arsenic is particularly dangerous as it behaves chemically similarly to analogue of phosphate (both As and P share the same element characteristics; they are above each other in the periodic table), and therefore, it finds its entry to the plant through canonical phosphate transporters (Cao & Ma, 2004; Meharg & Macnair, 1990; Wu et al., 2011). High arsenic concentrations can typically lead to the formation of reactive oxygen species (ROS) with consequent damage to proteins, nucleic acid, and generate oxidative degradation of lipids in the cell membranes (Møller et al., 2007). These changes cause harmful effects on plant growth by inducing cell necrosis and chlorosis (Ci et al., 2012; Singh et al., 2006; Stoeva et al., 2004). Arsenic can therefore be fatal to plants. The two dominant inorganic As species in soils are arsenate (AsV) and arsenite (AsIII). Although arsenite represents only 1-30% of inorganic arsenic in the soil, it is significantly more toxic than arsenate (Hong et al., 2014).

Plants have evolved several detoxification mechanisms that allow them to cope with arsenic. For example, maize plants are able to reduce arsenate to arsenite (Ci et al., 2012; Xu et al., 2007), and can thus evacuate it more efficiently out of the plant. In addition, it has recently been proposed that plants can tolerate arsenic contamination in soil by enhancing the exudation of phytochelatins and coumarins (Frémont et al., 2022). Thus, plant secondary metabolites may function in heavy metal tolerance and detoxification in the soil-plant system.

Soil is the most abundant and diverse sink of microorganisms present on Earth. Soil is also the foundation of our food chain; therefore, its health needs to be taken into consideration when assessing human health (Banerjee & Heijden, 2022). Microbiomes are the link between the different compartments of the food chain (soil, plant animals and humans) and they allow the interactions between interfaces of the same trophic level (e.g., soil-plant interface). Almost all microbes are resistant to inorganic arsenic (AsV and AsIII) (David Paez-Espino et al., 2009) and have the potential to transform it or influence its mobility (Sessitsch et al., 2013; Ström et al., 2002) and bioavailability (Ma et al., 2016). Thus, they may help plants to grow in arsenic contaminated soils. Effects are likely dynamics, as root exudates may influence the species and availability of metal(loid)s, while the rhizosphere microbial processes may affect the assimilation of metal(loid)s into the plants (Badri et al., 2009).

However, the interactions among plants, rhizosphere microbiota, and heavy metal contamination have not been sufficiently documented. A proper understanding of environment-microbe interactions, especially the metal-microbe interactions in contaminated arable soils, is a prerequisite for metal(loid) stabilization and detoxification, which consequently contribute to food security and safety.

Benzoxazinoids (BXs) are major secondary metabolites produced by cereals such as wheat and maize. They have been shown to be crucial in both insect herbivore and pathogen resistance (Maag et al., 2016; Oa et al., 2011), and their production and accumulation are enhanced upon attack (Köhler et al., 2015; Tzin et al., 2017). In addition, benzoxazinoids have been shown to trigger callose formation upon herbivore feeding and pathogen infection on maize plants (Ahmad et al., 2011). Furthermore, they also play a role in allelopathy, by influencing neighbouring plant performance (Hussain et al., 2022). Finally, benzoxazinoids have also been discussed for their metal-chelating properties (Zhou et al., 2018). In fact, benzoxazinoids are secreted into the rhizosphere and are known to have high affinity for iron (Tipton & Buell, 1970) and it has been shown their ability in forming complexes with iron to favour its uptake (Hu et al., 2018a). Similarly, it was shown that benzoxazinoids have also the capability of binding aluminium and reducing its toxicity effects in maize roots (Poschenrieder et al., 2005). Besides their role in pest and pathogen resistance, micronutrients uptake and allelopathy, benzoxazinoids have been proposed to reduce metal toxicity in maize.

Here, we studied the potential role of benzoxazinoids in arsenic tolerance of maize. As benzoxazinoids interact with metalloids, we hypothesized that they may also influence arsenic availability. We therefore tested if wild-type maize plants producing benzoxazinoids will cope better with arsenic in soil compared to benzoxazinoid-deficient mutants that exude less benzoxazinoids into the soil. We expected greater plant performance of the WT (plant height, chlorophyll content and dry leaves biomass) and less arsenic uptake in plant tissue of maize exuding benzoxazinoids compared to the mutant. Concerning the uptake, we expected that WT would take up more arsenic than the *bx1* mutant knowing the chelation properties of BXs; this could be a way to sequestrate arsenic from soil where it is a threat to plant health and store it safely in the plant (e.g., vacuoles).

Here, we studied the potential role of benzoxazinoids in arsenic tolerance of maize. As benzoxazinoids interact with metalloids, we hypothesized that they may also influence arsenic tolerance. We thus investigated the interaction between benzoxazinoids (plant roots exudates) and arsenic in soil. To understand the role of benzoxazinoids in this interaction, we used wild type and BX-deficient mutant plants. To evaluate the possible effect of benzoxazinoids on arsenic toxicity and therefore the feedbacks on the crop plant, we measured plant height, chlorophyll content and shoot biomass as plant performance parameters. We performed a series of experiments to confirm the dependency of the phenotype on benzoxazinoids. We also assessed whether the effect is agriculturally relevant in a field experiment and determined potential advantages for other plants in a plant-soil feedback setup.

Furthermore, we tested potential mechanisms that can mediate this observation such as the evaluation of changes in rhizosphere microbial communities and changes in arsenic uptake and speciation. With our study, we demonstrate that arsenic increase maize tolerance to benzoxazinoids. Our experiments also provide evidence that benzoxazinoids act in the soil and reduce arsenic uptake.

Methods

Greenhouse experiments

Soil spiking with arsenic and incubation: In spring 2018, we screened numerous agricultural soils for experiments. We chose to work with the silty loam soil (**Table S1**) from 'Q-Matte' in Frauenkappelen, Switzerland (46°57'20.5''N, 7°19'58.3''E), as it contained low levels of arsenic (2.9 ± 0.5 mg/kg). The A-horizon was collected and stored *en bloc* (24 m^3) next to our research facility in Ostermundigen (Bern, Switzerland). In June 2020, we collected soil and sieved it to 1 cm. We measured the soil water content by weighing a precise amount of soil (n = 3) and then drying it. Twenty kg of moist soil were decanted in 5 transparent plastic boxes (REGALUX Clear Box L, 54.8 x 38.4 x 28.3 cm, Bauhaus, Switzerland) and kept semi-closed with the respective plastic lid (REGALUX Clear Box-Deckel, 54.8 x 38.4 cm, Bauhaus, Switzerland). The soil in each box was watered with arsenic salt (Sodium arsenate dibasic heptahydrate, ≥ 98%, Sigma-Aldrich, Switzerland) dissolved in MilliQ water to reach 100 ppm of arsenic and 60% water content following the method of Guan et al (unpublished). The soil was watered with arsenic solution in 4 steps and in-between we mixed it thoroughly to spread the solution homogeneously. For the control treatment without arsenic, we applied the same procedure, just without adding the sodium arsenate to the MilliQ water. The soil was incubated at room temperature (**Fig. S1A**) for six weeks at 60% water holding capacity (WHC), allowing As equilibration between soil water and soil phases and simulating aging (Song et al., 2009). We merged and homogenized again the soil of the 5 plastic boxes before decanting it in the pots.

Greenhouse experiments: All the greenhouse experiments were prepared in the same way as described below. We used the maize (*Zea mays L.*) inbred line W22 and *bx1* and *bx2* mutant plants in the W22 background (Tzin et al., 2015) The experimental design included soil spiked with arsenic (100 ppm) and the control soil (0 ppm) prepared as explained before. We added a precise amount of As spiked or control soil (1.8 kg) at 60% WHC in each pot (Rosentopf Soparco 2.0 L, Hortima AG, Switzerland). Each pot had a underpot (Untersetzer mit flachem Rand 16 cm, Hortima AG, Switzerland) to avoid cross-contamination during watering. Maize seeds (W22 and *bx1*) were surface sterilized by soaking them in commercial bleach containing 5 % active hypochlorite (Potz Javel-Wasser Natur, Migros, Switzerland) for 6 minutes, mixing from time to time. Afterwards, the bleach was removed, and the seeds were washed 5-6 times with autoclaved MilliQ water. For pre-germination, the seeds were soaked in autoclaved MilliQ water in the dark for 8h. After 8h, the seeds were placed on a moist filter paper (Rundfilter Sorte 1 Whatman, 90mm, Huberlab, Switzerland) in plastic Petri dishes (Petri dish 94x16mm, without vents, sterile, Greiner Bio-One, Switzerland) overnight, before sowing them. All the work was performed under the clean bench with air flow.

We sowed three surface sterilized and pre-germinated seeds per pot to ensure germination. After germination, we removed the extra seedling and left only one seedling per pot. Maize plants grew for 6 weeks

under greenhouse conditions (ranges: light/dark 14h:10h, daily temperature 14°C-22°C, night temperature 10°C-14°C, humidity 50%-70%) and the pots were randomized weekly in the greenhouse (**Fig. S1.B**). We fertilized maize plants from week 2 with Plantaktiv Typ K (concentration 2 g/L, Hauert, Switzerland) by dissolving 50 g of nutrient salt in 1 L of water (stock solution). We transferred 80 mL of the stock solution in 2 L container, and we diluted by making the volume with water. At weeks 2 and 3 after sowing, plants were complemented with 100 mL of nutrients solution and from week 4 with 200 mL. We measured plant height and chlorophyll content weekly or every two weeks. Plant height was determined by stretching the leaves and measuring the highest point; chlorophyll content was determined with SPAD meter (Chlorophyll meter SPAD-502, Minolta Camera CO., LTD., Japan) by doing the average of nine measurements on the youngest fully open leaf (three on the tip, three in the middle, and three at the base of the leaf). Leaves were dried in an oven (UF 1060 Plus, Memmert Experts in Thermostatics, Hettich Laborapparate, Switzerland) at 70°C for 72h to obtain the dry biomass.

Bx2 genotype for phenotype confirmation: Soil spiking, soil water content and plant growing conditions were done as described previously. In this experiment, we grew W22 wild-type, *bx1* mutant and *bx2* mutant (Tzin et al., 2015) side by side in arsenic-free and arsenic-contaminated soils. Plant height and chlorophyll content were measured every two weeks and dry leaves biomass was dried and weighed as described in the previous section.

Field experiment: The test for the importance of benzoxazinoids in arsenic tolerance in the field, we chose an agricultural area with geogenic arsenic (Schmutz Daniel, 2018a). The contaminated region is situated in the Canton Basel, Switzerland, and in a well constrained area in Liesberg. In these soils, arsenic concentrations in soils have been found to exceed the remediation value. The Canton of Basel-Landschaft has measured the arsenic concentration of food crops (potato, lettuce, and cabbage stalk) growing on the contaminated soil (Schmutz Daniel, 2018a) and grass from permanent grassland, which is used as feed (Schmutz Daniel, 2018b). We grew plants in a heavily contaminated field (430 mg/Kg) and a poorly contaminated field (43 mg/Kg) (**Fig. S2**) in proximity, managed by the same farmer. In each field, we had 6 subplots where we planted both genotypes (W22 and *bx1*, n = 6 per subplot) in a line. The other lines were planted with a commercial maize hybrid using normal agricultural practice (**Fig. S3.A, B**). Seeds were planted on the 1st of June 2021 and the plants harvested on the 21st of September 2021 before the farmer's automated harvest (**Fig. S3.C**). The fields were managed by the farmer following the details in **Table S2**, and so were our plants. Plant height and chlorophyll content were measured 4 times during the period of growth (2, 5, 9 and 14 weeks after sowing). Plant height was determined by stretching the leaves and measuring the highest point. Chlorophyll content

was determined with the SPAD meter (Chlorophyll meter SPAD-502, Minolta Camera CO., LTD., Japan) by taking the average among nine measurements (three at the base, three in the middle and three on the tip of the leaf). After 14 weeks of growth, plants were harvested by cutting the aboveground part 1 cm from the crown roots to separate it from the roots that were sampled and washed with tap water. Both aboveground and belowground parts were dried in an oven (UF 1060 Plus, Memmert Experts in Thermostatics, Hettich Laborapparate, Switzerland) at 70°C for 72h.

Total arsenic analysis in soil, roots and leaves: After sampling the fresh samples, we freeze dried them for 72h in a lyophilizer (Swiss Vacuum Technologies SA, Telstar LyoQuest, Switzerland) then ground with a Retsch MM400 Mixer Mill (Fisherbrand™, Waltham, MA) at 30 Hz for 2 min. We weighed one 50 mL falcon tube with lid (Sigma Corning® 50 mL centrifuge tubes, polypropylene, conical bottom w/ CentriStar cap, sterile, Sigma-Aldrich, Switzerland) per sample and noted down the exact weight. Then we weighed directly in the centrifuge tubes, 0.25 g of freeze-dried material, recording the exact weight. As a control, we also included triplicates for blanks and tomato leaves (1573a) as certified reference material (certified arsenic concentration of 0.112 ± 0.004 mg kg^{-1}) for each microwave run. Under the chemical hood, we added 4 mL of self-distilled concentrated HNO_3 (65%) self-distilled to each sample and we allowed it to sit overnight with the cap not tightly closed. We added 2 mL of 30% (w/w) peroxide (Suprapur H_2O_2; Sigma-Aldrich®, Switzerland) to each tube in 5 min intervals to prevent over frothing according to the following sequence: 200 µl, 800 µl and 1 mL. The samples were left to sit at room temperature under the chemical hood for 30 minutes until the frothing subsided. We vortexed each sample before the microwave run. For the microwave digestion (Microwave Digestion System MARS™ 6; CEM GmbH, Kamp-Lintfort, Deutschland) (Norton et al., 2013). We distributed the tubes evenly on the tray, leaving the caps loose. The temperature probe was added into one of the samples and the program "Open Basic Full" was selected (55°C for 10 min, 75°C for 10 min and 95°C for 30 min). After digestion, the samples were diluted to 50 mL with MilliQ water, weighed again (tube + lid) and stored at 4°C until analysis. Before diluting the samples for analysis, we centrifuged them at 2500 rpm for 5 min (Multifuge™ X1 Centrifuge Series, Thermo Scientific™, Reinach, Switzerland). For dilution, we transferred an aliquot to 15 mL centrifuge tubes (Sigma Corning® 15 mL centrifuge tubes, polypropylene, conical bottom w/ CentriStar cap, sterile, Sigma-Aldrich, Switzerland) and added 1% HNO_3 for the analysis by inductively coupled plasma mass spectrometer (ICP-MS; 7700x Agilent Technologies, Santa Clara, California). For the soil samples, minor elements were diluted with a dilution factor of 5, major elements were diluted with a dilution factor of 200 and the CRM with a dilution factor of 2. Roots and leaves samples were diluted with a dilution factor of 5 for minor elements, a dilution factor of 100 for major elements and CRM with a dilution factor of 2. The samples that exceeded the calibration curve of the major elements were further diluted with a dilution factor of 2 (in total DF200) and

remeasured. The multielement analysis included as minor elements As, B, Cu, Mn, Na, Zn, Al, V, Ba, Co, Ni, Se, Sr, Cd, Pb, Cs, Tl and as major elements P, Fe, Mg, Ca, K.

DNA extraction and library preparation: DNA extraction was based on the EMP DNA Extraction Protocol (Berg-Lyons et al., 2018; Marotz et al., 2017). DNA was prepared using the DNeasy PowerSoil Pro Kit (QIAGEN, Beverly, MA, USA) following the manufacturer`s instructions The mix of 200 mg of rhizosphere material and 800 µL of Solution CD1 was homogenized with a Retsch Mixer Mill at 25 Hz for 10 minutes. DNA was eluted from the MB Spin Column with 100 µL of Solution C6. The DNA concentrations were quantified with the AccuClear® Ultra High Sensitivity dsDNA quantification kit (Biotium, Fremont, CA, USA) and diluted to 2 ng μL^{-1} using a Myra Liquid Handler (Bio Molecular Systems, Upper Coomera, Australia). During that step, the DNA was distributed into two 96-well plates in a random and equal manner.

For the bacterial library, a first PCR reaction was performed with the 16S rRNA gene primers CS1-515-F (ACACTGACGACATGGTTCTACAGTGYCAGCMGCCGCGGTAA – CS1 tagged version of Parada et al. (Parada et al., 2016)) and CS2-806-R (TACGGTAGCAGAGACTTGGTCTGGACTACNVGGGTWTCTAAT – CS2 tagged version of Apprill et al. (Apprill et al., 2015)). A second PCR tagged the PCR product with Access Array™ Barcode Library for Illumina® Sequencers—384, Single Direction (Fluidigm, South San Francisco, CA, USA). The first PCR program consisted of an initial denaturation step of 3 minutes at 94°C, 25 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 60 seconds, elongation at 72°C for 90 seconds, and a final elongation at 72°C for 10 minutes. The second PCR program was similar, with the difference that the annealing temperature was increased to 60°C and that the number of cycles was reduced to 10. For the fungal library, first a PCR reaction was performed with the internal transcribed spacer (ITS) region primers CS1-ITS1f-F (ACACTGACGACATGGTTCTACACTTGGTCATTTAGAGGAAGTAA – CS1tagged version of Gardes and Bruns 1993 (Gardes & Bruns, 1993)) and CS2-ITS2-R (TACGGTAGCAGAGACTTGGTCTGCTGCGTTCTTCATCGATGC – CS2tagged version of White et al. (White et al., 1990)). A second PCR tagged the PCR product with Access Array™ Barcode Library for Illumina® Sequencers—384, Single Direction (Fluidigm, South San Francisco, CA, USA). The first PCR program consisted of an initial denaturation step of 3 minutes at 94°C, 25 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 60 seconds, elongation at 72°C for 90 seconds, and a final elongation at 72°C for 10 minutes. The second PCR program was similar, with the difference that the annealing temperature was increased to 60°C and that the number of cycles was reduced to 10.

All PCR reactions were performed with NGS grade Oligos (Eurofins Genomics, Ebersberg, Germany) and the Platinum Hot Start PCR MM (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA). All PCR products and pooled libraries were purified with SPRIselect beads (Beckman Coulter, Brea, CA, USA) according to the manufacturer`s protocol with a ratio of 1:1; and were quantified with the AccuClear® Ultra High Sensitivity

dsDNA quantification kit (Biotium, Fremont, CA, USA). Sub pools were assembled by library type using a Myra Liquid Handler by adding an equal mass of each PCR product. All samples were paired end sequenced (v3 chemistry, 300 bp paired end) on an Illumina MiSeq instrument at the NGS platform of the University of Bern.

Bioinformatics: First, we performed a quality control using FastQC (V0.11.8, (Andrews, 2010)). Barcodes were previously removed and written to the sequence headers by the NGS platform. We removed primers with cutadapt (V3.4, (Martin, 2011)) and used sequence headers information to demultiplex the data. Nine samples were discarded due to a very low number of sequences or because they were detected as outliers. With the same methods as used in Gfeller et al. (unpublished), we used dada2 (V1.16.0, (Callahan et al., 2016)) in R (V4.0.0, (R Core Team, 2022)) to infer exact sequences variants and assign taxonomies. The sequencing data and bioinformatic code is publicly available (see below).

Complementation of BX to the mutant

UHPLC-MS Analysis: The analysis was adapted from a previous protocol (Robert et al., 2017). Briefly, an Acquity UHPLC system coupled to a G2-XS QTOF mass spectrometer equipped with an electrospray source and piloted by the software MassLynx 4.1 (Waters AG, Bade-Dättwil, Switzerland) was used. Gradient elution was performed on an Acquity BEH C18 column (2.1 x 50 mm i.d., 1.7 mm particle size) at 90-70% A over 3 min, 70-60% A over 1 min, 40-100% B over 1 min, holding at 100% B for 2.5 min, holding at 90% A for 1.5 min where A = 0.1% formic acid/water and B = 0.1% formic acid/acetonitrile. The flow rate was 0.4 mL/min. The temperature of the column was maintained at 40 °C, and the injection volume was 1 µL. The QTOF MS was operated in positive mode. The data were acquired over an m/z range of 50-1200 with scans of 0.15 s at a collision energy of 4 V and 0.2 s with a collision energy ramp from 10 to 40 V. The capillary and cone voltages were set to 2 kV and 20 V, respectively. The source temperature was maintained at 140 °C, the desolvation was 400 °C at 1000 L/hr and cone gas flow was 50 L/hr. Accurate mass measurements (< 2 ppm) were obtained by infusing a solution of leucin encephalin at 200 ng/mL and a flow rate of 10 mL/min through the Lock Spray probe.

Chemicals: Optima LC-MS formic acid (FA) and acetonitrile (ACN), as well as HPLC grade methanol were purchased from Fisher Scientific AG (Reinach, Switzerland). BOA (benzoxazolin-2(3*H*)-one) and MBOA (6-methoxy-benzoxazolin-2(3*H*)-one) were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). HMPMA (*N*-(3-methoxy-2-hydroxyphenyl)malonamic acid) was received as a gift from Prof. Dr. Francisco A. Macías (University of Cádiz, Spain). DIMBOA-Glc (2-O-β-D-glucopyranosyl-2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one) and HDMBOA-Glc (2-O-β-D-glucopyranosyl-2-hydroxy-4,7-dimethoxy-2*H*-1,4-benzoxazin-3(4*H*)-one) were isolated from maize plants in our laboratory. DIMBOA (2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one), DIMBOA-d_3 (2,4-dihydroxy-7-(methoxy-d_3)-2*H*-1,4-benzoxazin-3(4*H*)-one), MBOA-Glc (3-β-D-glucopyranosyl-6-methoxy-benzoxazolin-2(3*H*)-one), HMBOA (2-hydroxy-7-methoxy-2*H*-

1,4-benzoxazin-3(4*H*)-one), APO (2-amino-3*H*-phenoxazin-3-one), AMPO (9-methoxy-2-amino-3*H*-phenoxazin-3-one), AAMPO (9-methoxy-2-acetylamino-3*H*-phenoxazin-3-one), were synthesized in our laboratory.

Complementation bx1 mutant: The greenhouse experiment was performed in the same conditions as explained before and lasted 6 weeks. Crystallized benzoxazinoids were dissolved in MilliQ water and complemented to the mutant every three days for a total amount of 1.6 mg/pot per week of BXs, which correspond to the physiological levels for a wild-type plant.

Context-dependency and feedback experiment: Following the same instructions for spiking the soil with sodium arsenate and growing the plants in the same conditions as explained before, the feedback experiment consisted of two phases. During the first phase (conditioning phase) we grew W22 and *bx1* mutant in both arsenic-free and contaminated soils for 6 weeks. The conditioning phase, in addition to be used as a soil conditioning time for the feedback phase, was also used to test the context-dependency of the observed phenotype. In fact, we used two additional agricultural soils, Changins and Posieux, coming from different areas of Switzerland (Fig. S4) and having different characteristics (Table S3). After the conditioning phase, we harvested the plants including the root system, we sieved the soil of each pot through a 1 cm sieve and decanted it again in the same pot. In the second phase (feedback phase), we only considered Q-Matte soil and we planted in all pots only wild-type W22 seeds (following surface sterilization and pre-germination protocols explained previously). The feedback phase also lasted 6 weeks, during which plants were measured for height and chlorophyll content every two weeks. After 6 weeks, plants were harvested, and the leaves were dried for 72h at 70°C in a dry oven to get the dry biomass. The soil water content was kept at 60% during the entire experiment.

Arsenic speciation in rhizosphere: At harvesting, a root section from -2 till -7 cm was put into a 50 mL centrifuge tube (Sigma Corning® 50 mL centrifuge tubes, polypropylene, conical bottom w/ CentriStar cap, sterile, Sigma-Aldrich, Switzerland), with 25 mL of autoclaved Q-MilliQ water. The tube was shaken 10 times, afterwards we added again 25 mL of autoclaved MilliQ water, and we repeated the shaking. We removed the roots with tweezers, and we centrifuged the tubes at 3220xg for 5 minutes (Sorvall Legend XTR centrifuge, Unity Lab Services, Thermo Scientific, Switzerland) and removed the supernatant as previously described (Lingfei Hu et al., 2018b). We freeze dried the material for 72h in a lyophilizer (Swiss Vacuum Technologies SA, Telstar LyoQuest, Switzerland). After drying, the rhizosphere was homogenized with mortar and pestle and stored at room temperature before analysis. To proceed with the extraction, we worked under anaerobic conditions with oxygen concentration below 5 ppm (Glovebox, Systemtechnik, Germany).

We made the buffer with 5 mM tetrabutylammonium hydroxide (TBAH; Sigma-Aldrich, Switzerland), 5 % (v/v) methanol and 3 mM malonic acid (Sigma-Aldrich, Switzerland) and we adjusted the pH to 5.9. The buffer was deoxygenated by bubbling nitrogen during 30 min. We weighed 2-mL Eppendorf tubes (Safe-Lock Eppenforf tubes, Eppendorf AG, Germany) containing the 5 acid-washed zirconium oxide beads before transferring 60 mg of material (dry weight). We added 2 mL of buffer previously deoxygenated, closed the tubes and weighed the weight again (tube and beads + sample + buffer). We shook the samples with the buffer at 30 rpm for 10 minutes (Mixer Miller RETSCH MM 400, Fisherbrand™, Waltham, MA). We centrifuged the samples after extraction at 2500 rpm for 5 min (Multifuge™ X1 Centrifuge Series, Thermo Scientific™, Reinach, Switzerland), filtered with a 0.22 µm hydrophilic polytetrafluoroethylene filter (13 mm syringe filter, BGB®, Switzerland) and transferred them 500 – 700 µL of supernatant to a 0.7 mL PP HPLC vials (BGB®, Switzerland). As speciation analysis was done by high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) with a 1260 Infinity HPLC coupled to a 7700 ICP-MS (both Agilent, Switzerland). As species were separated by ion-pairing chromatography using a Zorbax SB-C18 column (150 mm × 4.6 mm, 5 µm particle size, Agilent, Switzerland) and the previously described buffer as eluent. The column was maintained at 50 °C and the flow rate was set as 1.2 mL min^{-1}. Calibration standards using DMA were prepared in the buffer solution at arsenic concentrations ranging from 100 to 0.1 µg kg^{-1}.

Statistical analysis

All statistical analyses were performed in R (version 4.1.2). All datasets were checked for normality with the Shapiro-Wilk test ($p < 0.05$) and transformed as necessary (square-root, log, or rank) to meet normality assumption. Eventual data transformations are indicated in the statistics tables for each figure. Such a general approach in treating the data permitted us to assess all the data with the same statistical test (ANOVA and pairwise test), using the following model: data ~ *Genotype* (G) * *Arsenic* (A) * (*Week* (W)). The factor 'Week' was only considered with parameters taken through time (plant height and chlorophyll content), while ignored when a parameter was taken at a single timepoint. ANOVA tables and data transformation can be found in the Supplementary Information (Tables S4, S5). Differences between treatments were further tested with the pairwise TukeyHSD test (Tables S6).

In the microbiome analysis, one fungal sample was removed due to a too low sequencing yield. Further, we excluded four bacterial and four fungal samples which were detected with the CLOUD method as outliers. Data were normalized by total sum scaling. Bray-Curtis (BC) distances were calculated and a permutation analysis of variance (PERMANOVA, ~ genotype * arsenic, 999 permutations) were performed to conduct for differences in phylum-abundances. The alpha diversity of each sample was determined by calculating Shannon diversity. An ANOVA with the factors *genotype* and *arsenic* (~ genotype * arsenic) was performed to test for alpha diversity differences.

BC distances were calculated as an index for beta-diversity and a PERMANOVA (~ genotype * arsenic; 999 permutations) was used to test for differences. Beta diversity was illustrated ordinations. Differential abundance analyses (DAA) were performed to find ASVs differing in abundance between the maize-genotypes in arsenic-contaminated soil. Four DAA-tools (aldex2, acombc, maaslin2 and metagenomeSeq) were used and predict ASVs to be different in abundance if they were detected by at least two tools.

Results

Benzoxazinoid producing plants are more tolerant to arsenic contamination than BX-deficient mutant plants

To test whether benzoxazinoids enhance arsenic tolerance, we grew wild type and BX-deficient *bx1* mutant maize plants in control and arsenic contaminated soil and measured their performance. The two genotypes grew equally well in the control soil (**Fig. 1A**). In soil contaminated with arsenic, both genotypes grew smaller ($p = 3.98e-05$). This suppression was more pronounced in the *bx1* mutant than the wild type plants ($p = 1.04e-05$). Shoot chlorophyll content varied with time but was independent of genotype or arsenic contamination (**Fig. 1B**). The differential growth observed in plant height was also manifested in the shoot biomass (dry weight) at the end of the experiment, where we found higher dry biomass of wild type plants compared to the *bx1* mutant ($p = 0.002$; **Fig. 1C**). No differences in shoot biomass were found between the two genotypes in the control soil. This finding was supported in the ANOVA with the interaction term between arsenic and genotype being significant ($p = 0.03$), showing that plant performance in arsenic-contaminated soil depended on BXs biosynthesis. Enhanced performance of wild type plants in As contaminated conditions was observed also in two other soils with different characteristics (**Fig. 2A, B**), suggesting that this phenotype is conserved across soil types.

To corroborate the benzoxazinoid dependency of the above-described finding, we repeated the experiment with a *bx2* mutant. Similar to the first experiments, we did not observe differences in plant height or shoot biomass among the three genotypes in the control soil (**Fig. 3A, B**). In arsenic-contaminated soil (100 ppm), the suppression of growth ($p = 0.02$) and shoot biomass ($p = 0.07$) was more pronounced in both *bx1* and *bx2* mutants compared to wild type plants and shoot biomass. Thus, the capacity to produce benzoxazinoids enhances maize tolerance to arsenic.

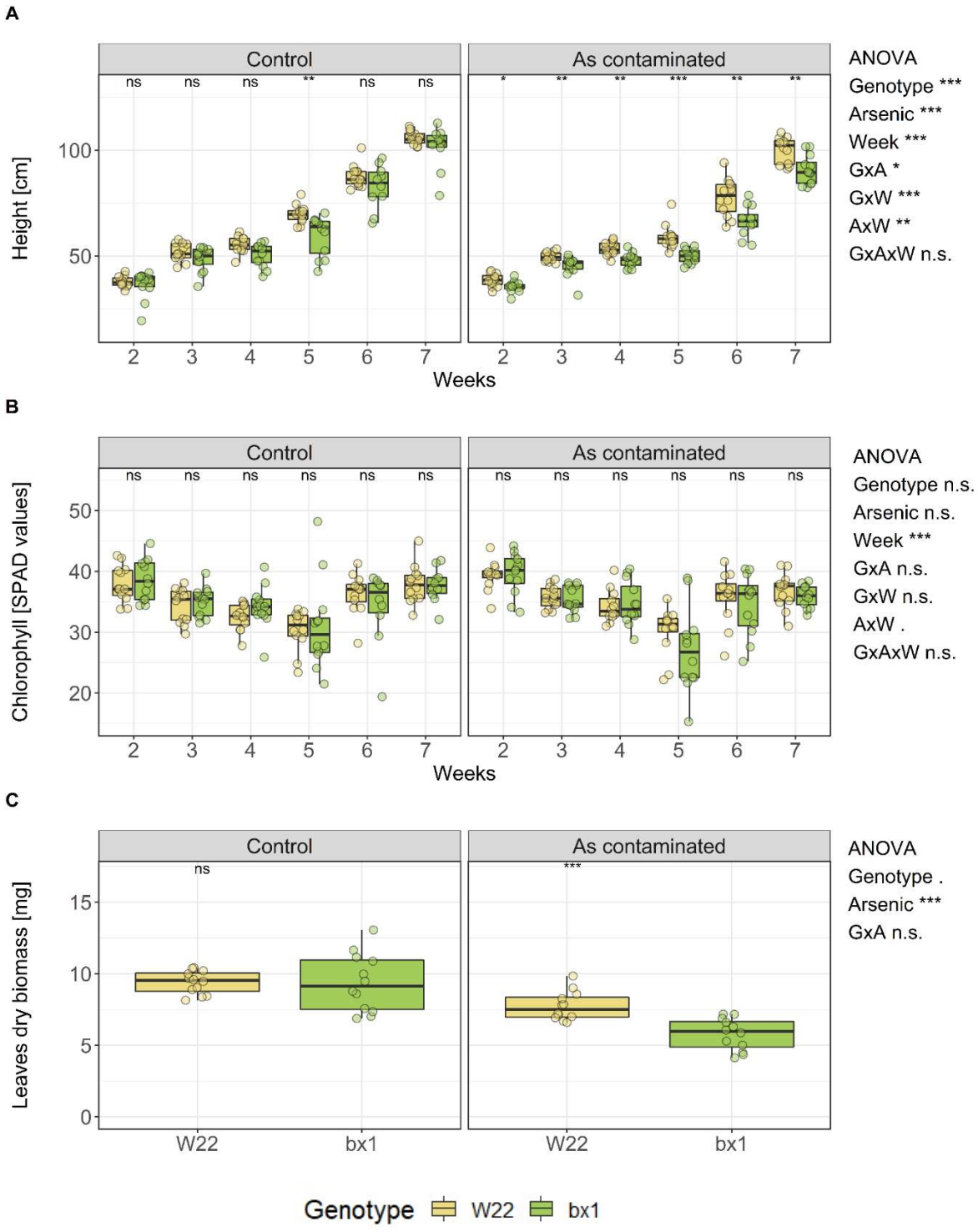


Figure 1. Maize plants phenotyping to determine the interaction between benzoxazinoids and arsenic in soil. (A) Plant height and (B) chlorophyll content of W22 and *bx1* measured in both arsenic-free (0 ppm) and arsenic-contaminated soil (100 ppm) during 7 weeks of growth. (C) Leaves dry biomass sampled at the end of the experiment. P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001.

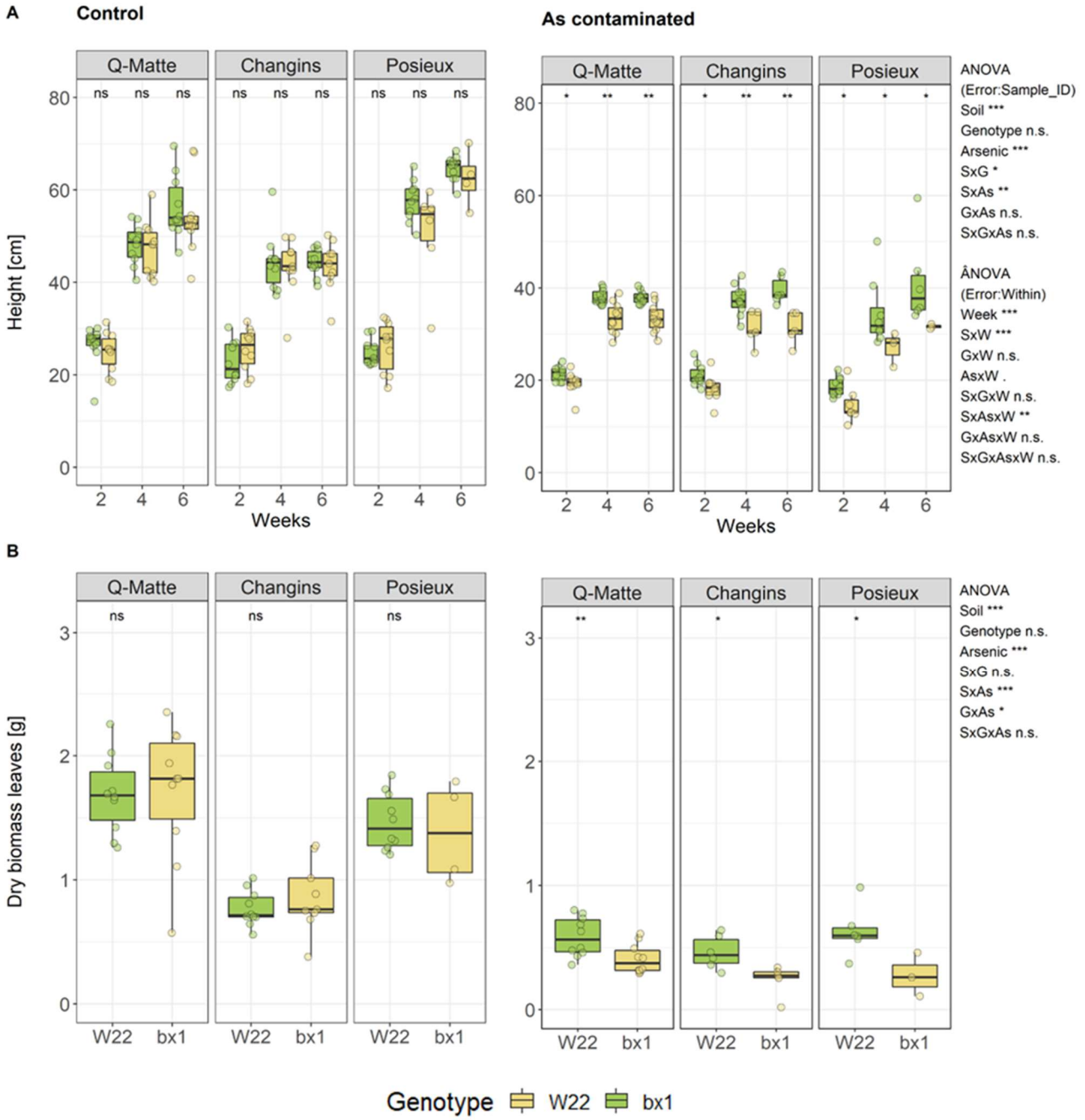


Figure 2. Comparison of the response in Q-Matte soil with two additional agricultural soils (Changing and Posieux). (A) Plant height and (B) dry leaves biomass of the two genotypes compared between control and contaminated-soil in three different soils. P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001.

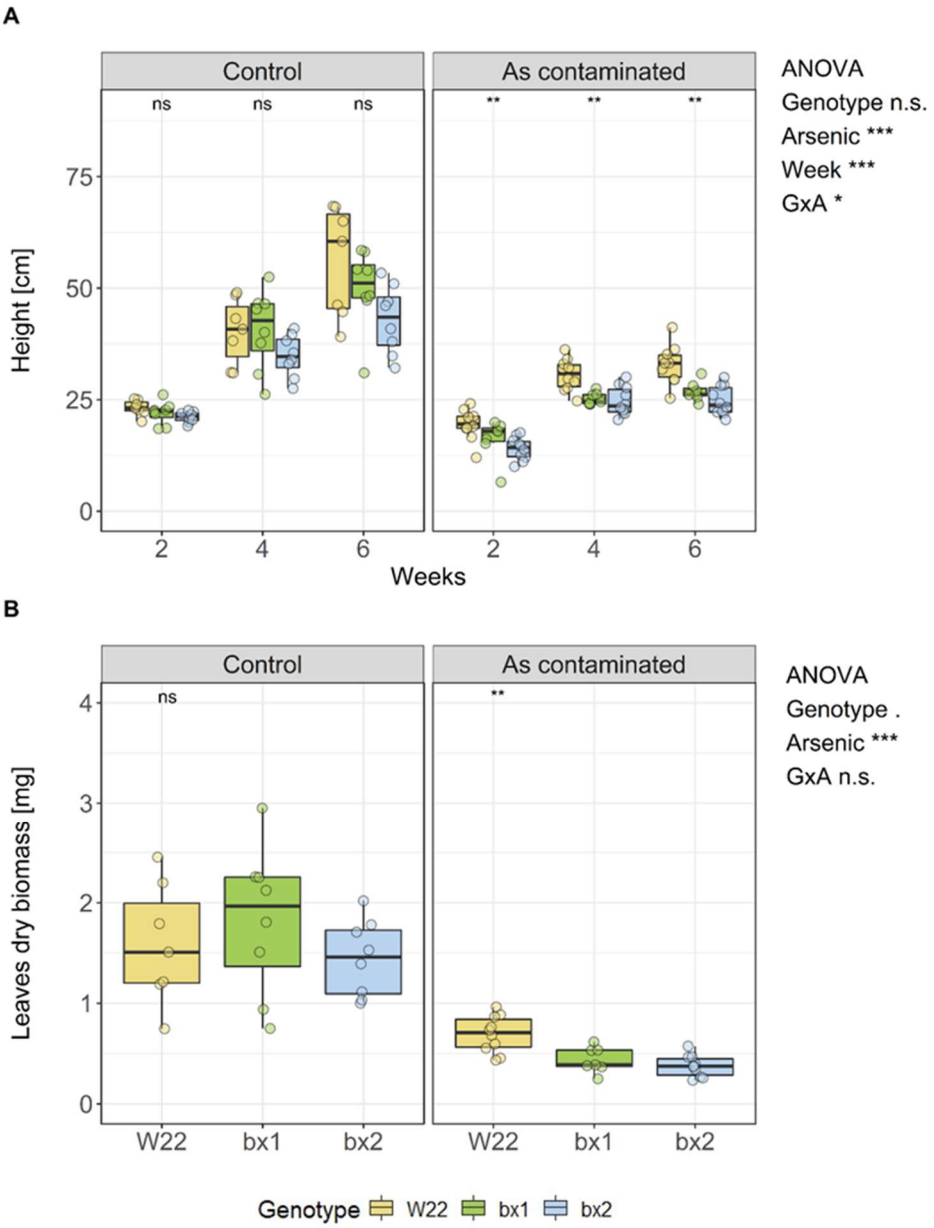


Figure 3. Another W22 mutant (*bx2*) was used to confirm the observed phenotype. (A) Plant height and (B) leaves dry biomass were recorded to confirm that the phenotype we observed is BX-dependent (left-hand panels: 0 ppm, right-hand panels: 100 ppm). P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001.

Soil complementation with benzoxazinoids enhances arsenic tolerance of benzoxazinoid-deficient maize

To test if benzoxazinoids that are released into the soil are sufficient to enhance benzoxazinoid tolerance, we complemented arsenic-contaminated soils with purified benzoxazinoids and measured plant performance of benzoxazinoid-deficient mutant plants.

Again, wild type plants grew taller than *bx1* mutant plants in the contaminated soil. Complementation of the soil with benzoxazinoid enhanced the growth of the *bx1* mutant in this soil, and partially rescued the wild-type phenotype (Fig. 4A). The same pattern was observed for biomass accumulation (Fig. 4B). No performance differences between genotypes and treatments were observed in non-contaminated soil. Thus, benzoxazinoids in the soil contribute to arsenic tolerance of maize.

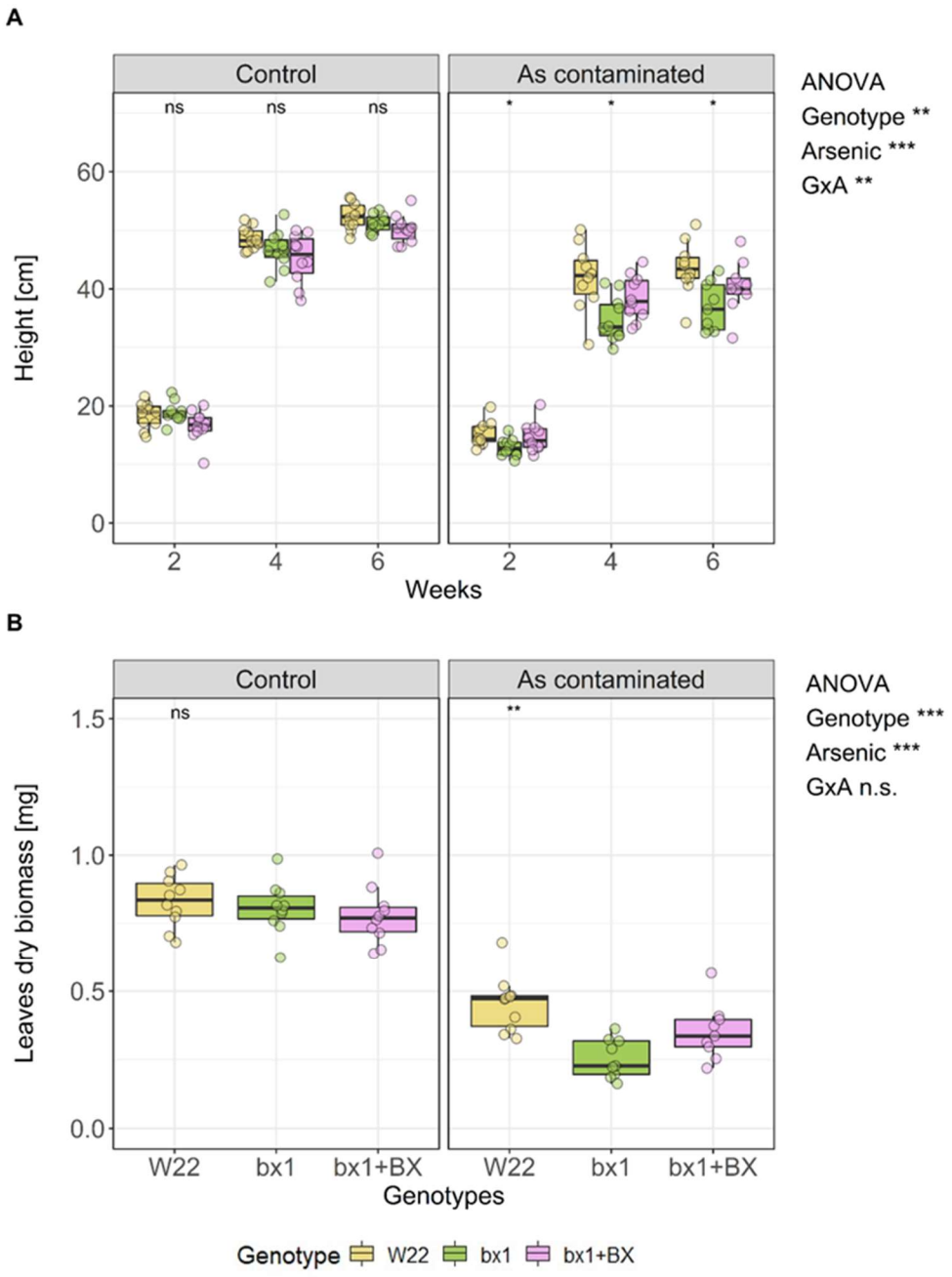


Figure 4. Benzoxazinoids complementation to the *bx1* mutant. (A) Plant height and (B) leaves dry biomass were measured for W22, *bx1* and *bx1*+BX in arsenic-free and arsenic-contaminated soil (left-hand panels: 0 ppm, right-hand panels: 100 ppm). P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001.

Field grown plants benefit from benzoxazinoids when growing in an arsenic contaminated environment

To test whether benzoxazinoids benefit maize plants growing in an arsenic contaminated field under realistic agricultural conditions, we grew wild type and *bx1* mutant plants in a maize field with high biogenic arsenic levels (430 mg/kg, 'As+' field). We also included a field nearby that contains 10 times lower concentrations of arsenic (43 mg/kg; termed 'As-' field). In both fields, wild type plants grew significantly better than the *bx1* mutant. While this effect was subtle in the As- field, it was stronger in the As+ field, with wild type plants growing 22 to 40% taller than *bx1* mutant plants (Fig. 5A). Shoot biomass showed the same pattern, albeit a bit less pronounced (Fig. 5B). Thus, benzoxazinoids promote plant performance in the field, and this effect is stronger when arsenic is present at high levels.

Arsenic tolerance to benzoxazinoids can be transmitted to other plants via the soil

To further investigate the importance of benzoxazinoids that are released into the soil for arsenic tolerance, we performed a plant-soil feedback experiment. As benzoxazinoids can have feedback effects independently of arsenic in certain soils (Hu et al., 2018b), we chose a soil (Q-matte) that does not show such feedback effects. We grew wild type plants in control and arsenic contaminated soils in which wild type or *bx1* mutant plants had been growing previously. As expected, we did not observe any differences in height or shoot biomass of wild type plants growing in wild type or *bx1* conditioned control soils (Fig. 6A, B) In arsenic contaminated soil, plants grew taller and accumulated more biomass when wild type plants had been growing before compared to when *bx1* mutants had been growing in this soil. Thus, arsenic tolerance conveyed by benzoxazinoids can be transmitted to other plants via the soil.

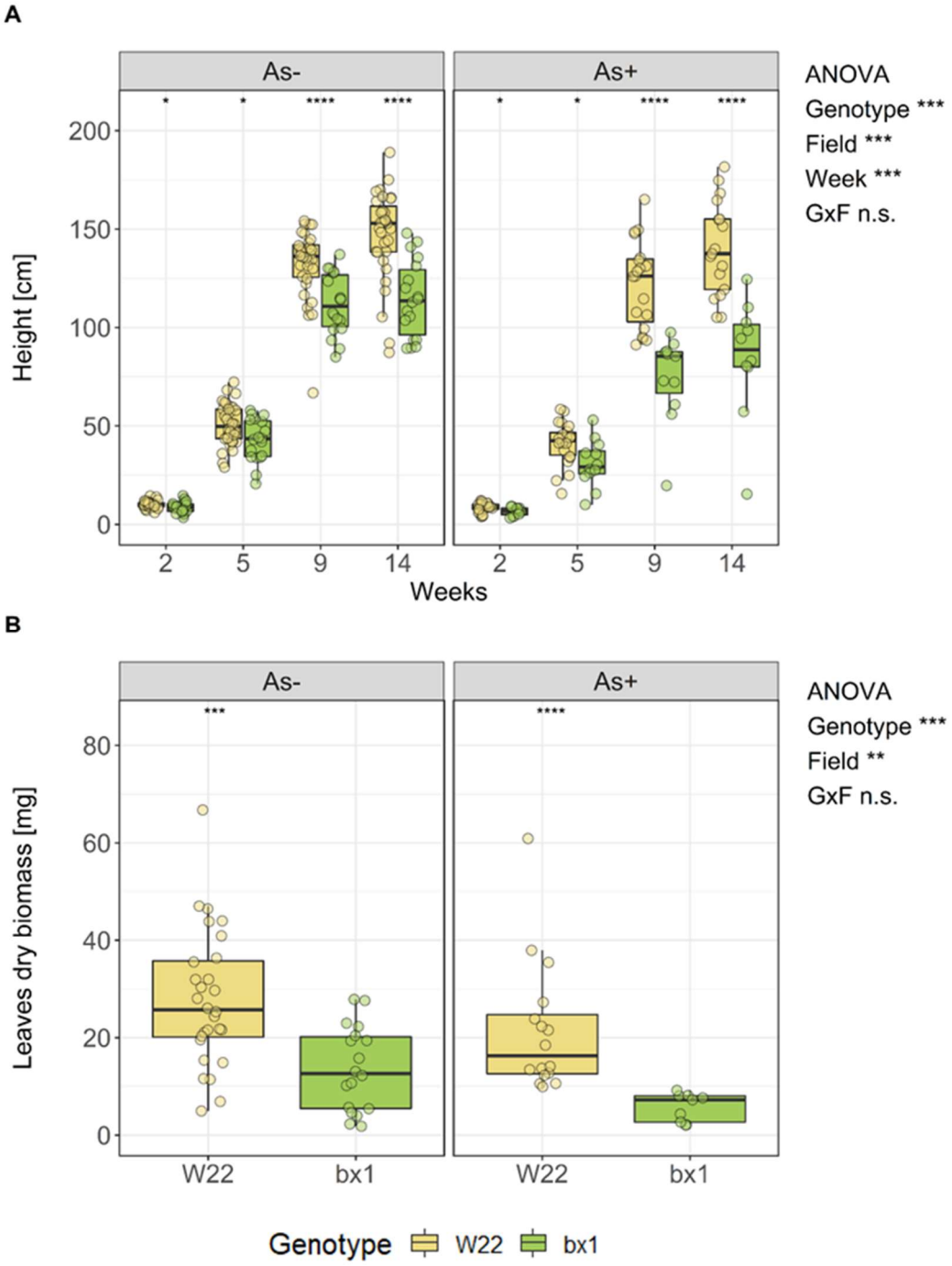


Figure 5. Field experiment in Liesberg (Basel, Switzerland) where geogenic arsenic is present in cultivated fields. (A) Plant height was recorded during maize growth season by 4 timepoints (2, 5, 9 and 14 weeks after sowing) and (B) leaves dry biomass was measured after harvesting to set/confirm the agricultural relevance of the observed phenotype. Left-hand panels represent the As- field (43 mg As/kg) and the right-hand panels represent the As+ field (430 mg As/kg). P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001.

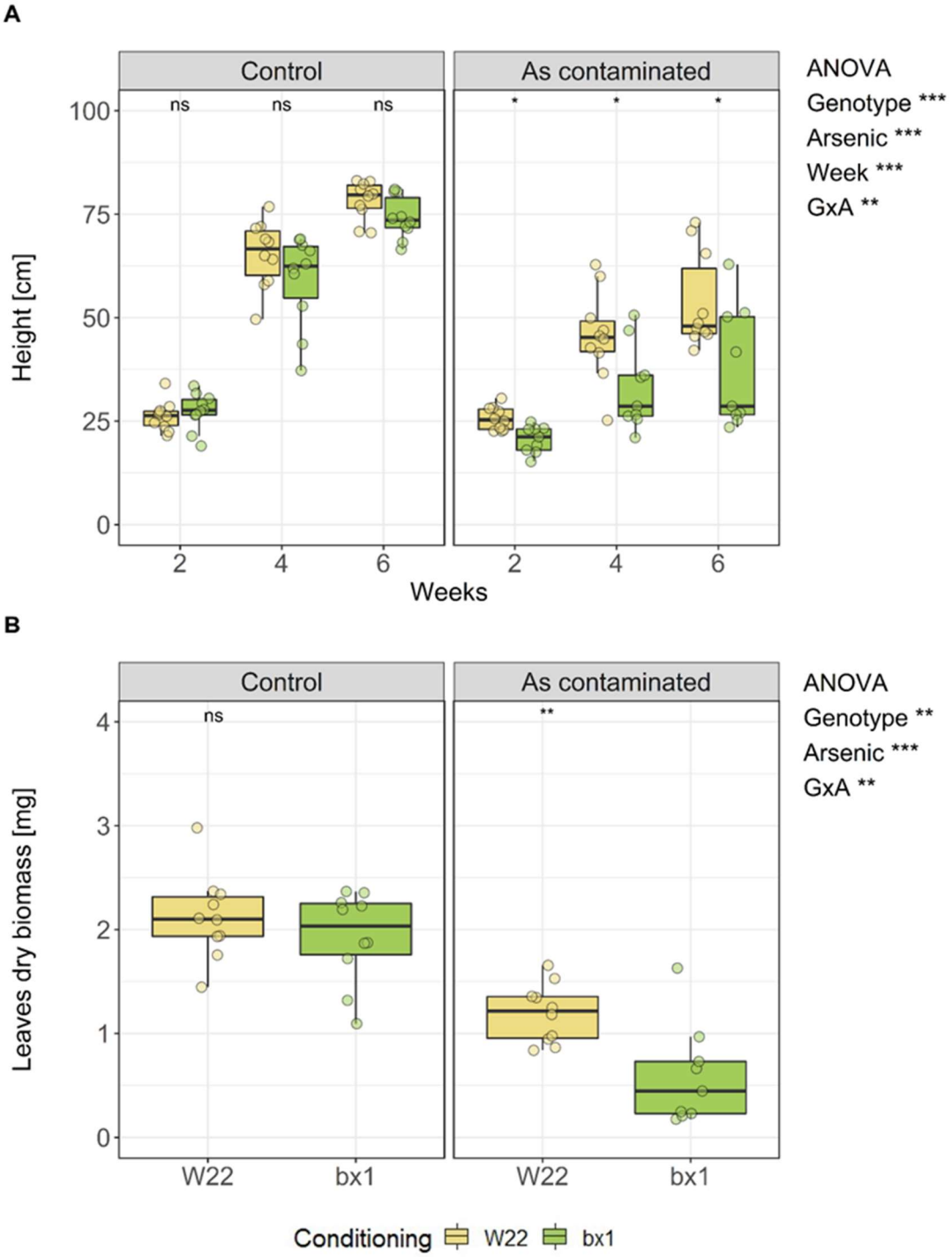


Figure 6. Feedback on W22 with W22 ± As and *bx1* ± As conditioned soils. W22 response in function of the soil conditioning was measured with (A) plant height during the experiment and (B) leaves dry biomass at the end. P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001.

Plant growth patterns are not associated with major changes in rhizosphere microbial communities

Benzoxazinoids may change the microbial composition of the rhizosphere, and thereby modify arsenic dynamics (Cadot et al., 2021; Lingfei Hu et al., 2018b). Thus, we screened microbiota of wild type and *bx1* mutant plants in control and arsenic contaminated soil for genotype*environment interactions. The rhizosphere microbiota was determined based on the 16S rRNA gene of bacteria and first internal transcribed spacer region of fungi using short read sequencing. Bacterial and fungal profiles contained between 37'055 and 77'593 (median: 49'508) and between 2'172 and 5'112 (median: 3'151) high-quality sequences per samples, respectively. Rarefaction analysis confirmed that the applied sequencing depth captured the microbial diversity present in these samples (**Fig. S5**). Taxonomically the bacterial communities were mainly composed of Verrucomicrobia, Firmicutes, Alphaproteobacteria and Actinobacteria; all in similar abundances. Acidobacteria, Gammaproteobacteria, Planctomycetes, Deltaproteobacteria, Chloroflexi, Thaumarchaeota, Gemmatimonadetes, Rokubacteria and others were also detected but at lower abundance. Fungal communities mainly consisted of Ascomycota, Mortierellomycota, Olpidiomycota and Basidomycota (**Fig. S6**). At the coarse level of taxonomy, we did not find statistical evidence for any abundance differences between benzoxazinoid exudation, arsenic conditions, or their interaction (**Table S6**).

We then investigated at the high resolution of individual amplicon sequence variants (ASVs) whether the maize rhizosphere microbiota was affected by BXs and/or the arsenic contamination in soil. Microbial Shannon diversity was unaffected by benzoxazinoid exudation, arsenic contamination, or their interaction (**Table S7**). However, microbiome composition changed slightly but significantly due to benzoxazinoid exudation and arsenic contamination (**Fig. 7**). PERMANOVA quantified effect sizes of 3.3% (both bacteria and fungi) for benzoxazinoid exudation and for arsenic contamination effect sizes of 3.64% and 5.3% for bacteria and fungi, respectively. Interestingly, a small (4%) but significant effect was found for the interaction between benzoxazinoid exudation and arsenic contamination (**Fig. 7**). CAP analysis visualized this finding: while bacterial communities of wild-type plants differed between control and contaminated soils, this was not seen for the mutant. In contrast to the fungi, arsenic had a stronger impact on the communities where both wild-type and mutant become similar in contaminated soil. Despite these subtle changes on community composition, we did not detect individual bacterial or fungal ASV which changed in abundance as a function of benzoxazinoid exudation and arsenic contamination (**Table S8**). Thus, we find subtle, but not very strong changes in the microbiome composition that could explain the observed plant phenotype, and our analysis did not reveal any candidate taxa for further functional analyses.

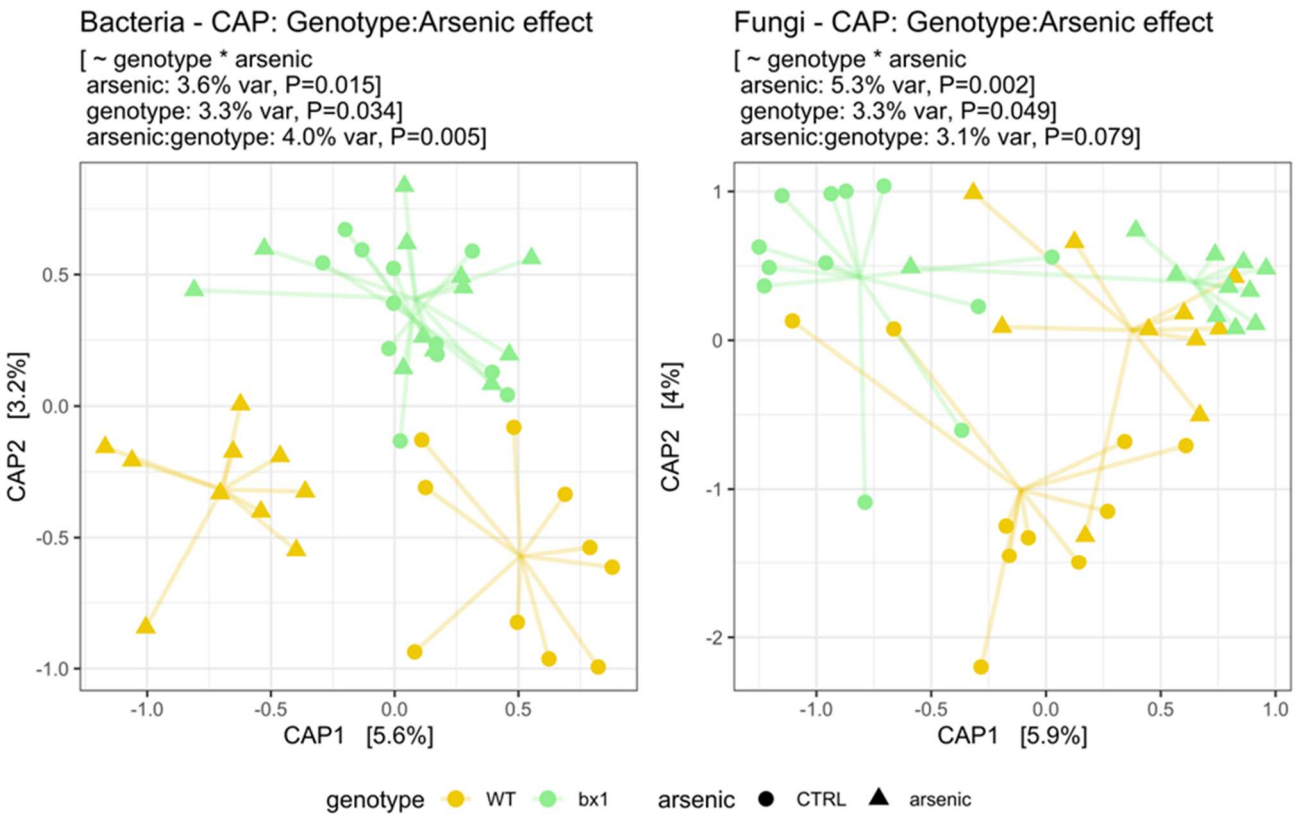


Figure 7. Constrained Analysis of Principal Coordinates (CAP) for bacteria (A) and fungi (B) computing dissimilarities with the model *Bray-Curtis-distance ~ arsenic * genotype*. Variances and P-values were computed by a PERMANOVA with 999 repetitions. P-values: *p <.05, **p <.01, ***p <.001.

Roots of BX-exuding plants take up less arsenic

Benzoxazinoids may reduce arsenic uptake and translocation. To test for such effects, we measured total arsenic levels in roots and leaves of wild type and *bx1* mutant plants. As expected, no arsenic was detected in plants growing in control soils. In arsenic-contaminated soil, we measured significantly lower arsenic concentrations in roots of wild type plants (~70 mg/kg) compared to the *bx1* mutant (~90 mg/kg; **Fig. 8A**). Leaves contained only small amounts of arsenic (~1 mg/kg), and no differences between genotypes were found (**Fig. 8B**). Thus, benzoxazinoids reduce arsenic uptake into the roots.

To test whether the reduced arsenic uptake and/or the enhanced plant growth is associated with differential arsenic speciation, we quantified the different arsenic species in the rhizosphere. Most of the arsenic was present as AsV, the arsenic species that we spiked into the soil (**Fig. S7**). We found a significantly higher concentration of AsV in the rhizosphere of the *bx1* mutant (~12,5 mg/kg) compared to the rhizosphere of the

wild-type (~7.5 mg/kg). We also detected small amounts of AsIII and two unknown arsenic species, without any differences between genotypes (Fig. S7).

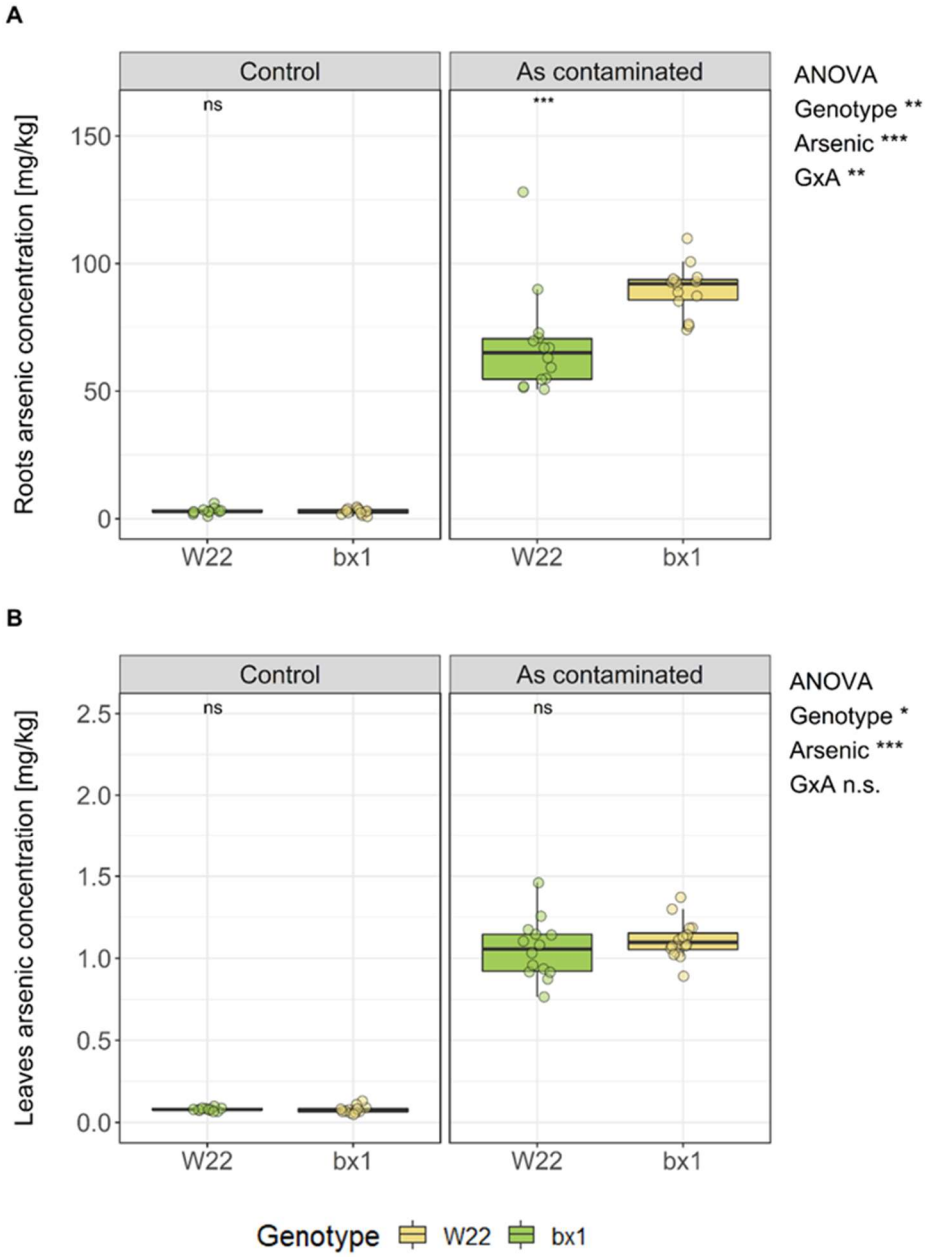


Figure 8. Total arsenic uptake of the different genotypes (W22 and *bx1*) in two plant compartments. (A) Roots and (B) leaves arsenic concentration of wildtype and mutant growing in arsenic-free and arsenic-contaminated soil (left-hand panels: 0 ppm, right-hand panels: 100 ppm). P-values: n.s. non-significant, . = marginally significant, *p <.05, **p <.01, ***p <.001.

Discussion

Exuded plant secondary metabolites may help plants to deal with adverse abiotic conditions such as heavy metal contamination. Despite the substantial potential for agriculture, few studies have investigated this phenomenon and its underlying mechanisms. Here, we demonstrate that benzoxazinoids improve arsenic tolerance in the greenhouse and the field, and that the improved plant performance is associated with direct interactions between benzoxazinoids and the soil environment. Below, we discuss the underlying mechanisms and agricultural implications of this finding.

Exuded plant secondary metabolites can improve plant growth by mobilizing micronutrients, suppressing pathogens and pests, attracting beneficial microbes, and attracting herbivore natural enemies (Dakora & Phillips, 2002; Kudjordjie et al., 2019; Mikic & Ahmad, 2018; Neal et al., 2012; Zhang et al., 2019). Several studies also suggest that they may help plants to cope with heavy metal toxicity (Frémont et al., 2022), but evidence remains scant. A maize cultivars that exude benzoxazinoids from their root tips in response to Aluminium for instance are more resistant to aluminium toxicity than another cultivar, suggesting a link between benzoxazinoid exudation and heavy metal tolerance (Zhao et al., 2019). Here, we performed genetically and environmentally controlled experiments to demonstrate that benzoxazinoids enhance maize tolerance to arsenic, an important metalloid whose toxicity affects at least 140 million people worldwide (WHO, 2018). We show that BX-deficiency conferred by mutations in two different benzoxazinoid biosynthesis genes reduces growth and biomass accumulation in different soils, both in the greenhouse and the field. These effects are contingent on toxic arsenic levels in the soil. The link between benzoxazinoids and arsenic tolerance is corroborated by the fact that soil spiking with purified benzoxazinoids enhances arsenic tolerance of the mutants. By demonstrating their involvement in arsenic tolerance, our study expands our view on the multifunctionality of benzoxazinoids and secondary metabolites in general (Erb & Kliebenstein, 2020).

Plant secondary metabolites can influence metabolic patterning in the plant itself as well as in the rhizosphere and soil. In fact, plant can change characteristics of the soil (chemical, physical and biological) through specialized metabolites (Bezemer et al., 2006), which could potentially influence the mobility of toxic metals present in soil (Lee et al., 2015; Pigna et al., 2015). It was also shown that that aluminium-resistant maize variety accumulate less aluminium in the roots tip, that are highly sensitive to Al, but also that benzoxazinoids accumulated mostly in the most Al sensitive part in maize (Poschenrieder et al., 2005; Sivaguru & Horst, 1998). Taken together these observations let us think that BXs can act directly in favouring higher effluxion of the toxic molecules, but also in internal detoxication. In fact, performing the complementation experiment, where we complemented the *bx1* mutant with exogenous BXs, we observed a tendency in restoring the phenotype. In fact, *bx1* mutant complemented with BXs was doing better than *bx1* mutant under arsenic conditions. However, despite the complementation, we were not able to

completely restore the phenotype, as W22 wild-type was still the genotype growing more in arsenic conditions. The composition of BXs that was added was not 100% pure (**Table S9**), and this may explain why we observed only a tendency and not a total re-establishment of the wild-type phenotype. However, we also performed a feedback experiment where we grew W22 plants in both W22- and *bx1*-conditioned soils. As a result, we can see that W22 growing on W22-conditioned soil, grew better than W22 plants growing on *bx1*-conditioned soil, in presence of arsenic. This shows that BXs exuded into the soil leave a legacy, with regards to arsenic, for the next generation of plants. With the help of these experiments, we can state that benzoxazinoids indeed have a direct effect on arsenic toxicity, and this can be seen both by complementing the mutant with BXs and by evaluating the feedbacks on the next plant generation.

After testing the agricultural relevance of our observation, we wanted to understand the mechanism underlying the arsenic tolerance conveyed by benzoxazinoid exudation. The arsenic form we used in our experiments is arsenate (AsV), which is a chemical analog of phosphate and can be therefore taken up by P-transporters. In fact, it has been proved that there is competition between P and arsenate in plant uptake (Abedin et al., 2002). This effect could be mediated by rhizosphere microorganisms shaped by BXs exudation, with their characteristics of mobilizing nutrients for the plant (Prakash et al., 2014). In our study we sequenced the rhizosphere of the wild-type and the mutant, both in control and arsenic-contaminated soil to determine eventual shifts in the bacterial and fungal communities. The bacterial composition significantly changed between genotypes, while the fungal community only marginally differed. Particularly, we observed a shift of the bacterial community composition in the control soil compared to the contaminated soil for the W22 genotype. However, the change was very subtle which points out that the beneficial effect of BXs for the plant performance in arsenic conditions, is probably not mediated by the soil microbes. There is a change in the community composition, but this only represents a small percentage of the entire community, and therefore this change is less likely to have a major influence in the plant response to arsenic in soil. Benzoxazinoids can directly act on arsenic without having the microbial community as a mediator of this effect. We can therefore speculate that benzoxazinoids can favour phosphate uptake outcompeting arsenic uptake in wild-type plants without microbial mediation.

We measured a greater total arsenic concentration in the mutant roots than in the wildtype. However, both genotypes had a similar arsenic concentration in leaves, that was roughly 100 times lower than in the roots. The higher concentration of arsenic in the roots of the *bx1* mutant can explain why it is suffering more than the W22 wildtype, as it must cope with As toxicity in its physiology. Arsenic availability depends on soil properties, environmental conditions and on the changes of physical-chemicals properties in the rhizosphere (Bianucci et al., 2020). Benzoxazinoids, in our case, can be responsible for the physical-chemical changes in the rhizosphere, that allow the wildtype to take up less arsenic. Both arsenate (AsV) used here and arsenite (AsIII) are forms of inorganic arsenic easily taken up by the roots` cells. AsV is rapidly converted to AsIII once

it reaches the cell. Both forms disrupt the plant metabolism, but AsIII is the more toxic of the two forms (Finnegan & Chen, 2012). Also, the speciation measurements in the rhizosphere confirm these results, as they showed that there is no major speciation and AsV is the species retrieved with the highest concentration. This result points out a possible mechanism through which BXs can help the maize plants avoid the formation of the more toxic species (AsIII) and interfering with the uptake of AsV. Since the wild-type takes up less arsenic from soil, benzoxazinoids may interfere with the uptake through some transporters (e.g., P-transporters (Zhang et al., 2021)). BXs can act as phytosiderophores and help the plant with the nutritional uptake of limiting micronutrients, such as iron (Zhou et al., 2018); therefore they might also trigger the competition between phosphate and arsenic at the entrance to the plant.

In conclusion, we demonstrate that benzoxazinoids that are released into the soil broadly enhance arsenic tolerance. From an ecological point of view, this finding underscores how a single class of bioactive secondary metabolites can provide a wide range of benefits to plants, the composition of which varies with environmental conditions. From an applied point of view, our work suggests that employing BXs releasing genotypes may be a useful strategy to maintain agricultural yields in heavy metal contaminated areas. The biosynthesis of benzoxazinoids is well understood, and proof-of-concept experiments show that benzoxazinoid production can be enhanced through genetic engineering (Zheng et al., 2015). As soon as the mechanisms of benzoxazinoid exudation are elucidated, hyper releasing maize plants could be employed to support areas of the world where arsenic contamination constrains food production.

Author statement

VC conceived, planned, performed the experiments. Co-authors contributed to preparing the DNA library, performing the microbiome analysis (Fig. 7), helping with measuring arsenic in soil, leaves and roots, and supporting with BX analytics.

Acknowledgments

We thank Antoine Baud for his precious help in setting up and harvesting the greenhouse experiments and Nichole Wetter for proofreading the English. We acknowledge the University of Bern for supporting the Interfaculty Research Cooperation (IRC) 'One Health' (www.onehealth.unibe.ch).

Supplementary information

Table S1. Soil characterization for Q-Matte

pH	6.61 ± 0.04
Clay (%)	10.2 ± 0.8
Silt (%)	53 ± 2
Sand (%)	37 ± 3
Plant available P (mg/kg)	2.41 ± 0.02
Total carbon (g/kg)	26.49 ± 0.07
Nitrogen (g/kg)	2.91 ± 0.01
Sulfur (g/kg)	0.35 ± 0.03
Total organic carbon (g/kg)	25.41 ± 0.09
Arsenic (mg/kg)	2.9 ± 0.5
Magnesium (g/kg)	4.1 ± 0.5
Potassium (g/kg)	1.5 ± 0.1
Iron (g/kg)	17.7 ± 0.7
Manganese (g/kg)	0.74 ± 0.02

Figure S1. (A) Soil incubation setup in plastic boxes with plastic lid in the corridor of the greenhouse. (B) Greenhouse setting of experiments with randomized design and weekly randomization.

A

B

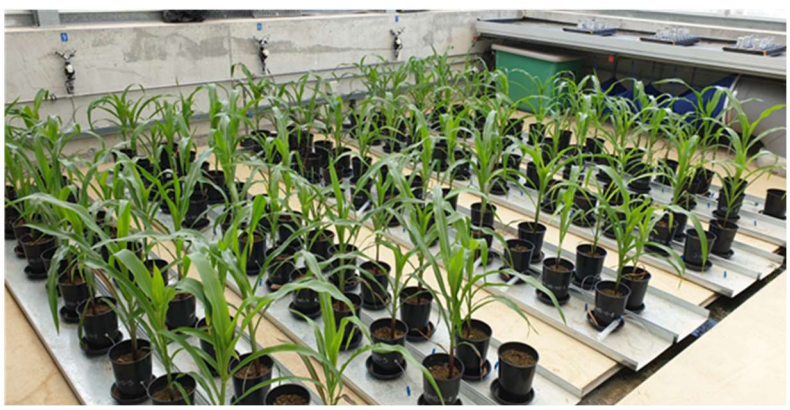


Figure S2. Map of the arsenic-contaminated area in Liesberg, Basel-Landschaft, Switzerland. In green, the least contaminated field and in red the heavily contaminated field. Both fields are owned and managed by the same farmer.

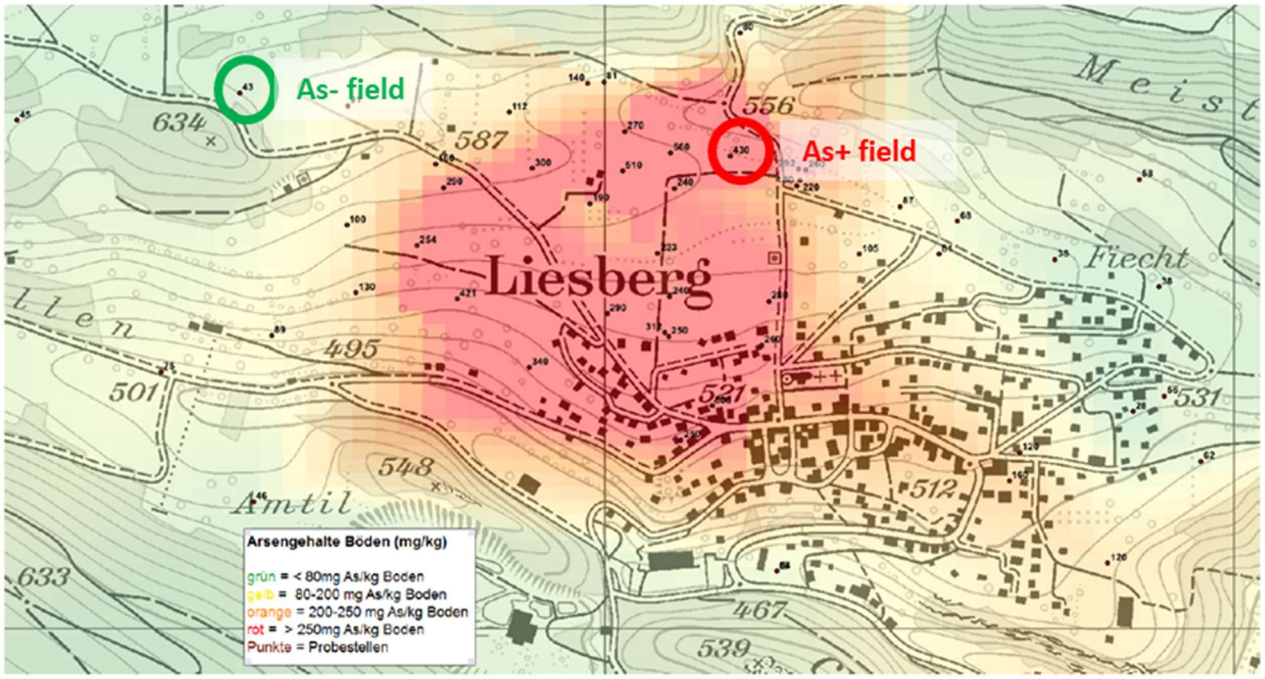


Figure S3. (A) Our seeds were planted, after removal of the farmer's seeds, in the field lines. (B) Subplot of 6 plants W22 and 6 plants *bx1* (W22 background). (C) Field just before harvesting.

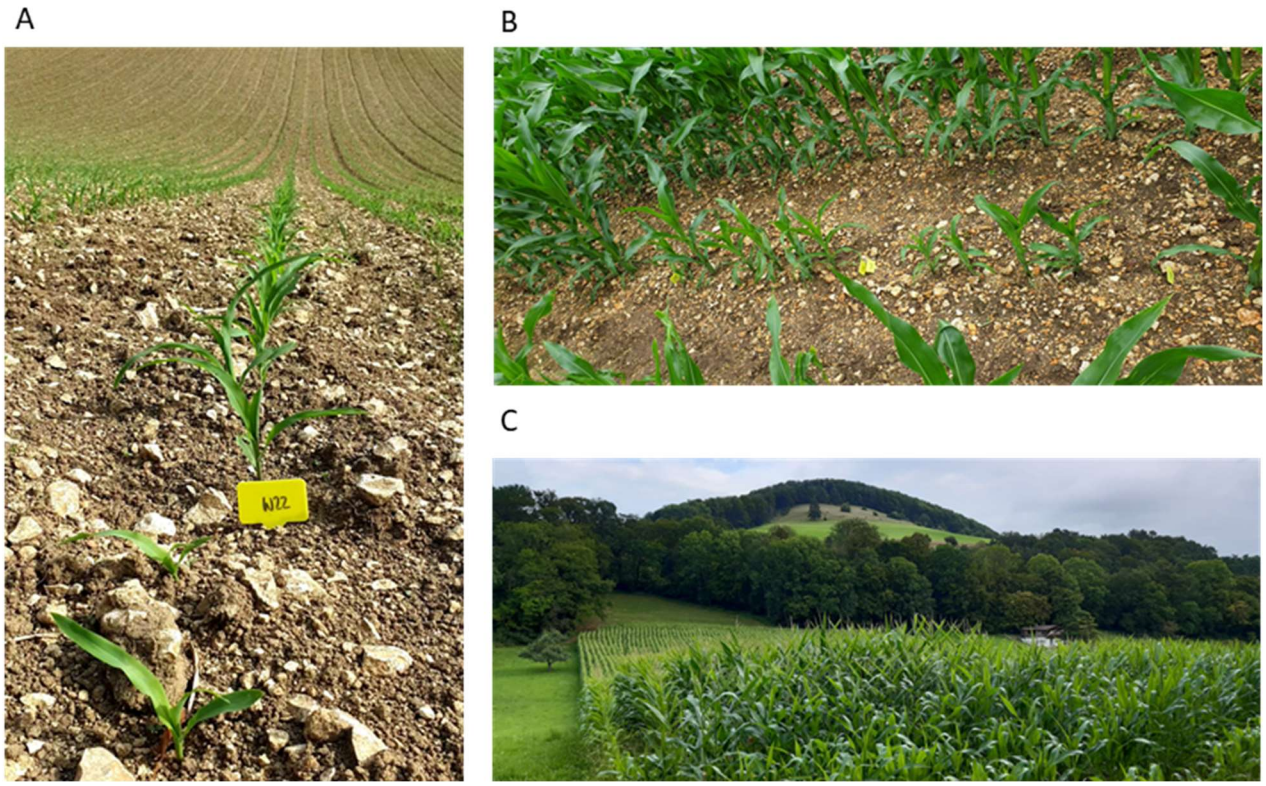


Table S2. Dates and treatments that the farmer applied in its fields. Therefore, also our plants were treated in the same way during the growth period.

Type of management	Date	Comments
Fertilization	before plowing	30 m3 liquid manure
Pre-crop (plow)	24.05.2021	
Pre-crop (arrow)	26.05.2021	
Sowing maize	01.06.2021	Amarock (As+), Robertino (As-)
Fertilization	16.06.2021	NPK 3x15% approx. 200 Kg/ha
Herbicide	26.06.2021	1.5 L/ha EquipPower + 0.5L Bavel
Fertilization	28.06.2021	Urea 150 Kg/ha

Figure S4. Switzerland map with the origin of the three used agricultural soils: Frauenkappelen (Canton Bern), Posieux (Canton Fribourg) and Changings (Canton Vaud).

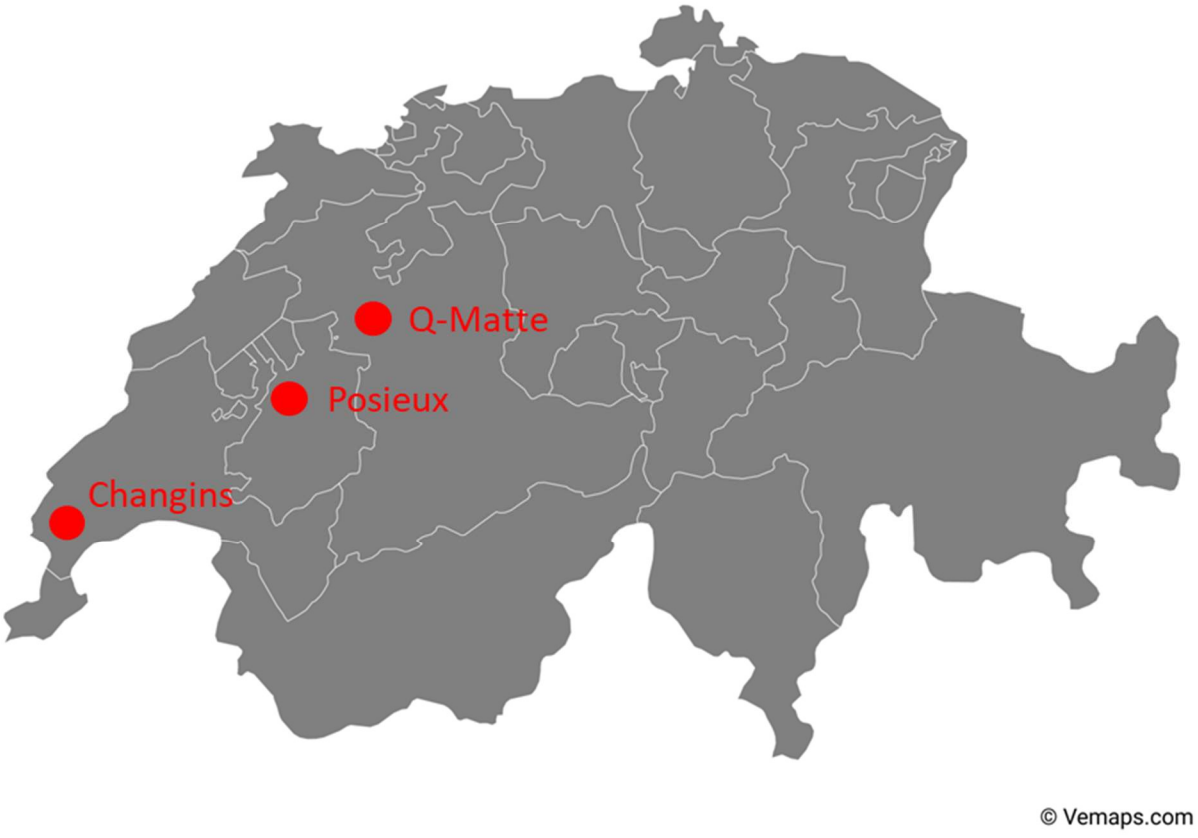


Table S3. Characteristics of Changins and Posieux soils

Parameters	Changins	Posieux
clay %	27.5	16
sand %	60.635	72.2
pH	7.043	6.775
phosphorus-CO2 (mg/kg)	5.96	12.675
potassium-CO2 (mg/kg)	1.395	1.6
magnesium-CO2 (mg/kg)	11.64	6.3625
nitrate-H2O (mg/kg)	11.1565	33.35625
phosphorus-H2O (mg/kg)	4.301	7.265
potassium-H2O (mg/kg)	17.327	16.6125
calcium-H2O (mg/kg)	77.146	68.7575
magnesium-H2O (mg/kg)	9.189	7.67125
iron-H2O (mg/kg)	15.43455	7.197875
phosphorus-AAE (mg/kg)	34.5015	41.56125
potassium-AAE (mg/kg)	159.79	77.78625
magnesium-AAE (mg/kg)	189.16	85.34375
manganese-AAE (mg/kg)	260.9	217.625
boron-AAE (mg/kg)	0.7	0.1375
copper-AAE (mg/kg)	5.57	3.9625
iron-AAE (mg/kg)	270.45	200

Table S4. ANOVA tables of plant height and chlorophyll content measured over time. Variables were untransformed (§), log transformed (¥), sqrt transformed (∞) or ranked transformed (†) to meet the requirements to perform the analysis. F-values and significance levels for a three-way analysis of variance with herbicide (Hc), application (Ap) and time (Ti) as separate factors and their interaction term are shown. P-values: ns not significant, . 0.1 < P < 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0 < P < 0.001.

	Error:Sample_ID			Error:Within			
	Genotype	Arsenic	G*A	Week	G*W	A*W	G*A*W
Variable	F	F	F	F	F	F	F
Figure 1							
Plant height †	1.04e-05 ***	3.98e-05 ***	0.154	< 2e-16***	0.000000148***	0.000115***	0.898617
Chlorophyll †	0.668	0.977	0.265	<2e-16 ***	0.7309	0.0578 .	0.998
Figure 2							
Plant height †	0.0298 *	3.07e-06 ***	0.2073	<2e-16 ***	8.08E-01	0.0392 *	0.0710 .
Figure 7							
Plant height †	0.00714 **	5.55e-15 ***	0.05258 .	< 2e-16 ***	0.286	6.61e-07 ***	0.998

	Error:Sample_ID			Error:Within			
	Genotype	Field	G*F	Week	G*W	F*W	G*F*W
Variable	F	F	F	F	F	F	F
Figure 3							
Plant height †	0.002208 **	0.000225 ***	0.725803	< 2e-16 ***	5.12e-05 ***	0.128	0.516

	Error:Sample_ID			Error:Within			
	Conditioning	Arsenic	C*A	Week	C*W	A*W	C*A*W
Variable	F	F	F	F	F	F	F
Figure 8							
Plant height §	0.00663 **	8.04e-10 ***	0.08010 .	< 2e-16 ***	0.00393 **	< 2e-16 ***	0.38233

Table S5. ANOVA tables of leaves biomass and arsenic uptake in roots and leaves. Variables were untransformed (§), log transformed (¥), sqrt transformed (∞) or ranked transformed (†) to meet the requirements to perform the analysis. F-values and significance levels for a three-way analysis of variance with herbicide (Hc), application (Ap) and time (Ti) as separate factors and their interaction term are shown. P-values: ns not significant, . 0.1 < P < 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0 < P < 0.001.

	Genotype	Arsenic	G*A
Variable	F	F	F
Figure 1			
Leaves dry biomass †	0.00289 **	2.24e-09 ***	0.03787 *
Figure 2			
Leaves dry biomass §	0.0794 .	3.85e-12 ***	0.25
Figure 4			
As uptake roots †	0.00363 **	< 2e-16 ***	0.00210 **
As uptake leaves ¥	0.0406 *	<2e-16 ***	0.2455
Figure 7			
Leaves dry biomass §	0.00899 **	< 2e-16 ***	0.02049 *

	Genotype	Field	G*F
Variable	F	F	F
Figure 3			
Leaves dry biomass ∞	1.81e-07 ***	0.00946 **	0.41199

	Conditioning	Arsenic	C*A
Variable	F	F	F
Figure 8			
Leaves dry biomass §	0.00858 **	3.61e-10 ***	0.1235

Tables S6. Tukey tests to determine the difference among treatments for Figure 1, 3, 4, 5, 6, 8. P-values: ns not significant, . 0.1 < P < 0.05, * 0.05 < P < 0.01, ** 0.01 < P < 0.001, *** 0 < P < 0.001.

Figure 1	Plant height	Chlorophyll	Dry biomass
W22:0 ppm-bx1:0 ppm	0.5611	0.6598	0.890
bx1:100 ppm-bx1:0 ppm	0.1193	0.8294	0.0000001***
W22:100 ppm-bx1:0 ppm	0.9987	0.9865	0.018908*
bx1:100 ppm-W22:0 ppm	0.0027	0.9909	0***
W22:100 ppm-W22:0 ppm	0.6577	0.8502	0.002561**
W22:100 ppm-bx1:100 ppm	0.0841	0.9560	0.0028325**

Figure 3	Plant height	Dry biomass
W22:0 ppm-bx1:0 ppm	0.9690	0.8720
bx1:100 ppm-bx1:0 ppm	0.2948	0.0000009***
W22:100 ppm-bx1:0 ppm	0.0240278*	0.0000097***
bx1:100 ppm-W22:0 ppm	0.0496575*	0.0000651***
W22:100 ppm-W22:0 ppm	0.0016951**	0.0008968***
W22:100 ppm-bx1:100 ppm	0.9023	0.8053
bx2:0 ppm-bx1:0 ppm	0.9926	0.4574
bx2:100 ppm-bx1:0 ppm	0.0000004***	0***
W22:0 ppm-bx2:0 ppm	0.7486	0.9861
bx1:100 ppm-bx2:0 ppm	0.6515	0.0003106***
bx2:100 ppm-bx2:0 ppm	0.0000059***	0.0000264***
W22:100 ppm-bx2:0 ppm	0.1117	0.0045947**
bx2:100 ppm-W22:0 ppm	0***	0.0000053***
bx2:100 ppm-bx1:100 ppm	0.0032404**	0.9996
W22:100 ppm-bx2:100 ppm	0.0791736.	0.5212

Figure 4	Plant height	Dry biomass
bx1+BX:0 ppm-bx1:0 ppm	0.9755	0.9869
W22:0 ppm-bx1:0 ppm	0.9987	0.9932
bx1:100 ppm-bx1:0 ppm	0.0007081***	0***
bx1+BX:100 ppm-bx1:0 ppm	0.0195017*	0***
W22:100 ppm-bx1:0 ppm	0.1573	0***
W22:0 ppm-bx1+BX:0 ppm	0.8614	0.8324
bx1:100 ppm-bx1+BX:0 ppm	0.0103273*	0***
bx1+BX:100 ppm-bx1+BX:0 ppm	0.1420	0***
W22:100 ppm-bx1+BX:0 ppm	0.5565	0***
bx1:100 ppm-W22:0 ppm	0.0001413***	0***
bx1+BX:100 ppm-W22:0 ppm	0.0053593**	0***
W22:100 ppm-W22:0 ppm	0.0603534.	0***
bx1+BX:100 ppm-bx1:100 ppm	0.9328	0.2906
W22:100 ppm-bx1:100 ppm	0.5046	0.0005542***
W22:100 ppm-bx1+BX:100 ppm	0.9681	0.2109

Figure 5	Plant height	Dry biomass
W22:(As-)-bx1:(As-)	0.0011223**	0.0003338***
bx1:(As+)-bx1:(As-)	0.0012858**	0.1126
W22:(As+)-bx1:(As-)	0.8877	0.1084
bx1:(As+)-W22:(As-)	0***	0.0000011***
W22:(As+)-W22:(As-)	0.0000552***	0.3764
W22:(As+)-bx1:(As+)	0.0154284*	0.0005803***

Figure 6	Plant height	Dry biomass
W22:0 ppm-bx1:0 ppm	0.9182	0.7177
bx1:100 ppm-bx1:0 ppm	0.0000419***	0.0000001***
W22:100 ppm-bx1:0 ppm	0.0681427.	0.0016297**
bx1:100 ppm-W22:0 ppm	0.000003***	0***
W22:100 ppm-W22:0 ppm	0.0119816*	0.0000737***
W22:100 ppm-bx1:100 ppm	0.1059	0.0136562*

Figure 8	As uptake roots	As uptake leaves
W22:0 ppm-bx1:0 ppm	0.9897	0.1385
bx1:100 ppm-bx1:0 ppm	0***	0***
W22:100 ppm-bx1:0 ppm	0***	0***
bx1:100 ppm-W22:0 ppm	0***	0***
W22:100 ppm-W22:0 ppm	0***	0***
W22:100 ppm-bx1:100 ppm	0.0002867***	0.7630

Figure S5. Rarefaction plot with the rarefaction threshold labelled as red line for bacteria (A) and fungi (B).

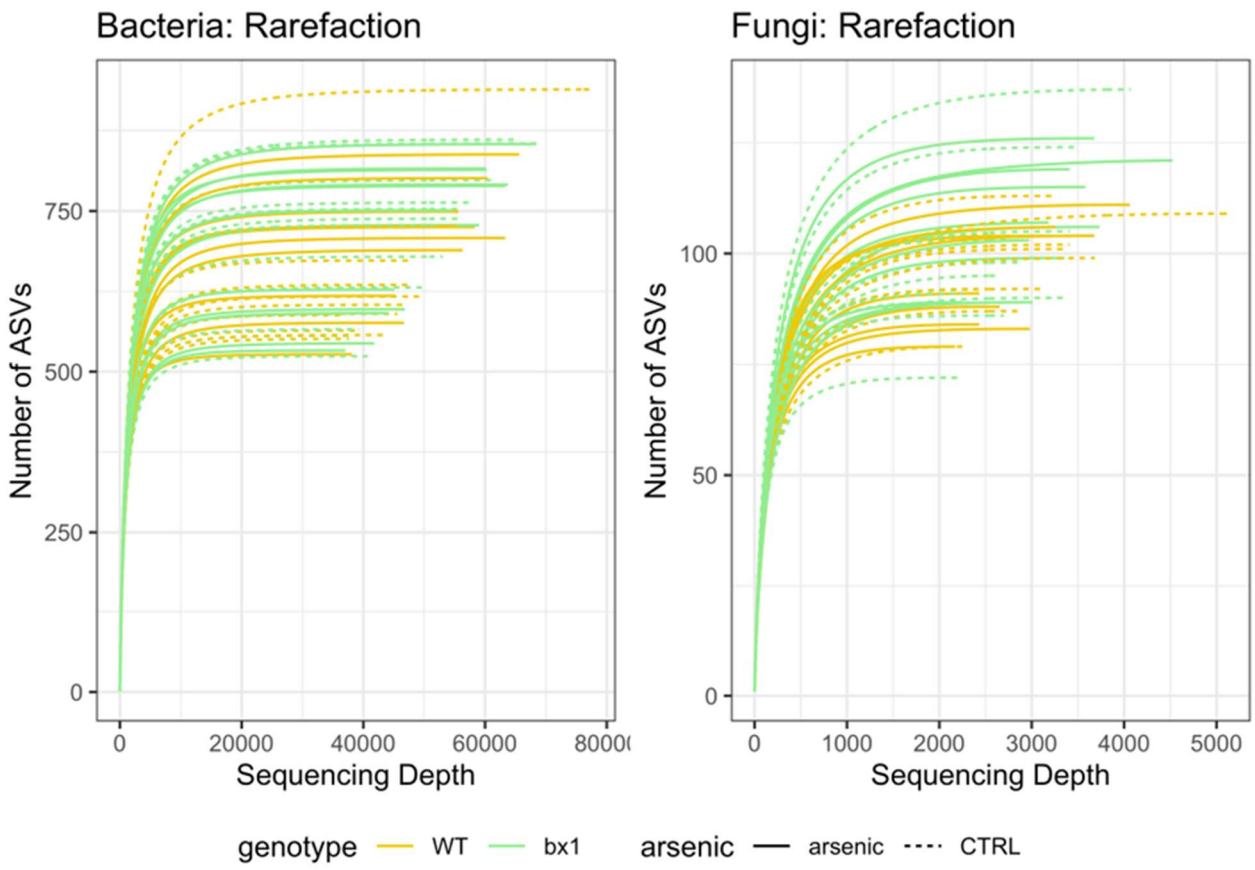


Figure S6. Relative abundance of different phyla for bacteria (A) and fungi (B).

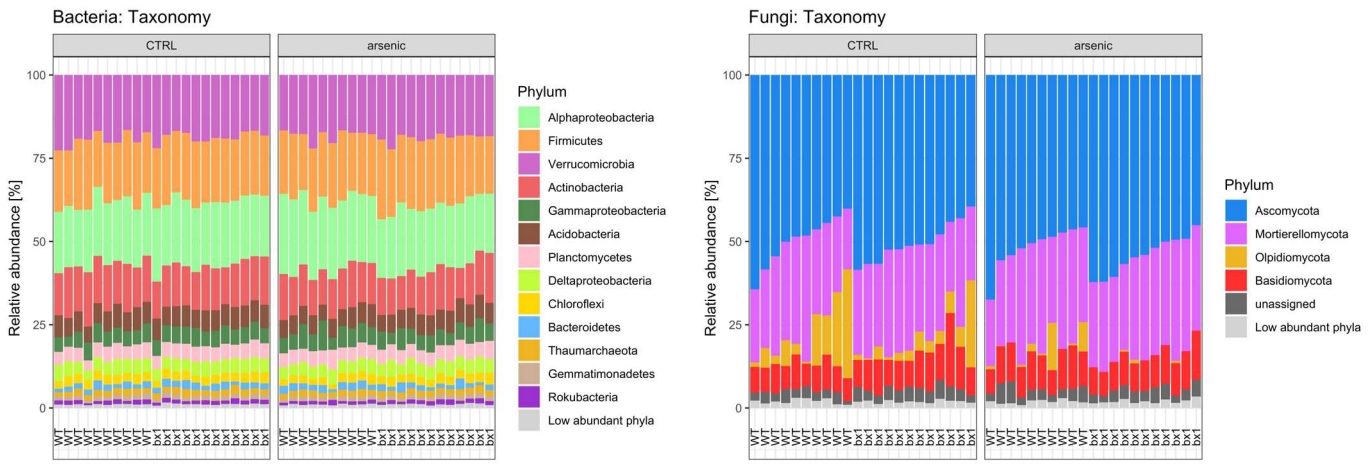


Table S7. PERMANOVA of phyla abundances (abundance ~ genotype * arsenic, 999 repetitions).

Taxa	Factor	Df	SumOfSqs	R2	F	Pr(>F)
Bacteria	arsenic	1	0.0038882	0.04372751	1.90381578	0.097
Bacteria	genotype	1	0.00297084	0.03341072	1.45464156	0.188
Bacteria	arsenic:genotype	1	0.00036702	0.00412761	0.17970875	0.957
Bacteria	Residual	40	0.08169277	0.91873415	NA	NA
Bacteria	Total	43	0.08891884	1	NA	NA
Fungi	arsenic	1	0.00397966	0.01001834	0.41468005	0.729
Fungi	genotype	1	0.0089264	0.02247121	0.93013019	0.427
Fungi	arsenic:genotype	1	0.01005068	0.02530145	1.04728	0.368
Fungi	Residual	39	0.37428052	0.942209	NA	NA
Fungi	Total	42	0.39723726	1	NA	NA

Table S8. ANOVA of alpha diversity (Shannon diversity ~ genotype * arsenic).

Taxa	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Bacteria	genotype	1	0.02808331	0.02808331	1.24414974	0.27132934
Bacteria	arsenic	1	0.00156474	0.00156474	0.06932106	0.79367946
Bacteria	genotype:arsenic	1	0.03245025	0.03245025	1.43761428	0.23758074
Bacteria	Residuals	40	0.9028917	0.02257229	NA	NA
Fungi	genotype	1	0.0478357	0.0478357	1.99925905	0.16530961
Fungi	arsenic	1	0.00489366	0.00489366	0.20452691	0.65359694
Fungi	genotype:arsenic	1	0.01818744	0.01818744	0.76013099	0.38862342
Fungi	Residuals	39	0.93314185	0.02392671	NA	NA

Table S9. The number of sensitive ASVs which have an altered relative abundance between the genotypes in arsenic-contaminated soil. The last column represents the relative abundance sum of all sensitive ASVs in the control treatment.

taxa	lower in CTRL	unchanged	higher in CTRL	rel. abu. of sens. ASVs
bacteria	0	1284	0	0%
fungi	0	176	0	0%

Figure S7. Arsenic speciation data in maize rhizosphere. The four panels represent four different measured compounds.

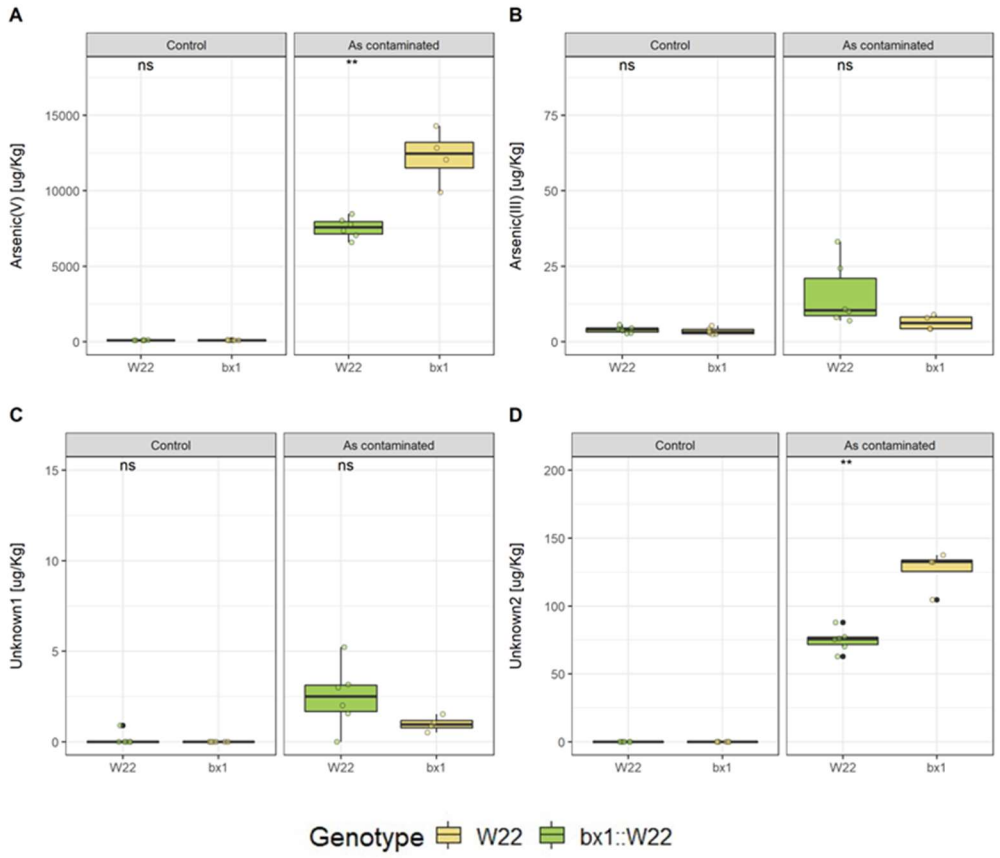


Table S10. Composition and purity of BXs used for complementation.

Compound	Conc_ug_ml
MBOA-Glc	0
HMBOA-Glc	0.514
HM2BOA-Glc	0
DIMBOA-Glc	24.019
DIM2BOA-Glc	0.409
HMBOA	0
DIMBOA	0.244
HDMBOA-Glc	2.229
HDM2BOA-Glc	0
MBOA	0
DIMBOA-2xHexose	0
DIMBOA-3xHexose	0.031
HMBOA-2xHexose	0.297
Total:	**27.743** **ug/ml**
Prepared for analysis:	50 ug/ml
Final BXs purity:	56%

General discussion

In this work, we investigated the feedbacks of chemically perturbated soil microbiomes on soil health and if this impacted plant performance. We selected three different chemical stress compounds to study the feedbacks: a metalloid, two herbicides, and a group of plant secondary metabolites. These chemicals represented three very different types of compounds typically found in soil: arsenic as a naturally geogenic source of pollution (can also be anthropogenic), herbicides as anthropogenic chemicals and benzoxazinoids as highly bioactive secondary metabolites that plants release to the surrounding soil as exudates. This constitutes of high interest and importance, first because human health is interconnected with environmental health, and secondly because agriculture is facing enormous challenges on the point of view of food safety and food security. In this thesis we particularly focused on the effects of arsenic and the herbicides glyphosate and terbuthylazine on the soil microbial communities and their functioning, and on maize health. In addition, we explored the possible interaction between arsenic and root exudates of plants (benzoxazinoids) in soil. We found that herbicides affected marginally the soil microbial communities and their functioning and that this was highly context-dependent (time and soil moisture). While this indicated that herbicide applications impacted soil health, we did not measure negative downstream consequences on crop performance; neither by the pre- nor post-emergence application of glyphosate and terbuthylazine, respectively. Similarly, studying arsenic as chemical stress in soil, we discovered that arsenic did not influence the enzyme activities and plant-associated microorganisms while direct toxicity was observed at the level of plant biomass. We only observed an increase in soil bacterial diversity in arsenic contaminated soils, three months after planting maize plants. The unexpected finding of this thesis was that benzoxazinoids can confer tolerance to the plant by mitigating the toxic effects of arsenic (**Figure 1**). Hereafter I discuss the importance of studying the interactions between the environment and chemical inputs, especially in systems such as agriculture that represent a possible entrance for these chemicals in the food chain.

Environmental chemicals have direct impacts on soil microbial communities

In this thesis, we investigated how chemical perturbations in the soil could negatively impact soil microbial communities and their functioning and that will also negatively affect plant performance. To this end, we have first profiled the soil bacterial and fungal communities to quantify the direct effects of the chemical perturbations on the soil microbiome. Regarding the direct effects of chemical soil stress, we have shown that soil microbial communities were mostly influenced by environmental conditions, such as soil moisture, rather than the direct or indirect applications of chemical stresses such as herbicides or soil pollution by arsenic. In addition, the herbicide treatment at recommended doses, regardless of if it was glyphosate or terbuthylazine, had a small but significant impact on the bacterial microbiome composition.

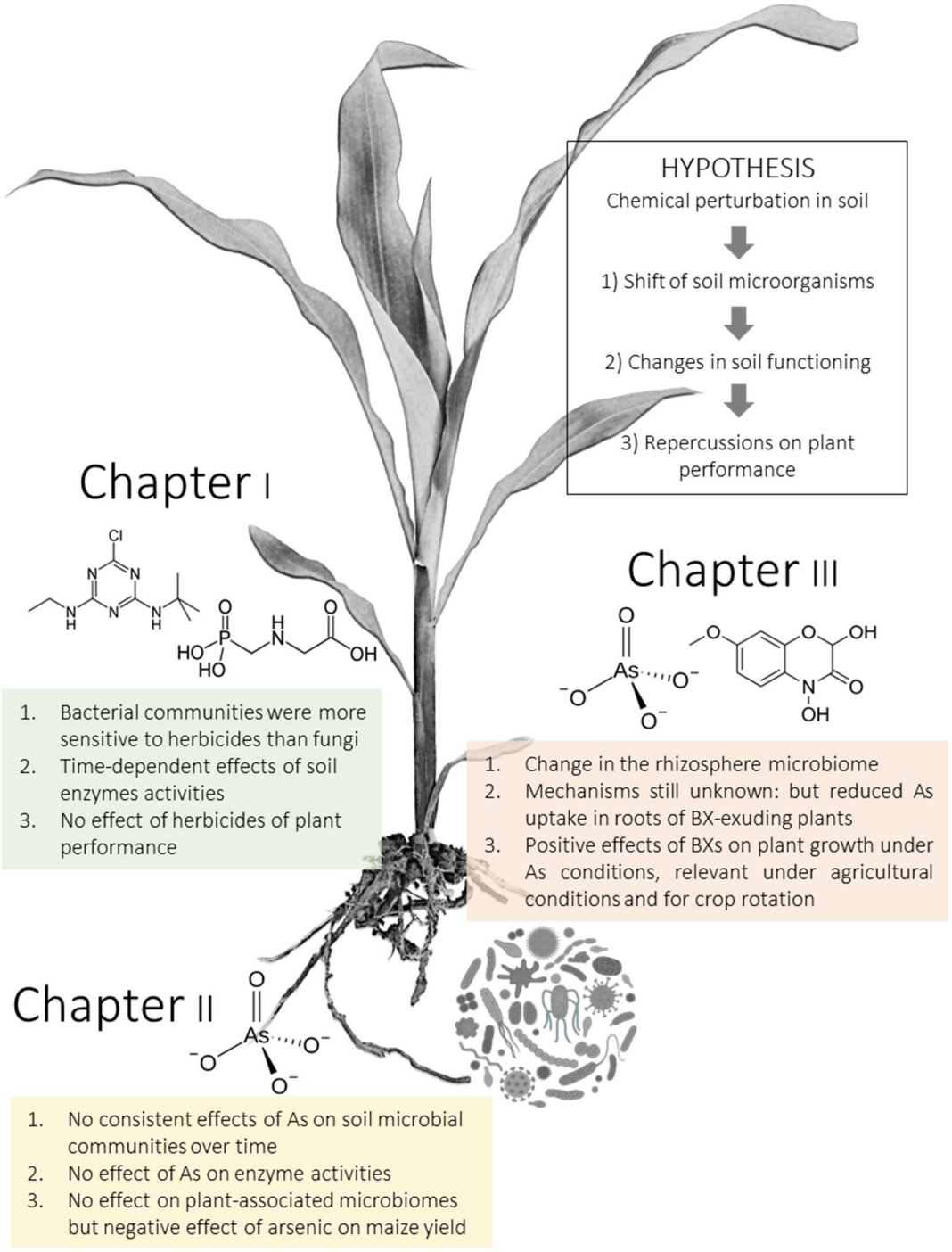


Figure 1. Summary of the main results of the thesis separated by chapters: Chapter I (glyphosate and terbuthylazine), Chapter II (arsenic) and Chapter III (arsenic and benzoxazinoids) studied the different environmental chemicals and their feedbacks on soil and plant health.

In the case of arsenic, soil bacterial diversity was affected but there was no constant effect of arsenic on soil microbial communities over time. It is curious that the application of recommended doses of glyphosate and terbuthylazine, and arsenic up to 200 mg/kg in soil have shown small shifts in the microbial communities, while arsenic did not affect soil microbial communities over time and plant-associated microbiomes (especially roots). This can either suggest that we were missing proper replication or that there is a high variation of

changes in microbiome shaping among situations. Sor sure, high variation can be triggered by a weak number of biological replicates and an heterogenous presence of arsenic in the soil. Concerning the last reason, our soil preparation with arsenic was done in several steps, trying to mix thoroughly after each watering with arsenic solution. Therefore, maybe the number of replicates in this case was not enough to represent the entire microbial community in the pot. For a next experiment we could think about repetitive sampling of soil in one single pot. It is also known that soil microbial communities can be already highly influenced by parameters such as soil characteristics, (Anandham & Sa, 2021; Custódio et al., 2022; Kim et al., 2021; Panax & Meyer, 2021) and that soil characteristic, such as pH or organic matter content among others, can highly affect arsenic bioavailability (Cao & Ma, 2004; Mandal & Suzuki, 2002; Tu & Ma, 2003). It is still very surprising that we could not observe changes in soil and roots microbiome upon arsenic contamination, and no constant effects on soil communities exposed to arsenic over time. Other studies have shown shifts in the microbiome, to help the plant with As remediation or metabolization (Ali et al., 2022; González Henao & Ghneim-Herrera, 2021; Xiao et al., 2021). However, it is less surprising that we did not observe differences in the leaf and kernel's microbiomes, as they are the plant compartment less connected to the soil. Overall, we observed small but significant effects on the soil microbiome upon both herbicides and arsenic presence, that can still have cascading effects on plant performance.

Soil functioning was not or only slightly changed by the chemical perturbations

Together with quantifying the chemical perturbations on the microbial communities, we also measured enzyme activities as a proxy for a healthy soil functioning. The activities of soil enzyme activities are commonly used indicators of soil health because of their rapid response to both natural and anthropogenic factors (Miller & Dick, 1995b; Theriot et al., 2013). These measurements complemented the data on microbial community composition as they would indicate if eventual changes in microbiome structure have led to altered functioning. Due to the nature of the two analyses, a direct connection, of which microbial groups should be responsible for certain altered enzymatic activities, is unfortunately impossible. In the case of soil perturbations by herbicides (Chapter I) we observed slight shifts in the microbial community that were context-dependent (for example to soil moisture or time, **Fig. 4**) and we also found altered enzyme activities. Hence, there were parallel effects, suggesting that the herbicide induced perturbation affected the composition of the soil microbiome and consequently also its functioning. Consistent with these parallel effects in case of our herbicide work, we also found paralleling effects of microbial communities and enzyme activities upon arsenic contamination (Chapter II). However, the main difference was that while we found soil perturbations by herbicides, this was not observed with soil pollution by arsenic (Chapter II). Consistency in parallel effects were that following the lack of soil and roots microbiome perturbations and also no constant arsenic-dependent changes in soil enzymatic activities were found. Our results are in contrast with other

studies that showed significant inhibition of enzymes activities (including β-glucosidase) in arsenic conditions (Das et al., 2013). Taken together, our work confirms the literature that soil enzyme activity measurements present good first proxies that the composition of soil microbial communities was influenced e.g., by environmental chemicals. Nevertheless, more in-depth follow up work is needed to pin-point the specific functions of the microbial groups but also more systematic approaches studying the ecosystems functions of these microbes on the soil-plant system. Future studies could go back to *in vitro* experiments to see which bacterial or fungal groups are more sensitive to arsenic presence, for later built a gnotobiotic system including a plant and further test *in vivo* those interactions.

Overall, our results about the impact of herbicides and arsenic on soil microbial communities and soil functioning revealed a very strong and important context-dependency. Even though there are probably greater drivers for the soil ecosystem composition and functioning than environmental chemicals, soil contaminants still show induced microbiome changes that can play a role in plant performance.

Arsenic negatively affected maize biomass while herbicides did not have any impact on plant performance

Downstream of quantifying the direct effects of the chemical perturbations on the soil microbiome and to test the third part of the main underlying hypothesis, we studied the indirect feedback effects on plant performance. The two study cases with herbicides and arsenic yielded opposing findings: while we didn't detect negative chemical feedbacks on maize performance after application of the herbicides glyphosate and terbuthylazine (Chapter I), we found a negative feedback in form of reduced maize growth in As polluted soil (Chapter II). When maize was exposed to 100 mg/kg of inorganic arsenic in soil, height and yield was reduced but the plants were still able to expand and carry out a normal development though a bit slower and delayed. However, under 200 mg/kg of inorganic arsenic in soil, plants were highly affected in their development and growth. Especially their yield was reduced more than twice compared to the control treatment.

With reference to the main underlying hypothesis the two study cases illustrate that negative plant performance do not need to be caused by soil microbiome changes. In the case of herbicide applications, we did not detect feedbacks on plant performance but the applied chemically still perturbated the soil microbial community and impacted their functioning. This clearly shows how well these chemicals are designed to strongly sorb to the soil particles, which ends up by increasing their persistence in the environment (Dollinger et al., 2015; Ronka & Bodylska, 2021). Future work needs to show whether similar effects are present beyond recommended doses and repetitive applications.

Taken together these results pointed out that complete incorporation of a chemical into the soil system is much more impactful and dangerous than an intermittent addition to the studies system. This suggests that a

forced and thorough introduction of chemical into the environment causes many more damages than an occasional entrance.

Root exuded benzoxazinoids increase arsenic tolerance of maize as well as on the next plant generation

Because benzoxazinoids in maize root exudates change the surrounding soil microbiome that then affect plant performance (Hu et al., 2018b) and because benzoxazinoids mitigate aluminium toxicity in plants (Poschenrieder et al., 2005), we tested here the hypothesis that they also facilitate plant growth in arsenic-contaminated soil, possibly via microbiome-mediate feedbacks on plant growth (Chapter III). We explored the potential of maize-exuded benzoxazinoids in mitigating arsenic toxicity in soil, by comparing wild-type and mutant plants growing in arsenic-free or contaminated soil. We found that the benzoxazinoid-exuding wild-type maize coped better with the arsenic pollution than its respective benzoxazinoid-deficient mutant (**Fig. 1**). We further corroborate by exogenous additions of benzoxazinoids to the mutant, that this compound in the maize exudates was sufficient to restore the better growth phenotype on arsenic polluted soil similar to the wild-type (**Fig. 3**).

Towards unravelling the underlying tolerance or detoxification mechanism by which benzoxazinoids may mediate better plant growth on arsenic contaminated soil, we measured arsenic levels in different plant tissues. The finding that wild-type maize plants take up less arsenic in their roots compared to the mutant (**Fig. 8**) suggesting a possible benzoxazinoid-mediated exclusion or low accumulation mechanisms, as it is happening with aluminium resistant maize variety (Poschenrieder et al., 2005). In contrast to a possible exclusion mechanism, benzoxazinoids are known for facilitating iron uptake to the plant (Hu et al., 2018a). Mechanistically, iron uptake functions by chelating the metal ions for assimilation as an important plant nutrient. In the study of Poschenrieder et al. (2005), they could show DIMBOA (a benzoxazinoid) binding to aluminium and as a consequence, indicators for Al toxicity demonstrated that benzoxazinoids can protect the plant. However together with our observation of lower arsenic uptake in the benzoxazinoid-producing plant compared to the mutant, we speculate the underlying mechanism must be a different one (rather exclusion or efficient effluxion), unless we can prove the formation of complexes with arsenic inside the plant that suggest chelation.

Interestingly, we discovered a shift in the microbial composition in the rhizosphere of the benzoxazinoid-exuding plants in arsenic polluted soil compared to the microbiome present under control conditions (**Fig. 7**). Maize-exuded benzoxazinoids are well-known to shape the host-associated microbiome (Cadot et al., 2021; Hu et al., 2018b; Kudjordjie et al., 2019) and the altered microbiome is known for feedbacks on plant performance (Hu et al., 2018b). Hence, our new result may indicate that the benzoxazinoid-shaped rhizosphere microbiome may help mediating the arsenic exclusion or interfere with its uptake and that may

explain the enhanced tolerance of benzoxazinoid-exuding plants. This is probably not the main reason and therefore future work is needed to show to which extend the rhizospheric microbial community plays a role in arsenic detoxification, for example by comparing wild-type and mutant plants growing in native or sterilized soil conditions.

With our study we further provided evidence that benzoxazinoids are plant secondary metabolites extremely useful for adaptation to the environment. Although, most of the studies focus on the potential of benzoxazinoids for pest control and plant-soil feedbacks in cropping system (Cadot et al., 2021; Hu et al., 2018b; Pineda et al., 2020), we explored the potential of benzoxazinoids in conferring to the plant tolerance against arsenic toxicity in soil. Our work further pointed to the importance of benzoxazinoid exudation in arsenic contaminated soils for the next plant generation (Fig. 6). We found that benzoxazinoid exudation in a first generation will benefit a next generation of both wild-type and mutant plants in arsenic polluted soil. Because benzoxazinoids cause feedbacks on multiple generations in soil they probably have affect crop productivity in agriculture. Therefore, plant secondary metabolites, beyond their importance in adaptation and defense responses in the plant (Isah, 2019), they may also present an interesting breeding possibility for sustainable agriculture. What we discovered with this thesis – benzoxazinoid exuding plants cope better with soil arsenic pollution – presents a new finding also with a perspective for agriculture. Our discovery could present the rational basis to cultivate maize also on (mildly) As contaminated soils in a safe manner. As benzoxazinoids biosynthesis is well understood and it has been shown that they production could be enhanced by genetic engineering (Zheng et al., 2015), over-producing maize plants can be used in extensively contaminated areas to ensure food production. Especially that the potential risk of high arsenic levels in groundwater (Podgorski & Berg, 2020), that could be used to irrigate or that directly affect crops, is spread worldwide (Figure 2) and includes areas where maize is staple food. Therefore, the mechanism behind benzoxazinoids alleviating arsenic toxicity must be further studied to be able to completely unleash the potential of benzoxazinoids against As toxicity as a tool to ensure food security and safety.

Context-dependence in biological systems

With a series of experiments, we tried to unravel the impact of diverse environmental chemical on the soil microbial communities and soil functioning, and to evaluate the consequences trying to disentangle the direct and mediated effects on the crop performance. Having relatively complex systems that involved different chemicals, type of application, concentrations, soil parameters and the temporal variable, we realized how difficult it is to have consistent observations. In fact, by investigating diverse environmental chemicals (Chapter I and II), different entrance in the ecosystem and a variety of soil and plant health-related parameters, we brought up a crucial topic: the context-dependence. Context-dependence emerge when ecological relationships vary in their magnitude or signal depending on the conditions in the moment of the observation

(Catford et al., 2022). Since this is the result of multiple factors and processes, concluding that relationships are context dependent provides only limited understanding of the biological phenomenon, unless we can identify the underlying causes.

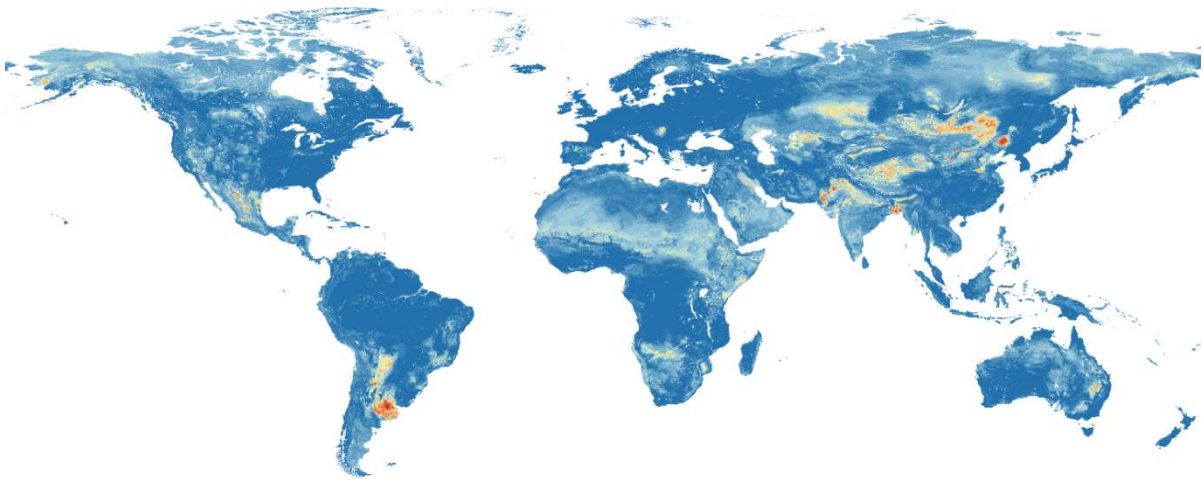


Figure 2. World map taken from Podgorski & Berg (2020) illustrating the regions around the world with potential groundwater arsenic pollution.

The context-dependence in nature has been found and studied in a huge variety of systems. In fact, it has been found in ecological studies to have a role in species interactions (Chamberlain et al., 2014), as well as in ecological risk assessments of contaminated sites with bioavailability, microbial transformations, chemical and physical characteristics (Liu et al., 2015). It has also been detected in microbial communities assembly (Bittleston et al., 2020) and even in gene regulation (Gamazon, 2021). It is well proved that context-dependence is present and has a crucial impact in predicting biological interactions (Catanach et al., 2018). This means that we should more and more include modelling in our research to be able to point which parameters or conditions are the most important for the phenomenon we are studying. At last, being able to recognize and address the different origins of context dependence should ease a better understanding, prediction, generalisation, and application of these studies (Catford et al., 2022). The application of biological solutions for a problem requires to understand its context dependency, while chemicals are more likely to work in many different situations (a pesticide, or fertilisers), with the related environmental health consequences, this is not the case for nature-based solutions. If we want to go toward sustainable agriculture exploiting microbial functions, we need to understand the exact conditions in which we are working and develop adapted tools.

Outlook and open questions

Investigating the impact of herbicides, arsenic and the interaction between arsenic and plant secondary metabolites on soil and plant-associated microbial communities, soil functioning, and plant performance we revealed important context-dependencies and new chemical interactions of these environmental chemicals. Nevertheless, results in this thesis pointed out new research questions that are worth to be tackled to know even more about the mechanisms behind the interactions within the soil-plant system challenged with chemical perturbations.

Chapter I:

How do glyphosate and terbuthylazine influence the soil-plant system with repetitive applications or beyond recommended doses?

In our experiments we applied the herbicides only once and only in recommended doses. However, it would be important to test the effects of repeated herbicide applications as it is normally done in agriculture, and to evaluate the effects of higher doses as well of herbicides. In this case, it would be possible as well to test the factor "time" and evaluate the short and long-term effects of herbicides input in agricultural system on soil microorganisms, soil functioning and crop performance.

What could be the relationship of plant secondary metabolites in enhancing herbicides' tolerance in both soil microorganisms and plant?

We already know that plant secondary metabolites play an important role in reducing biotic and abiotic stresses for plants. By using wild-type and mutant plants and i*n vitro* assays, we could further unravel the importance of root exuded secondary metabolites in microbiome shaping and resistance against herbicides present in soil. Benzoxazinoids could work as mediator and recruiter of herbicide-tolerant genus conferring the plant the resistance to such environmental chemical.

Chapter II:

What would be the impact of other arsenic species on the soil-plant system?

We focused our experiments using the inorganic species AsV as it is the most abundant in soils. However, it would be worth to compared as well the effects of more toxic arsenic species, starting from AsIII (more toxic than AsV), in the exact same soil-plant system. This could help understanding

the microorganisms and plant strategies to tolerate, detoxify or transform different arsenic species in their system.

Is there enhancement of gene expression related to arsenic detoxification?

A wide array of arsenic-related genes in microorganisms are known. Since we did not observe any differences in the soil microbial communities exposed to arsenic, it would be interesting to test the gene expression of microorganisms being expose or not to arsenic in soil. As most of the microbes have the potential to transform and detoxify arsenic, the enhancement of arsenic-related gene expression in contaminated soil could be a confirmation of the adaptation of the soil microbial communities.

What are the potential downstream effects of consumers eating leaves of maize growing in arsenic?

It would be for sure an interesting follow-up experiment within the One Health concept, to feed cows with leaves of maize growing in arsenic contaminated soil and see if the arsenic transferred from the plant to the animal is responsible for changes in the ruminants' gut microbiome. I would like to clarify that I do not know if this would be possible and how, since I do not know requirements and possibility in animal research. It would therefore be wise to first discuss this idea with expert in ruminant's gut microbiome research.

Chapter III:

What is the exact mechanism of arsenic tolerance or detoxification by mean of benzoxazinoids?

The phenotype we observed is clearly benzoxazinoids-mediated. However, we are still missing the exact mechanisms behind this. Arsenic complexation is suggested, and therefore it would be useful to develop and analytical method that can detect As-BXs complexes which could be measured into the soil. It is also worth to eventually work and set up experiments to understand if we are observing an exclusion mechanism where arsenic is blocked at the entrance or excreted by the plant (transporters).

Which benzoxazinoids are responsible for the beneficial effects under arsenic contaminated soils?

Future studies should tease apart which benzoxazinoids, if there is one, is mainly responsible for the interaction with arsenic in soil and that confers to the plant the wanted tolerance.

This can be done by using different maize mutants that show differences in benzoxazinoids production and amounts. This strategy can put beside the previous research questions that aims to develop an analytical procedure to be able to detect and measure complexes metalloid-BXs.

Do other benzoxazinoid-exuding species show the same phenotype as maize?

Maize is not the only BX-exuding plant, as this is a feature of grasses. It would be therefore compelling if other species other than maize show the same tendency to be more arsenic-tolerant than their respective mutants or compared to comparable crops. In addition, it would be interesting to know if this phenomenon also exists under natural conditions (and not only in agriculture), which can be an important step toward understanding other ecological functions of benzoxazinoids.

Can this be implemented in agriculture as a crop rotation system/strategy?

Depending on the exact mechanism behind the positive interaction between arsenic and benzoxazinoids, it would be interesting to test how this can be practically used by farmers. For sure, it is now advisable to use BX-exuding cultivars in arsenic contaminated sites as they cope better against the metalloid. In addition, if the mechanism results in detoxification, immobilization, or sequestration by the plant, this can be implemented in crop rotation systems to ensure a greater food safety and security even in contaminated areas.

Altogether, this thesis highlighted the strong context-dependence of herbicides and arsenic effects on soil microbial communities and soil functioning. We observed small changes in soil microbial communities and soil functioning when soil was challenged with different environmental chemicals, suggesting that despite other major drivers, these chemicals induced changes that can have cascading effects on plant performance. In addition, we observed a novel function of a class of specialized metabolites, that were able to confer arsenic tolerance to the plant, but also to next plant generation and complemented mutant. If we aim to go towards nature-based solutions for the future of sustainable agriculture and to counteract the negative effects of chemical inputs, it is necessary to understand every context-dependency related to targeted system. Future studies addressing the open questions mentioned above could lead to deeper insights into the protecting mechanisms and interactions between the soil-plant system and chemical inputs in agriculture, as this represents a possible route for these chemicals into the food chain.

References

Abedin, M. J., Feldmann, J., & Meharg, A. A. (2002). Uptake kinetics of arsenic species in rice plants. *Plant Physiology*, *128*(3), 1120–1128. https://doi.org/10.1104/pp.010733

Abid, N., Ansari, A., & Singh, S. (2020). *Contaminants in Agriculture*.

Adriano, D. (2001). *Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals*.

Ahmad, S., Veyrat, N., Gordon-Weeks, R., Zhang, Y., Martin, J., Smart, L., Glauser, G., Erb, M., Flors, V., Frey, M., & Ton, J. (2011). Benzoxazinoid metabolites regulate innate immunity against aphids and fungi in maize. *Plant Physiology*, *157*(1), 317–327. https://doi.org/10.1104/pp.111.180224

Ali, S., Tyagi, A., Mushtaq, M., Al-Mahmoudi, H., & Bae, H. (2022). Harnessing plant microbiome for mitigating arsenic toxicity in sustainable agriculture. *Environmental Pollution*, *300*(February), 118940. https://doi.org/10.1016/j.envpol.2022.118940

Allegrini, M., Gomez, E. del V., & Zabaloy, M. C. (2017). Repeated glyphosate exposure induces shifts in nitrifying communities and metabolism of phenylpropanoids. *Soil Biology and Biochemistry*, *105*, 206–215. https://doi.org/10.1016/j.soilbio.2016.11.024

Amaibi, P. M., Entwistle, J. A., Kennedy, N., Cave, M., Kemp, S. J., Potgieter-Vermaak, S., & Dean, J. R. (2019). Mineralogy, solid-phase fractionation and chemical extraction to assess the mobility and availability of arsenic in an urban environment. *Applied Geochemistry*, *100*(December 2018), 244–257. https://doi.org/10.1016/j.apgeochem.2018.12.004

Amini, M., Mueller, K., Abbaspour, K. C., Rosenberg, T., Afyuni, M., Møller, K. N., Sarr, M., & Johnson, C. A. (2008). Statistical modeling of global geogenic fluoride contamination in groundwaters. *Environmental Science and Technology*, *42*(10), 3662–3668. https://doi.org/10.1021/es071958y

Anandham, R., & Sa, T. (2021). Editorial for special issue "environmental factors shaping the soil microbiome." *Applied Sciences (Switzerland)*, *11*(21). https://doi.org/10.3390/app112110387

Anastassiadou, M., Bernasconi, G., Brancato, A., Carrasco Cabrera, L., Greco, L., Jarrah, S., Kazocina, A., Leuschner, R., Magrans, J. O., Miron, I., Nave, S., Pedersen, R., Reich, H., Rojas, A., Sacchi, A., Santos, M., Stanek, A., Theobald, A., Vagenende, B., & Verani, A. (2020). Review of the existing maximum residue levels for terbuthylazine according to Article 12 of Regulation (EC) No 396/2005. *EFSA Journal*, *18*(1), 1–48. https://doi.org/10.2903/j.efsa.2020.5980

Andres, J., & Bertin, P. N. (2016). The microbial genomics of arsenic. *FEMS Microbiology Reviews*, *40*(2), 299–322. https://doi.org/10.1093/femsre/fuv050

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data.* Babraham Bioinform. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Aon, M. A., Cabello, M. N., Sarena, D. E., Colaneri, A. C., Franco, M. G., Burgos, J. L., & Cortassa, S. (2001). Spatio-temporal patterns of soil microbial and enzymatic activities in agricultural soils. *Anatomical Sciences Education 18*.

Apprill, A., Mcnally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic Microbial Ecology*, *75*(2), 129–137. https://doi.org/10.3354/ame01753

B. J. Callahan, P. J. Mcmurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, & S. P. Holmes. (2016). *DADA2 : High-resolution sample inference from Illumina amplicon data*. *13*. https://doi.org/10.1038/nmeth.3869

Badri, D. V., Weir, T. L., van der Lelie, D., & Vivanco, J. M. (2009). Rhizosphere chemical dialogues: plant-microbe interactions. *Current Opinion in Biotechnology*, *20*(6), 642–650. https://doi.org/10.1016/j.copbio.2009.09.014

Bai, J., Lin, X., Yin, R., Zhang, H., Junhua, W., Xueming, C., & Yongming, L. (2008). The influence of arbuscular mycorrhizal fungi on As and P uptake by maize (Zea mays L.) from As-contaminated soils. *Applied Soil Ecology*, *38*(2), 137–145. https://doi.org/10.1016/j.apsoil.2007.10.002

Bai, S. H., & Ogbourne, S. M. (2016). Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. *Environmental Science and Pollution Research*, *23*(19), 18988–19001. https://doi.org/10.1007/s11356-016-7425-3

Bali, A. S., & Sidhu, G. P. S. (2021). Arsenic acquisition, toxicity and tolerance in plants - From physiology to remediation: A review. *Chemosphere*, *283*(June), 131050. https://doi.org/10.1016/j.chemosphere.2021.131050

Banerjee, S., & Heijden, M. G. A. (2022). *Soil microbiomes and one health*. *19*. https://doi.org/10.1038/s41579-022-00779-w

Barriuso, J., Marín, S., & Mellado, R. P. (2010). Effect of the herbicide glyphosate on glyphosate-tolerant maize rhizobacterial communities: A comparison with pre-emergency applied herbicide consisting of a combination of acetochlor and terbuthylazine. *Environmental Microbiology*, *12*(4), 1021–1030. https://doi.org/10.1111/j.1462-2920.2009.02146.x

Bartucca, M. L., Celletti, S., Mimmo, T., Cesco, S., Astolfi, S., & Del Buono, D. (2017). Terbuthylazine interferes with iron nutrition in maize (Zea mays) plants. *Acta Physiologiae Plantarum*, *39*(10), 1–8. https://doi.org/10.1007/s11738-017-2537-z

Bartucca, M. L., Di Michele, A., & Del Buono, D. (2018). Interference of three herbicides on iron acquisition in maize plants. *Chemosphere*, *206*, 424–431. https://doi.org/10.1016/j.chemosphere.2018.05.040

Becerril, J. M., Duke, S. O., & Lydon, J. (1989). Glyphosate effects on shikimate pathway products in leaves and flowers of velvetleaf. *Phytochemistry*, *28*(3), 695–699. https://doi.org/10.1016/0031-9422(89)80095-0

Bentley, R., & Chasteen, T. G. (2002). Microbial Methylation of Metalloids: Arsenic, Antimony, and Bismuth. *Microbiology and Molecular Biology Reviews*, *66*(2), 250–271. https://doi.org/10.1128/mmbr.66.2.250-271.2002

Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, *17*(8), 478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Berg-Lyons, D., Lauber, C. L., Humphrey, G., Luke Thompson, Jack A. Gilbert, Janet K. Jansson, & Knight, R. (2018). *EMP DNA Extraction Protocol*. https://www.protocols.io/view/emp-dna-extraction-protocol-4r3l2rkjv1y9/v1

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., & Maguin, E. (2020). *Microbiome definition re-visited : old concepts and new challenges*. 1–22.

Bezemer, T. M., Lawson, C. S., Hedlund, K., Edwards, A. R., Brook, A. J., Igual, J. M., Mortimer, S. R., & Van Der Putten, W. H. (2006). Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *Journal of Ecology*, *94*(5), 893–904. https://doi.org/10.1111/j.1365-2745.2006.01158.x

Bhattacharyya, P. N., & Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology and Biotechnology*, *28*(4), 1327–1350. https://doi.org/10.1007/s11274-011-0979-9

Bhatti, S. M., Anderson, C. W. N., Stewart, R. B., & Robinson, B. H. (2013). Risk assessment of vegetables irrigated with arsenic-contaminated water. *Environmental Sciences: Processes and Impacts*, *15*(10), 1866–1875. https://doi.org/10.1039/c3em00218g

Bianucci, E., Peralta, J. M., Furlan, A., & Hernández, L. E. (2020). Arsenic in Wheat, Maize, and Other Crops. *Arsenic in Drinking Water and Food*, *January*. https://doi.org/10.1007/978-981-13-8587-2

Bittleston, L. S., Gralka, M., Leventhal, G. E., Mizrahi, I., & Cordero, O. X. (2020). Context-dependent dynamics lead to the assembly of functionally distinct microbial communities. *Nature Communications*, *11*(1), 1–10. https://doi.org/10.1038/s41467-020-15169-0

Bodenhausen, N., Horton, M. W., & Bergelson, J. (2013). *Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana*. *8*(2). https://doi.org/10.1371/journal.pone.0056329

Borowik, A., Wyszkowska, J., Kucharski, J., Baćmaga, M., & Tomkiel, M. (2017). Response of microorganisms and enzymes to soil contamination with a mixture of terbuthylazine, mesotrione, and S-metolachlor. *Environmental Science and Pollution Research*, *24*(2), 1910–1925. https://doi.org/10.1007/s11356-016-7919-z

Bottoni, P., Grenni, P., Lucentini, L., & Caracciolo, A. B. (2013). Terbuthylazine and other triazines in Italian water resources. *Microchemical Journal*, *107*, 136–142. https://doi.org/10.1016/j.microc.2012.06.011

Brito, I. P. F. S., Tropaldi, L., Carbonari, C. A., & Velini, E. D. (2018). Hormetic effects of glyphosate on plants. *Pest Management Science*, *74*(5), 1064–1070. https://doi.org/10.1002/ps.4523

Brumovský, M., Bečanová, J., Kohoutek, J., Borghini, M., & Nizzetto, L. (2017). Contaminants of emerging concern in the open sea waters of the Western Mediterranean. *Environmental Pollution*, *229*, 976–983. https://doi.org/10.1016/j.envpol.2017.07.082

CABI. (2021). *Chenopodium album (fat hen)*. Invasive Species Compedium. https://www.cabi.org/isc/datasheet/12648#tosummaryOfInvasiveness

Cadot, S., Gfeller, V., Singh, N., Glauser, G., Croll, D., Sánchez-vallet, A., Erb, M., Heijden, M. G. A. Van Der, & Schlaeppi, K. (2021). *Soil composition and plant genotype determine benzoxazinoid- mediated plant – soil feedbacks in cereals*. *July*, 3732–3744. https://doi.org/10.1111/pce.14184

Cadot, S., Guan, H., Bigalke, M., Walser, J. C., Jander, G., Erb, M., van der Heijden, M. G. A., & Schlaeppi, K. (2021). Specific and conserved patterns of microbiota-structuring by maize benzoxazinoids in the field. *Microbiome*, *9*(1), 1–19. https://doi.org/10.1186/s40168-021-01049-2

Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). *DADA2 : High-resolution sample inference from Illumina amplicon data*. *13*(7). https://doi.org/10.1038/nmeth.3869

Cañero, A. I., Cox, L., Redondo-Gómez, S., Mateos-Naranjo, E., Hermosín, M. C., & Cornejo, J. (2011). Effect of the herbicides terbuthylazine and glyphosate on photosystem II photochemistry of young olive (Olea europaea) plants. *Journal of Agricultural and Food Chemistry*, *59*(10), 5528–5534. https://doi.org/10.1021/jf200875u

Cang, L., Zhou, D. M., Wang, Q. Y., & Wu, D. Y. (2009). Effects of electrokinetic treatment of a heavy metal contaminated soil on soil enzyme activities. *Journal of Hazardous Materials*, *172*(2–3), 1602–1607. https://doi.org/10.1016/j.jhazmat.2009.08.033

Cao, X., & Ma, L. Q. (2004). Effects of compost and phosphate on plant arsenic accumulation from soils near pressure-treated wood. *Environmental Pollution*, *132*(3), 435–442. https://doi.org/10.1016/j.envpol.2004.05.019

Cao, Y., Feng, H., Sun, D., Xu, G., Rathinasabapathi, B., Chen, Y., & Ma, L. Q. (2019). Heterologous Expression

of Pteris vittata Phosphate Transporter PvPht1;3 Enhances Arsenic Translocation to and Accumulation in Tobacco Shoots. *Environmental Science and Technology*, *53*(18), 10636–10644. https://doi.org/10.1021/acs.est.9b02082

Carding, S., Verbecke, K., Vipond, D. T., Corfe, B. M., & Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health and Disease*. https://doi.org/http://dx.doi.org/10.3402/mehd.v26.26191

Catanach, T. A., McCardell, R., Baetica, A. A., & Murray, R. M. (2018). Context dependence of biological circuits. *BioRxiv*, 1–24.

Catford, J. A., Wilson, J. R. U., Pyšek, P., Hulme, P. E., & Duncan, R. P. (2022). Addressing context dependence in ecology. *Trends in Ecology and Evolution*, *37*(2), 158–170. https://doi.org/10.1016/j.tree.2021.09.007

Ceci, A., Spinelli, V., Massimi, L., Canepari, S., & Persiani, A. M. (2020). Fungi and arsenic: Tolerance and bioaccumulation by soil saprotrophic species. *Applied Sciences (Switzerland)*, *10*(9). https://doi.org/10.3390/app10093218

Ceres. (2016). *Corn*. Non-Profit Organization. https://engagethechain.org/corn

Chamberlain, S. A., Bronstein, J. L., & Rudgers, J. A. (2014). How context dependent are species interactions? *Ecology Letters*, *17*(7), 881–890. https://doi.org/10.1111/ele.12279

Chauhan, B. S. (2020). *Grand Challenges in Weed Management*. *1*(January), 1–4. https://doi.org/10.3389/fagro.2019.00003

Chelius, M. K., & Triplett, E. W. (2001). *The Diversity of Archaea and Bacteria in Association with the*. 252–263. https://doi.org/10.1007/s002480000087

Chen, Q. Y., & Costa, M. (2021). Arsenic: A Global Environmental Challenge. *Annual Review of Pharmacology and Toxicology*, *61*, 47–63. https://doi.org/10.1146/annurev-pharmtox-030220-013418

Chiaia-Hernandez, A. C., Keller, A., Wächter, D., Steinlin, C., Camenzuli, L., Hollender, J., & Krauss, M. (2017). Long-Term Persistence of Pesticides and TPs in Archived Agricultural Soil Samples and Comparison with Pesticide Application. *Environmental Science and Technology*, *51*(18), 10642–10651. https://doi.org/10.1021/acs.est.7b02529

Chiaia-Hernández, A. C., Scheringer, M., Müller, A., Stieger, G., Wächter, D., Keller, A., Pintado-Herrera, M. G., Lara-Martin, P. A., Bucheli, T. D., & Hollender, J. (2020). Target and suspect screening analysis reveals persistent emerging organic contaminants in soils and sediments. *Science of the Total Environment*, *740*, 140181. https://doi.org/10.1016/j.scitotenv.2020.140181

Choudhury, B., Chowdhury, S., & Biswas, A. K. (2011). Regulation of growth and metabolism in rice (Oryza sativa L.) by arsenic and its possible reversal by phosphate. *Journal of Plant Interactions*, *6*(1), 15–24. https://doi.org/10.1080/17429140903487552

Ci, X. ke, Liu, H. lin, Hao, Y. bo, Zhang, J. wang, Liu, P., & Dong, S. ting. (2012). Arsenic Distribution, Species, and Its Effect on Maize Growth Treated with Arsenate. *Journal of Integrative Agriculture*, *11*(3), 416–423. https://doi.org/10.1016/S2095-3119(12)60026-4

Cubadda, F., Jackson, B. P., Cottingham, K. L., Van Horne, Y. O., & Kurzius-Spencer, M. (2017). Human exposure to dietary inorganic arsenic and other arsenic species: State of knowledge, gaps and uncertainties. *Science of the Total Environment*, *579*, 1228–1239. https://doi.org/10.1016/j.scitotenv.2016.11.108

Curran, W. S. (2001). Persistence of herbicides in soil. *Agronomy Facts*. https://doi.org/10.1017/s0043174500046105

Custódio, V., Gonin, M., Stabl, G., Bakhoum, N., Oliveira, M. M., Gutjahr, C., & Castrillo, G. (2022). Sculpting

the soil microbiota. *Plant Journal*, *109*(3), 508–522. https://doi.org/10.1111/tpj.15568

Dakora, F. D., & Phillips, D. A. (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant and Soil*, *245*(1), 35–47. https://doi.org/10.1023/A:1020809400075

Das, S., Jean, J. S., Kar, S., & Chakraborty, S. (2013). Effect of arsenic contamination on bacterial and fungal biomass and enzyme activities in tropical arsenic-contaminated soils. *Biology and Fertility of Soils*, *49*(6), 757–765. https://doi.org/10.1007/s00374-012-0769-z

David Paez-Espino, Javier Tamames, Vı´ctor de Lorenzo, & David Ca´novas. (2009). Microbial responses to environmental arsenic. *Biometals*.

De, A., Bose, R., Kumar, A., & Mozumdar, S. (2014). *Targeted Delivery of Pesticides Using Biodegradable Polymeric Nanoparticles*.

De Cesare, F., Garzillo, A. M. V., Buonocore, V., & Badalucco, L. (2000). Use of sonication for measuring acid phosphatase activity in soil. *Soil Biology and Biochemistry*, *32*(6), 825–832. https://doi.org/10.1016/S0038-0717(99)00212-6

Dolaptsoglou, C., Karpouzas, D. G., Menkissoglu-Spiroudi, U., Eleftherohorinos, I., & Voudrias, E. A. (2007). Influence of Different Organic Amendments on the Degradation, Metabolism, and Adsorption of Terbuthylazine. *Journal of Environmental Quality*, *36*(6), 1793–1802. https://doi.org/10.2134/jeq2006.0388

Dollinger, J., Dagès, C., & Voltz, M. (2015). Glyphosate sorption to soils and sediments predicted by pedotransfer functions. *Environmental Chemistry Letters*, *13*(3), 293–307. https://doi.org/10.1007/s10311-015-0515-5

Druart, C., Millet, M., Scheifler, R., Delhomme, O., & de Vaufleury, A. (2011). Glyphosate and glufosinate-based herbicides: Fate in soil, transfer to, and effects on land snails. *Journal of Soils and Sediments*, *11*(8), 1373–1384. https://doi.org/10.1007/s11368-011-0409-5

Duke, S. O. (2020). Glyphosate: Environmental fate and impact. *Weed Science*, *68*(3), 201–207. https://doi.org/10.1017/wsc.2019.28

Erb, M., & Kliebenstein, D. J. (2020). Plant Secondary Metabolites as Defenses, Regulators, and Primary Metabolites: The Blurred Functional Trichotomy1[OPEN]. *Plant Physiology*, *184*(1), 39–52. https://doi.org/10.1104/PP.20.00433

Erenstein, O., Jaleta, M., Sonder, K., Mottaleb, K., & Prasanna, B. M. (2022). Global maize production, consumption and trade: trends and R&D implications. *Food Security*, 1295–1319. https://doi.org/10.1007/s12571-022-01288-7

FAO. (2017a). *Sustainable food and agriculture*. Agricultural Systems. https://doi.org/10.1016/j.agsy.2003.10.002

FAO. (2017b). The future of food and agriculture: trends and challenges. In *The future of food and agriculture: trends and challenges* (Vol. 4, Issue 4). www.fao.org/publications%0Ahttp://www.fao.org/3/a-i6583e.pdf%0Ahttp://siteresources.worldbank.org/INTARD/825826-1111044795683/20424536/Ag_ed_Africa.pdf%0Awww.fao.org/cfs%0Ahttp://www.jstor.org/stable/4356839%0Ahttps://ediss.uni-goettingen.de/bitstream/han

FAO. (2018). *Arsenic*.

FAOSTAT. (2004a). *Food and Agriculture Organization of the United Nations*. http://faostat.fao.org/site/613/DesktopDefault.aspx?PageID=613#ancor

FAOSTAT. (2004b). *Food and Agriculture Organization of the United Nations*.

http://faostat.fao.org/site/613/DesktopDefault.aspx?PageID=613#ancor

Finnegan, P. M., & Chen, W. (2012). Arsenic toxicity: The effects on plant metabolism. *Frontiers in Physiology*, *3 JUN*(June), 1–18. https://doi.org/10.3389/fphys.2012.00182

Foundation for Arable Research. (2007). *Weeds, pests and diseases - Terbuthylazine*.

Franz, J. E., Mao, M. K., & Sikorski, J. A. (1997). Glyphosate: A Unique Global Herbicide. *Am. Chem. Soc.*

Frémont, A., Sas, E., Sarrazin, M., Gonzalez, E., Brisson, J., Pitre, F. E., & Brereton, N. J. B. (2022). Phytochelatin and coumarin enrichment in root exudates of arsenic-treated white lupin. *Plant Cell and Environment*, *45*(3), 936–954. https://doi.org/10.1111/pce.14163

Fuchs, B., Saikkonen, K., & Helander, M. (2021). Glyphosate-Modulated Biosynthesis Driving Plant Defense and Species Interactions. *Trends in Plant Science*, *26*(4), 312–323. https://doi.org/10.1016/j.tplants.2020.11.004

Gamazon, E. R. (2021). Detecting context-dependent gene regulation. *Nature Computational Science*.

Garcia, S. N., Osburn, B. I., & Jay-Russell, M. T. (2020). One Health for Food Safety, Food Security, and Sustainable Food Production. *Frontiers in Sustainable Food Systems*, *4*(January), 1–9. https://doi.org/10.3389/fsufs.2020.00001

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology*, *2*(2), 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

German, D. P., Weintraub, M. N., Grandy, A. S., Lauber, C. L., Rinkes, Z. L., & Allison, S. D. (2011). Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry*, *43*(7), 1387–1397. https://doi.org/10.1016/j.soilbio.2011.03.017

González Henao, S., & Ghneim-Herrera, T. (2021). Heavy Metals in Soils and the Remediation Potential of Bacteria Associated With the Plant Microbiome. *Frontiers in Environmental Science*, *9*(April), 1–17. https://doi.org/10.3389/fenvs.2021.604216

Gowdy, J., & Baveye, P. (2018). An evolutionary perspective on industrial and sustainable agriculture. In *Agroecosystem Diversity: Reconciling Contemporary Agriculture and Environmental Quality*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-811050-8.00027-3

Guan, D. X., Dai, Z. H., Sun, H. J., & Ma, L. Q. (2021). Arsenic and selenium in the plant-soil-human ecosystem: CREST publications during 2018–2021. *Critical Reviews in Environmental Science and Technology*, *52*(20), 3567–3572. https://doi.org/10.1080/10643389.2021.2010836

Guerrieri, A., Dong, L., & Bouwmeester, H. J. (2019). Role and exploitation of underground chemical signaling in plants. *Pest Management Science*, *75*(9), 2455–2463. https://doi.org/10.1002/ps.5507

Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., Straif, K., Blair, A., Fritschi, L., McLaughlin, J., Sergi, C. M., Calaf, G. M., Le Curieux, F., Baldi, I., Forastiere, F., Kromhout, H., ’t Mannetje, A., … Zeise, L. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *The Lancet Oncology*, *16*(5), 490–491. https://doi.org/10.1016/S1470-2045(15)70134-8

Hacquard, S., Garrido-Oter, R., González, A., Spaepen, S., Ackermann, G., Lebeis, S., McHardy, A. C., Dangl, J. L., Knight, R., Ley, R., & Schulze-Lefert, P. (2015). Microbiota and host nutrition across plant and animal kingdoms. *Cell Host and Microbe*, *17*(5), 603–616. https://doi.org/10.1016/j.chom.2015.04.009

Hamidian, A. H., Razeghi, N., Zhang, Y., & Yang, M. (2019). Spatial distribution of arsenic in groundwater of Iran, a review. *Journal of Geochemical Exploration*, *201*(February), 88–98. https://doi.org/10.1016/j.gexplo.2019.03.014

Han, F. X., Su, Y., Monts, D. L., Plodinec, M. J., Banin, A., & Triplett, G. E. (2003). Assessment of global industrial-age anthropogenic arsenic contamination. *Naturwissenschaften*, *90*(9), 395–401. https://doi.org/10.1007/s00114-003-0451-2

Heap, I. (2011). *International Survey of Herbicide Resistant Weeds*.

Helander, M., Saloniemi, I., Omacini, M., Druille, M., Salminen, J. P., & Saikkonen, K. (2018). Decreases mycorrhizal colonization and affects plant-soil feedback. *Science of the Total Environment*, *642*, 285–291. https://doi.org/10.1016/j.scitotenv.2018.05.377

Hernandez, A., Garcia-Plazaola, J. I., & Becerril, J. M. (1999). Glyphosate effects on phenolic metabolism of nodulated soybean (Glycine max L. Merr.). *Journal of Agricultural and Food Chemistry*, *47*(7), 2920–2925. https://doi.org/10.1021/jf981052z

Hong, Y., Song, K., & Chung, J. (2014). *Health Effects of Chronic Arsenic Exposure*. 245–252.

Hu, L., Mateo, P., Ye, M., Zhang, X., Berset, J. D., Handrick, V., Radisch, D., Grabe, V., Köllner, T. G., Gershenzon, J., Robert, C. A. M., & Erb, M. (2018a). Plant iron acquisition strategy exploited by an insect herbivore. *Science*, *361*(6403), 694–697. https://doi.org/10.1126/science.aat4082

Hu, Lingfei, Robert, C. A. M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., Van Der Heijden, M. G. A., Schlaeppi, K., & Erb, M. (2018b). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications*, *9*(1), 1–13. https://doi.org/10.1038/s41467-018-05122-7

Hussain, M. I., Araniti, F., Schulz, M., Baerson, S., Vieites-Álvarez, Y., Rempelos, L., Bilsborrow, P., Chinchilla, N., Macías, F. A., Weston, L. A., Reigosa, M. J., & Sánchez-Moreiras, A. M. (2022). Benzoxazinoids in wheat allelopathy – From discovery to application for sustainable weed management. *Environmental and Experimental Botany*, *202*(March). https://doi.org/10.1016/j.envexpbot.2022.104997

IARC. (2004). *Some Drinking-Water Disinfectants and Contaminants, Including Arsenic*.

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biological Research*, *52*(1), 39. https://doi.org/10.1186/s40659-019-0246-3

IUSS Working Group WRB. (2022). *Worl Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps.* (4th editio).

Jia, T., Guo, T., Cao, M., & Chai, B. (2018). Effects of heavy metals on phyllosphere and rhizosphere microbial community of Bothriochloa ischaemum. *Applied Sciences (Switzerland)*, *8*(9). https://doi.org/10.3390/app8091419

Jiang, Y., Yi, X. T., Liu, M. Y., Liu, B. bei, Zhou, H., Zeng, P., Liao, B. H., & Gu, J. F. (2022). Dynamic responses of soil enzymes at key growth stages in rice after the in situ remediation of paddy soil contaminated with cadmium and arsenic. *Science of the Total Environment*, *830*, 154633. https://doi.org/10.1016/j.scitotenv.2022.154633

Johal, G. S., & Huber, D. M. (2009). Glyphosate effects on diseases of plants. *European Journal of Agronomy*, *31*(3), 144–152. https://doi.org/10.1016/j.eja.2009.04.004

John E Franz, Michael K Mao, & James A Sikorski. (1997). Glyphosate: a unique global herbicide. *American Chemical Society*.

John L. Vicini, Pamela K. Jensen, Bruce M. Young, & John T. Swarthout. (2021). Residues of glyphosate in food and dietary exposure. *COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY*. https://ift.onlinelibrary.wiley.com/doi/10.1111/1541-4337.12822

Johnson, J. A., Runge, C. F., Senauer, B., Foley, J., & Polasky, S. (2014). Global agriculture and carbon trade-offs. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(34), 12342–

12347. https://doi.org/10.1073/pnas.1412835111

Kafarski, P., Wieczorek, P., Bartela, I., Dabrowska, J., The, S., Biology, A., May, N., Effects, G. H., Wieczorek, P., & Ottenbreit, B. (1988a). *Glyphosate: Herbicidal Effects, Mode of Action and Degradation in Soil*. *50*(5), 296–299.

Kafarski, P., Wieczorek, P., Bartela, I., Dabrowska, J., The, S., Biology, A., May, N., Effects, G. H., Wieczorek, P., & Ottenbreit, B. (1988b). *How-To-Do-It*. *50*(5), 296–299.

Kahn, L. H. (2006). Confronting Zoonoses, linking human and veterinary medicine. *Emerging Infectious Diseases*, *12*. https://doi.org/10.3390/VETSCI6010011

Kamiya, T., Tanaka, M., Mitani, N., Jian, F. M., Maeshima, M., & Fujiwara, T. (2009). NIP1;1, an aquaporin homolog, determines the arsenite sensitivity of Arabidopsis thaliana. *Journal of Biological Chemistry*, *284*(4), 2114–2120. https://doi.org/10.1074/jbc.M806881200

Kandeler, E., Stemmer, M., & Klimanek, E. M. (1999). Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. *Soil Biology and Biochemistry*, *31*(2), 261–273. https://doi.org/10.1016/S0038-0717(98)00115-1

Kepler, R. M., Epp Schmidt, D. J., Yarwood, S. A., Cavigelli, M. A., Reddy, K. N., Duke, S. O., Bradley, C. A., Williams, M. M., & Maula, J. E. (2020). Soil microbial communities in diverse agroecosystems exposed to the herbicide glyphosate. *Applied and Environmental Microbiology*, *86*(5), 1–16. https://doi.org/10.1128/AEM.01744-19

Keshavarzi, B., Moore, F., Mosaferi, M., & Rahmani, F. (2011). The Source of Natural Arsenic Contamination in Groundwater, West of Iran. *Water Quality, Exposure and Health*, *3*(3–4), 135–147. https://doi.org/10.1007/s12403-011-0051-x

Kim, S., Samaddar, S., Chatterjee, P., Choudhury, A. R., Choi, J., Choi, J., & Sa, T. (2021). Structural and functional shift in soil bacterial community in response to long-term compost amendment in paddy field. *Applied Sciences (Switzerland)*, *11*(5), 1–10. https://doi.org/10.3390/app11052183

Köhler, A., Maag, D., Veyrat, N., Glauser, G., Wolfender, J., Turlings, T. C. J., & Erb, M. (2015). *Within-plant distribution of 1 , 4-benzoxazin-3-ones contributes to herbivore niche differentiation in maize*. 1081–1093. https://doi.org/10.1111/pce.12464

Kokubo, Y., Nishizaka, M., Ube, N., Yabuta, Y., Tebayashi, S. I., Ueno, K., Taketa, S., & Ishihara, A. (2017). Distribution of the tryptophan pathway-derived defensive secondary metabolites gramine and benzoxazinones in Poaceae. *Bioscience, Biotechnology and Biochemistry*, *81*(3), 431–440. https://doi.org/10.1080/09168451.2016.1256758

Kudjordjie, E. N., Sapkota, R., Steffensen, S. K., Fomsgaard, I. S., & Nicolaisen, M. (2019). *Maize synthesized benzoxazinoids affect the host associated microbiome*. 1–17.

Ladd, J. N. (1985). *Soil Enzymes Soil Organic Matter and Biological Activity*. https://doi.org/10.1007/978-94-009-5105-1_6

Laitinen, P., Rämö, S., & Siimes, K. (2007). Glyphosate translocation from plants to soil - Does this constitute a significant proportion of residues in soil? *Plant and Soil*, *300*(1–2), 51–60. https://doi.org/10.1007/s11104-007-9387-1

Lake, L. M., Basta, N. T., & Barker, D. J. (2021). Modifying effect of soil properties on bio-accessibility of as and pb from human ingestion of contaminated soil. *Geosciences (Switzerland)*, *11*(3), 1–15. https://doi.org/10.3390/geosciences11030126

Lancashire, P. D., Bleiholder, H., Van den Boom, T., Langelüddeke, P., Stauss, R., Weber, E., & Witzenberger, A. (1991). A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology*.

Lancaster, S. H., Hollister, E. B., Senseman, S. A., & Gentry, T. J. (2010). Effects of repeated glyphosate applications on soil microbial community composition and the mineralization of glyphosate. *Pest Management Science*, *66*(1), 59–64. https://doi.org/10.1002/ps.1831

Lebel, J. (2003). Health: An ecosystem approach. In *Journal of Chemical Information and Modeling* (Vol. 53).

Lee, K. S., Shim, H. Y., Lee, D. S., & Chung, D. Y. (2015). The Fate and Factors Determining Arsenic Mobility of Arsenic in Soil-A Review. *Korean Journal of Soil Science and Fertilizer*, *48*(2), 73–80. https://doi.org/10.7745/kjssf.2015.48.2.073

Li, X., Liu, X., Cao, N., Fang, S., & Yu, C. (2021). Adaptation mechanisms of arsenic metabolism genes and their host microorganisms in soils with different arsenic contamination levels around abandoned gold tailings. *Environmental Pollution*, *291*(April), 117994. https://doi.org/10.1016/j.envpol.2021.117994

Li, Y., Zhang, M., Xu, R., Lin, H., Sun, X., Xu, F., Gao, P., Kong, T., Xiao, E., Yang, N., & Sun, W. (2021). Arsenic and antimony co-contamination influences on soil microbial community composition and functions: Relevance to arsenic resistance and carbon, nitrogen, and sulfur cycling. *Environment International*, *153*, 106522. https://doi.org/10.1016/j.envint.2021.106522

Linan, C. (2009). Vademecum of phytosanitary and nutritionals products.

Liu, J., Ma, K., & Qu, L. (2015). Ecological risk assessments and context-dependence analysis of heavy metal contamination in the sediments of mangrove swamp in Leizhou Peninsula, China. *Marine Pollution Bulletin*, *100*(1), 224–230. https://doi.org/10.1016/j.marpolbul.2015.08.046

Loeppmann, S., Blagodatskaya, E., Pausch, J., & Kuzyakov, Y. (2016). Substrate quality affects kinetics and catalytic efficiency of exo-enzymes in rhizosphere and detritusphere. *Soil Biology and Biochemistry*, *92*, 111–118. https://doi.org/10.1016/j.soilbio.2015.09.020

López-Roldán, R., Jubany, I., Martí, V., González, S., & Cortina, J. L. (2013). Ecological screening indicators of stress and risk for the Llobregat river water. *Journal of Hazardous Materials*, *263*, 239–247. https://doi.org/10.1016/j.jhazmat.2013.07.008

Lorenz, N., Hintemann, T., Kramarewa, T., Katayama, A., Yasuta, T., Marschner, P., & Kandeler, E. (2006). Response of microbial activity and microbial community composition in soils to long-term arsenic and cadmium exposure. *Soil Biology and Biochemistry*, *38*(6), 1430–1437. https://doi.org/10.1016/j.soilbio.2005.10.020

Lui, J.-X., Li, C., Jing, J.-H., Jia, T., Liu, X.-G., Wang, X.-Y., & Chai, B.-F. (2017). *Composition and Environmental Adaptation of Microbial Community in Shibahe Copper Tailing in Zhongtiao Mountain in Shanxi*.

Lydon, J., & Duke, S. O. (1988). Glyphosate induction of elevated levels of hydroxybenzoic acids in higher plants. *Journal of Agricultural and Food Chemistry*, *36*(4), 813–818. https://doi.org/10.1021/jf00082a036

Ma, Y., Oliveira, R. S., Freitas, H., & Zhang, C. (2016). *Biochemical and Molecular Mechanisms of Plant-Microbe-Metal Interactions : Relevance for Phytoremediation*. *7*(June), 1–19. https://doi.org/10.3389/fpls.2016.00918

Maag, D., Köhler, A., Robert, C. A. M., Frey, M., Wolfender, J. L., Turlings, T. C. J., Glauser, G., & Erb, M. (2016). Highly localized and persistent induction of Bx1-dependent herbivore resistance factors in maize. *Plant Journal*, *88*(6), 976–991. https://doi.org/10.1111/tpj.13308

Macherey & Nagel GmbH & Co. KG. (2017). *Determination of Glyphosate, AMPA and Glufosinate with FMOC Derivatisation*. Application No. 120490. https://chromaappdb.mn-net.com/hplc/120490/determination-of-glyphosate-ampa-and-glufosinate-with-fmoc-derivatisation

Maggi, F., la Cecilia, D., Tang, F. H. M., & McBratney, A. (2020). The global environmental hazard of

glyphosate use. *Science of the Total Environment*, *717*, 137167. https://doi.org/10.1016/j.scitotenv.2020.137167

Majumder, S., Powell, M. A., Biswas, P. K., & Banik, P. (2022). The impact of Arsenic induced stress on soil enzyme activity in different rice agroecosystems. *Environmental Technology and Innovation*, *26*, 102282. https://doi.org/10.1016/j.eti.2022.102282

Mandal, B. K., & Suzuki, K. T. (2002). *Arsenic round the world: a review*. https://doi.org/10.1016/S0065-2113(08)70013-0

Marcel Martin. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *Technical Notes*.

Marotz, C., Amir, A., Humphrey, G., Gaffney, J., Gogul, G., & Knight, R. (2017). DNA extraction for streamlined metagenomics of diverse environmental samples. *BioTechniques*, *62*(6), 290–293. https://doi.org/10.2144/000114559

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *Technical Notes*.

Marx, M. C., Wood, M., & Jarvis, S. C. (2001). A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry*, *33*(12–13), 1633–1640. https://doi.org/10.1016/S0038-0717(01)00079-7

Matschullat, J. (2000). Arsenic in the geosphere {\textemdash} a review. *Science of the Total Environment, The*, *249*(1{\textendash}3), 297–312.

Mclean, J. E., & Bledsoe, B. E. (1992). Ground Water Issue Behavior of Metals in Soils. *Director*, 1–25. https://doi.org/10.1056/NEJMoa030660

Meftaul, I. M., Venkateswarlu, K., Dharmarajan, R., Annamalai, P., Asaduzzaman, M., Parven, A., & Megharaj, M. (2020). Controversies over human health and ecological impacts of glyphosate: Is it to be banned in modern agriculture? *Environmental Pollution*, *263*, 114372. https://doi.org/10.1016/j.envpol.2020.114372

Meharg, A. A., & Macnair, M. R. (1990). An altered phosphate uptake system in arsenate-tolerant Holcus lanatus L. *New Phytologist*, *116*(1), 29–35. https://doi.org/10.1111/j.1469-8137.1990.tb00507.x

Meharg, ANDREW A., & Macnair, M. R. (1990). An altered phosphate uptake system in arsenate-tolerant Holcus lanatus L. *New Phytologist*, *116*(1), 29–35. https://doi.org/10.1111/j.1469-8137.1990.tb00507.x

Mensah, A. K., Marschner, B., Shaheen, S. M., Wang, J., Wang, S. L., & Rinklebe, J. (2020). Arsenic contamination in abandoned and active gold mine spoils in Ghana: Geochemical fractionation, speciation, and assessment of the potential human health risk. *Environmental Pollution*, *261*. https://doi.org/10.1016/j.envpol.2020.114116

Mestrot, A., Feldmann, J., Krupp, E. M., Hossain, M. S., Roman-Ross, G., & Meharg, A. A. (2011). Field fluxes and speciation of arsines emanating from soils. *Environmental Science and Technology*, *45*(5), 1798–1804. https://doi.org/10.1021/es103463d

Mijangos, I., Becerril, J. M., Albizu, I., Epelde, L., & Garbisu, C. (2009). Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies. *Soil Biology and Biochemistry*, *41*(3), 505–513. https://doi.org/10.1016/j.soilbio.2008.12.009

Mikic, S., & Ahmad, S. (2018). Benzoxazinoids - protective secondary metabolites in cereals: The role and application. *Ratar. Povrt.*

Miller, M., & Dick, R. P. (1995). Thermal stability and activities of soil enzymes as influenced by crop

rotations. *Soil Biology and Biochemistry*, *27*(9), 1161–1166. https://doi.org/10.1016/0038-0717(95)00045-G

Mohammed Abdul, K. S., Jayasinghe, S. S., Chandana, E. P. S., Jayasumana, C., & De Silva, P. M. C. S. (2015). Arsenic and human health effects: A review. *Environmental Toxicology and Pharmacology*, *40*(3), 828–846. https://doi.org/10.1016/j.etap.2015.09.016

Møller, I. M., Jensen, P. E., & Hansson, A. (2007). Oxidative modifications to cellular components in plants. *Annual Review of Plant Biology*, *58*, 459–481. https://doi.org/10.1146/annurev.arplant.58.032806.103946

Nachman, K. E., Punshon, T., Rardin, L., Signes-Pastor, A. J., Murray, C. J., Jackson, B. P., Guerinot, M. Lou, Burke, T. A., Chen, C. Y., Ahsan, H., Argos, M., Cottingham, K. L., Cubadda, F., Ginsberg, G. L., Goodale, B. C., Kurzius-Spencer, M., Meharg, A. A., Miller, M. D., Nigra, A. E., … Karagas, M. R. (2018). Opportunities and challenges for dietary arsenic intervention. *Environmental Health Perspectives*, *126*(8), 6–11. https://doi.org/10.1289/EHP3997

Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M. C., & Marinari, S. (2012). Soil enzymology: Classical and molecular approaches. *Biology and Fertility of Soils*, *48*(7), 743–762. https://doi.org/10.1007/s00374-012-0723-0

Naomi, M. R., Supriyono, Nurmalasari, I. A., & Pardono. (2021). Role of phosphate fertilizer on growth and yield of hybrid maize (Zea mays L.). *IOP Conference Series: Earth and Environmental Science*, *637*(1). https://doi.org/10.1088/1755-1315/637/1/012070

Navarro, S., Vela, N., José Giménez, M., & Navarro, G. (2004). Persistence of four s-triazine herbicides in river, sea and groundwater samples exposed to sunlight and darkness under laboratory conditions. *Science of the Total Environment*, *329*(1–3), 87–97. https://doi.org/10.1016/j.scitotenv.2004.03.013

Neal, A. L., Ahmad, S., Gordon-Weeks, R., & Ton, J. (2012). Benzoxazinoids in root exudates of maize attract pseudomonas putida to the rhizosphere. *PLoS ONE*, *7*(4). https://doi.org/10.1371/journal.pone.0035498

Nguyen, D. B., Rose, M. T., Rose, T. J., & van Zwieten, L. (2018). Effect of glyphosate and a commercial formulation on soil functionality assessed by substrate induced respiration and enzyme activity. *European Journal of Soil Biology*, *85*(December 2017), 64–72. https://doi.org/10.1016/j.ejsobi.2018.01.004

Niemeyer, H. M. (2009). Hydroxamic acids derived from 2-hydroxy-2h-1,4-benzoxazin-3(4h)-one: Key defense chemicals of cereals. *Journal of Agricultural and Food Chemistry*, *57*(5), 1677–1695. https://doi.org/10.1021/jf8034034

Norton, G., Deacon, C., Mestrot, A., Feldmann, J., Jenkins, P., Baskaran, C., & Meharg, A. A. (2013). Arsenic speciation and localization in horticultural produce grown in a historically impacted mining region. *Environmental Science and Technology*, *47*(12), 6164–6172. https://doi.org/10.1021/es400720r

Nriagu, J., Bhattacharya, P., Mukherjee, A., Bundschuh, J., Zevenhoven, R., & Loeppert, R. (2007). Arsenic in soil and groundwater: an overview. *Arsenic in Soil and Groundwater Environment - Biogeochemical Interactions, Health Effects and Remediation*, *9*(06), 3–60. https://doi.org/10.1016/s0927-5215(06)09001-1

O. Akinbil, C., & Haque, A. M. M. (2012). Arsenic Contamination in Irrigation Water for Rice Production in Bangladesh: A Review. In *Trends in Applied Sciences Research* (Vol. 7, Issue 5, pp. 331–349). https://doi.org/10.3923/tasr.2012.331.349

O Duke, S., & B Powles, S. (2008). Glyphosate: a once-in-a-century herbicide. *Pest Management Science*, *63*(11), 1100–1106. https://doi.org/10.1002/ps

Oa, W., Huffaker, A., Dafoe, N. J., & Schmelz, E. A. (2011). *Regulates Maize Innate Immunity and Enhances*. *155*(March), 1325–1338. https://doi.org/10.1104/pp.110.166710

Paez-Espino, D., Tamames, J., de Lorenzo, V., & Canovas, D. (2009). *Microbial responses to environmental arsenic*. 117–130. https://doi.org/10.1007/s10534-008-9195-y

Palma, P., Köck-Schulmeyer, M., Alvarenga, P., Ledo, L., Barbosa, I. R., López de Alda, M., & Barceló, D. (2014). Risk assessment of pesticides detected in surface water of the Alqueva reservoir (Guadiana basin, southern of Portugal). *Science of the Total Environment*, *488–489*(1), 208–219. https://doi.org/10.1016/j.scitotenv.2014.04.088

Panax, W. G., & Meyer, C. A. (2021). *Correlation between Soil Bacterial Community Structure and Soil Properties in Cultivation Sites of 13-Year-Old*.

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Parween, T., & Jan, S. (2019). Pesticide consumption and threats to biodiversity. In *Ecophysiology of Pesticides*. https://doi.org/10.1016/b978-0-12-817614-6.00002-0

Pasaribu, A., Mohamad, R. B., Awang, Y., Othman, R., & Puteh, A. (2011). Growth and development of symbiotic Arbuscular mycorrhizal fungi, Glomus mossea (Nicol. and Gerd.), in alachlor and glyphosate treated soils. *African Journal of Biotechnology*, *10*(55), 11520–11526. https://doi.org/10.5897/AJB11.1200

Pearce, D. C., Dowling, K., & Sim, M. R. (2012). Cancer incidence and soil arsenic exposure in a historical gold mining area in Victoria, Australia: A geospatial analysis. *Journal of Exposure Science and Environmental Epidemiology*, *22*(3), 248–257. https://doi.org/10.1038/jes.2012.15

Pierce, M. L., & Moore, C. B. (1980). Adsorption of Ca2+ on amorphous iron hydroxide from aqueous electrolyte solution. *Environmental Technology Letters*, *9*(4), 311–316. https://doi.org/10.1080/09593338809384571

Pigna, M., Caporale, A. G., Cavalca, L., Sommella, A., & Violante, A. (2015). Arsenic in the soil environment: Mobility and phytoavailability. *Environmental Engineering Science*, *32*(7), 551–563. https://doi.org/10.1089/ees.2015.0018

Pineda, A., Kaplan, I., Hannula, S. E., Ghanem, W., & Bezemer, T. M. (2020). *Conditioning the soil microbiome through plant – soil feedbacks suppresses an aboveground insect pest*. 595–608. https://doi.org/10.1111/nph.16385

Podgorski, J., & Berg, M. (2020). Global threat of arsenic in groundwater. *Science*, *368*(6493), 845–850. https://doi.org/10.1126/science.aba1510

Porras-Alfaro, A., & Bayman, P. (2011). Hidden fungi, emergent properties: Endophytes and microbiomes. *Annual Review of Phytopathology*, *49*, 291–315. https://doi.org/10.1146/annurev-phyto-080508-081831

Poschenrieder, C., Tolrà, R. P., & Barceló, J. (2005). A role for cyclic hydroxamates in aluminium resistance in maize? *Journal of Inorganic Biochemistry*, *99*(9 SPEC. ISS.), 1830–1836. https://doi.org/10.1016/j.jinorgbio.2005.05.017

Powell, J. R., Campbell, R. G., Dunfield, K. E., Gulden, R. H., Hart, M. M., Levy-Booth, D. J., Klironomos, J. N., Pauls, K. P., Swanton, C. J., Trevors, J. T., & Antunes, P. M. (2009). Effect of glyphosate on the tripartite symbiosis formed by Glomus intraradices, Bradyrhizobium japonicum, and genetically modified soybean. *Applied Soil Ecology*, *41*(1), 128–136. https://doi.org/10.1016/j.apsoil.2008.10.002

Powles, S. B., & Yu, Q. (2010). Evolution in action: Plants resistant to herbicides. In *Annual Review of Plant Biology* (Vol. 61). https://doi.org/10.1146/annurev-arplant-042809-112119

Prakash, O., Sharma, R., Rahi, P., & Karthikeyan, N. (2014). *Nutrient Use Effciency : from Basics to Advances*.

Punshon, T., Jackson, B. P., Meharg, A. A., Warczack, T., Scheckel, K., Guerinot, M. Lou, Hampshire, N., Hampshire, N., Kingdom, U., Risk, N., Luther, M., & Drive, K. (2018). *HHS Public Access*. 209–220. https://doi.org/10.1016/j.scitotenv.2016.12.111.Understanding

Qi, Y., Li, J., Guan, X., Yan, B., Fu, G., He, J., Du, L., Zhao, C., & Zhang, D. (2020). Effects of herbicides on non-target plant species diversity and the community composition of fallow fields in northern China. *Scientific Reports*, *10*(1), 1–10. https://doi.org/10.1038/s41598-020-67025-2

Qiu, Z., Egidi, E., Liu, H., Kaur, S., & Singh, B. K. (2019). New frontiers in agriculture productivity: Optimised microbial inoculants and in situ microbiome engineering. *Biotechnology Advances*, *37*(6), 107371. https://doi.org/10.1016/j.biotechadv.2019.03.010

R-bloggers. (2021). *How to Remove Outliers in R*. https://www.r-bloggers.com/2021/09/how-to-remove-outliers-in-r-3/

R Core Team. (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/

R Core Team. (2022). R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. https://www.r-project.org/

Ramirez-Villacis, D. (2020). *Root Microbiome Modulates Plant Growth Promotion Induced by Low Doses of Glyphosate*. *August*, 1–11. https://doi.org/10.46678/pb.20.1383160

Ranum, P., & Pe, J. P. (2014). *Global maize production , utilization , and consumption*. *1312*, 105–112. https://doi.org/10.1111/nyas.12396

Rapport, D. J., Bohm, G., Buckingham, D., Cairns, J., Costanza, R., Karr, J. R., de Kruijf, H. A. M., Levins, R., McMichael, A. J., Nielsen, N. O., & Whitford, W. G. (1999). Ecosystem Health: The concept, the ISEH, and the important tasks ahead. *Ecosystem Health*, *5*(2), 82–90. https://doi.org/10.1046/j.1526-0992.1999.09913.x

Reddy, K. N., Rimando, A. M., & Duke, S. O. (2004). Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. *Journal of Agricultural and Food Chemistry*, *52*(16), 5139–5143. https://doi.org/10.1021/jf049605v

Retta, A., Vanderlip, R. L., Higgins, R. A., Moshier, L. J., & Feyerherm, A. M. (1991). Suitability of Corn Growth Models for Incorporation of Weed and Insect Stresses. *Agronomy Journal*, *83*(4), 757–765. https://doi.org/10.2134/agronj1991.00021962008300040021x

Robert, C. A. M., Zhang, X., Machado, R. A. R., Schirmer, S., Lori, M., Mateo, P., Erb, M., & Gershenzon, J. (2017). Sequestration and activation of plant toxins protect the western corn rootworm from enemies at multiple trophic levels. *ELife*, *6*, 1–17. https://doi.org/10.7554/eLife.29307

Ronka, S., & Bodylska, W. (2021). Sorption properties of specific polymeric microspheres towards desethyl-terbuthylazine and 2-hydroxy-terbuthylazine: Batch and column studies. *Materials*, *14*(11). https://doi.org/10.3390/ma14112734

Rosas-Castor, J. M., Guzmán-Mar, J. L., Hernández-Ramírez, A., Garza-González, M. T., & Hinojosa-Reyes, L. (2014). Arsenic accumulation in maize crop (Zea mays): A review. *Science of the Total Environment*, *488–489*(1), 176–187. https://doi.org/10.1016/j.scitotenv.2014.04.075

Rose, M. T., Ng, E. L., Weng, Z. (Han), Wood, R., Rose, T. J., & Van Zwieten, L. (2018). Minor effects of herbicides on microbial activity in agricultural soils are detected by N-transformation but not enzyme

activity assays. *European Journal of Soil Biology*, *87*(April), 72–79. https://doi.org/10.1016/j.ejsobi.2018.04.003

Rosen, B. P. (2002). Biochemistry of arsenic detoxification. *FEBS Letters*, *529*(1), 86–92. https://doi.org/10.1016/S0014-5793(02)03186-1

Sahid, I. B., & Teoh, S. S. (1994). Persistence of terbuthylazine in soils. *Bulletin of Environmental Contamination and Toxicology*, *52*(2), 226–230. https://doi.org/10.1007/BF00198492

Schlaeppi, K., & Bulgarelli, D. (2015). The plant microbiome at work. *Molecular Plant-Microbe Interactions*, *28*(3), 212–217. https://doi.org/10.1094/MPMI-10-14-0334-FI

Schmutz Daniel. (2018a). *Gemeinde Liesberg: Arsen in Gartenböden und in angebautem Gemüse, Bau- und Umweltschutzdirektion*.

Schmutz Daniel. (2018b). *Gemeinde Liesberg: Arsen in Landwirtschaftsböden und Futterpflanzen, Bau- und Umweltschutzdirektion*.

Serna-Saldivar, S. O., Amaya Guerra, C. A., Herrera Macias, P., Melesio Cuellar, J. L., Preciado Ortiz, R. E., Terron Ibarra, A. D., & Vazquez Carrillo, G. (2008). Evaluation of the lime-cooking and tortilla making properties of quality protein maize hybrids grown in Mexico. *Plant Foods for Human Nutrition*, *63*(3), 119–125. https://doi.org/10.1007/s11130-008-0080-1

Sessitsch, A., Kuffner, M., Kidd, P., Vangronsveld, J., Wenzel, W. W., Fallmann, K., & Puschenreiter, M. (2013). The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. *Soil Biology and Biochemistry*, *60*, 182–194. https://doi.org/10.1016/j.soilbio.2013.01.012

Seulki, J., Hong, J. K., Jho, E. H., & Nam, K. (2019). Interaction among soil physicochemical properties, bacterial community structure, and arsenic contamination: Clay-induced change in long-term arsenic contaminated soils. *Journal of Hazardous Materials*, *378*(June), 120729. https://doi.org/10.1016/j.jhazmat.2019.06.006

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Preet, G., Sidhu, S., & Handa, N. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, *1*(11), 1–16. https://doi.org/10.1007/s42452-019-1485-1

Sharma, A., Sidhu, G. P. S., Araniti, F., Bali, A. S., Shahzad, B., Tripathi, D. K., Brestic, M., Skalicky, M., & Landi, M. (2020). The role of salicylic acid in plants exposed to heavy metals. *Molecules*, *25*(3), 1–22. https://doi.org/10.3390/molecules25030540

Sheng, M., Hamel, C., & Fernandez, M. R. (2012). Cropping practices modulate the impact of glyphosate on arbuscular mycorrhizal fungi and rhizosphere bacteria in agroecosystems of the semiarid prairie. *Canadian Journal of Microbiology*, *58*(8), 990–1001. https://doi.org/10.1139/W2012-080

Sherlyn, T., Truu, J., Samaddar, S., Truu, M., Preem, J. K., Oopkaup, K., Espenberg, M., Chatterjee, P., Kang, Y., Kim, K., & Sa, T. (2018). The bacterial community structure and functional profile in the heavy metal contaminated paddy soils, surrounding a nonferrous smelter in South Korea. *Ecology and Evolution*, *8*(12), 6157–6168. https://doi.org/10.1002/ece3.4170

Shibata, T., Meng, C., Umoren, J., & West, H. (2016). Risk assessment of arsenic in rice cereal and other dietary sources for infants and toddlers in the U.S. *International Journal of Environmental Research and Public Health*, *13*(4). https://doi.org/10.3390/ijerph13040361

Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, *3*(3), 307–327. https://doi.org/10.1007/s12571-011-0140-5

Singh, N., Ma, L. Q., Srivastava, M., & Rathinasabapathi, B. (2006). Metabolic adaptations to arsenic-induced oxidative stress in Pteris vittata L and Pteris ensiformis L. *Plant Science*, *170*(2), 274–282. https://doi.org/10.1016/j.plantsci.2005.08.013

Sinsabaugh, R. L., Carreiro, M. M., & Repert, D. A. (2002). Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry*, *60*(1), 1–24. https://doi.org/10.1023/A:1016541114786

Sinsabaugh, Robert L. (2010). Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology and Biochemistry*, *42*(3), 391–404. https://doi.org/10.1016/j.soilbio.2009.10.014

Sivaguru, M., & Horst, W. J. (1998). The distal part of the transition zone is the most aluminum-sensitive apical root zone of maize. *Plant Physiology*, *116*(1), 155–163. https://doi.org/10.1104/pp.116.1.155

Skrzypczak, G., & Waniorek, W. (2007). Assessment of the tank mixture of mesotrione and pethoxamid plus terbuthylazine efficacy for weed control in maize [Zea mays L.]. *Journal of Plant Protection Research*, *47*(4), 375–381.

Smith, P. (2013). Delivering food security without increasing pressure on land. *Global Food Security*, *2*(1), 18–23. https://doi.org/10.1016/j.gfs.2012.11.008

Song, J., Zhao, F., McGrath, S., & Luo, Y. (2009). Influence of soil properties and aging on arsenic phytotoxicity. *Environmental Toxicology and Chemistry*.

Song, Jing, Zhao, F. J., McGrath, S. P., & Luo, Y. M. (2006). Influence of soil properties and aging on arsenic phytotoxicity. *Environmental Toxicology and Chemistry*, *25*(6), 1663–1670. https://doi.org/10.1897/05-480R2.1

Speir, T. W., Kettles, H. A., Parshotam, A., Searle, P. L., & Vlaar, L. N. C. (1999). Simple kinetic approach to determine the toxicity of As[V] to soil biological properties. *Soil Biology and Biochemistry*, *31*(5), 705–713. https://doi.org/10.1016/S0038-0717(98)00169-2

Srivastava, S., Suprasanna, P., & D'Souza, S. F. (2012). Mechanisms of Arsenic Tolerance and Detoxification in Plants and their Application in Transgenic Technology: A Critical Appraisal. *International Journal of Phytoremediation*, *14*(5), 506–517. https://doi.org/10.1080/15226514.2011.604690

Stark, J. D., & Banks, J. E. (2003). P Opulation- L Evel E Ffects of P Esticides and. In *Most* (Vol. 33, Issue 0).

Stoeva, N., Berova, M., & Zlatev, Z. (2004). Stoeva2004.Pdf. In *Biologia Plantarum* (Vol. 3, pp. 449–452).

Ström, L., Owen, A. G., Godbold, D. L., & Jones, D. L. (2002). Organic acid mediated P mobilization in the rhizosphere and uptake by maize roots. *Soil Biology and Biochemistry*, *34*(5), 703–710. https://doi.org/10.1016/S0038-0717(01)00235-8

Sun, W., Xiao, E., Krumins, V., Häggblom, M. ., Dong, Y., Pu, Z., Li, B., Wang, Q., Xiao, T., & Li, F. (2018). *Rhizosphere Microbial Response to Multiple Metal(loid)s in Different Contaminated Arable Soils Indicates Crop-Specific Metal-Microbe Interactions*. *84*(24), 1–13.

Tang, Z., Chen, Y., Chen, F., Ji, Y., & Zhao, F. J. (2017). OsPTR7 (OsNPF8.1), a putative peptide transporter in rice, is involved in dimethylarsenate accumulation in rice grain. *Plant and Cell Physiology*, *58*(5), 904–913. https://doi.org/10.1093/pcp/pcx029

Tang, Z., Chen, Y., Miller, A. J., & Zhao, F. J. (2019). The C-type ATP-Binding Cassette Transporter OsABCC7 Is Involved in the Root-to-Shoot Translocation of Arsenic in Rice. *Plant and Cell Physiology*, *60*(7), 1525–1535. https://doi.org/10.1093/pcp/pcz054

Tejada, M. (2009). Evolution of soil biological properties after addition of glyphosate, diflufenican and glyphosate+diflufenican herbicides. *Chemosphere*, *76*(3), 365–373. https://doi.org/10.1016/j.chemosphere.2009.03.040

The Federal Council. (2021). *Popular initiative "For a Switzerland without artificial pesticides."*

Theriot, J. M., Conkle, J. L., Reza Pezeshki, S., DeLaune, R. D., & White, J. R. (2013). Will hydrologic restoration of Mississippi River riparian wetlands improve their critical biogeochemical functions? *Ecological Engineering*, *60*, 192–198. https://doi.org/10.1016/j.ecoleng.2013.07.021

Thiour-Mauprivez, C., Martin-Laurent, F., Calvayrac, C., & Barthelmebs, L. (2019). Effects of herbicide on non-target microorganisms: Towards a new class of biomarkers? *Science of the Total Environment*, *684*, 314–325. https://doi.org/10.1016/j.scitotenv.2019.05.230

Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(50), 20260–20264. https://doi.org/10.1073/pnas.1116437108

Tipton, L., & Buell, E. L. (1970). *Short Communication Ferric Iron Complexes of Hydroxamic From Maize * Acids*. *9*(1960), 1964–1966.

Toju, H., Peay, K. G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., Onoda, Y., Yoshida, K., Schlaeppi, K., Bai, Y., Sugiura, R., Ichihashi, Y., Minamisawa, K., & Kiers, E. T. (2018). Core microbiomes for sustainable agroecosystems. *Nature Plants*, *4*(5), 247–257. https://doi.org/10.1038/s41477-018-0139-4

Torsvik, V., Øvreås, L., & Thingstad, T. F. (2002). Prokaryotic diversity - Magnitude, dynamics, and controlling factors. *Science*, *296*(5570), 1064–1066. https://doi.org/10.1126/science.1071698

Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., & Singh, B. K. (2020). Plant–microbiome interactions: from community assembly to plant health. *Nature Reviews Microbiology*, *18*(11), 607–621. https://doi.org/10.1038/s41579-020-0412-1

Tsaboula, A., Papadakis, E. N., Vryzas, Z., Kotopoulou, A., Kintzikoglou, K., & Papadopoulou-Mourkidou, E. (2016). Environmental and human risk hierarchy of pesticides: A prioritization method, based on monitoring, hazard assessment and environmental fate. *Environment International*, *91*, 78–93. https://doi.org/10.1016/j.envint.2016.02.008

Tu, S., & Ma, L. Q. (2003). Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth of the arsenic hyperaccumulator Pteris vittata L. under hydroponic conditions. *Environmental and Experimental Botany*, *50*(3), 243–251. https://doi.org/10.1016/S0098-8472(03)00040-6

Tzin, V., Fernandez-Pozo, N., Richter, A., Schmelz, E. A., Schoettner, M., Schäfer, M., Ahern, K. R., Meihls, L. N., Kaur, H., Huffaker, A., Mori, N., Degenhardt, J., Mueller, L. A., & Jander, G. (2015). Dynamic maize responses to aphid feeding are revealed by a time series of transcriptomic and metabolomic assays. *Plant Physiology*, *169*(3), 1727–1743. https://doi.org/10.1104/pp.15.01039

Tzin, V., Hojo, Y., Strickler, S. R., Bartsch, L. J., Archer, C. M., Ahern, K. R., Zhou, S., Christensen, S. A., Galis, I., Mueller, L. A., & Jander, G. (2017). Rapid defense responses in maize leaves induced by Spodoptera exigua caterpillar feeding. *Journal of Experimental Botany*, *68*(16), 4709–4723. https://doi.org/10.1093/jxb/erx274

University of Hertfordshire. (2013). *The pesticide properties DataBase (PPDB)*. Agriculture & Environment Research Unit (AERU). http://sitem.herts.ac.uk/aeru/iupac/Reports/842.htm

Upadhyay, Munish K., Shukla, A., Yadav, P., & Srivastava, S. (2019). A review of arsenic in crops, vegetables, animals and food products. *Food Chemistry*, *276*(October 2018), 608–618. https://doi.org/10.1016/j.foodchem.2018.10.069

Upadhyay, Munish Kumar, Majumdar, A., Suresh Kumar, J., & Srivastava, S. (2020). Arsenic in Rice Agro-Ecosystem: Solutions for Safe and Sustainable Rice Production. *Frontiers in Sustainable Food Systems*, *4*(May). https://doi.org/10.3389/fsufs.2020.00053

Van Bruggen, A. H.C., He, M. M., Shin, K., Mai, V., Jeong, K. C., Finckh, M. R., & Morris, J. G. (2018). Environmental and health effects of the herbicide glyphosate. *Science of the Total Environment*, *616–617*, 255–268. https://doi.org/10.1016/j.scitotenv.2017.10.309

van Bruggen, Ariena H.C., Goss, E. M., Havelaar, A., van Diepeningen, A. D., Finckh, M. R., & Morris, J. G. (2019). One Health - Cycling of diverse microbial communities as a connecting force for soil, plant, animal, human and ecosystem health. *Science of the Total Environment*, *664*, 927–937. https://doi.org/10.1016/j.scitotenv.2019.02.091

van Wyk, D. A. B., Adeleke, R., Rhode, O. H. J., Bezuidenhout, C. C., & Mienie, C. (2017). Ecological guild and enzyme activities of rhizosphere soil microbial communities associated with Bt-maize cultivation under field conditions in North West Province of South Africa. *Journal of Basic Microbiology*, *57*(9), 781–792. https://doi.org/10.1002/jobm.201700043

Velini, E. D., Alves, E., Godoy, M. C., Meschede, D. K., Souza, R. T., & Duke, S. O. (2008). *Glyphosate applied at low doses can stimulate plant growth*. *496*(February), 489–496. https://doi.org/10.1002/ps

Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., Wu, D., Paulsen, I., Nelson, K. E., Nelson, W., Fouts, D. E., Levy, S., Knap, A. H., Lomas, M. W., Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., … Smith, H. O. (2004). Environmental Genome Shotgun Sequencing of the Sargasso Sea. *Science*, *304*(5667), 66–74. https://doi.org/10.1126/science.1093857

Vereecken, H. (2005). Mobility and leaching of glyphosate: A review. *Pest Management Science*, *61*(12), 1139–1151. https://doi.org/10.1002/ps.1122

Vorholt, J. A. (2012). Microbial life in the phyllosphere. *Nature Reviews Microbiology*, *10*(12), 828–840. https://doi.org/10.1038/nrmicro2910

Wallenstein, M. D., & Burns, R. G. (2015). Ecology of extracellular enzyme activities and organic matt er degradation in soil: A complex community-driven process. *Methods of Soil Enzymology*, *80523*(9), 35–55. https://doi.org/10.2136/sssabookser9.c2

Ware, G. W. (2007). *Reviews of environmental contamination and toxicology*.

Wasimuddin, Schlaeppi, K., Ronchi, F., Leib, S. L., Erb, M., & Ramette, A. (2020). Evaluation of primer pairs for microbiome profiling from soils to humans within the One Health framework. *Molecular Ecology Resources*, *20*(6), 1558–1571. https://doi.org/10.1111/1755-0998.13215

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J. R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E. R., & Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, *5*(1), 1–18. https://doi.org/10.1186/s40168-017-0237-y

Wenzel, W. (2013). *Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability* (Brian J. Alloway (Ed.)).

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and Direct Sequencing of Fungal Ribosomal Rna Genes for Phylogenetics. In *PCR Protocols* (Issue 1). Academic Press, Inc. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Whitman, W. B., Coleman, D. C., & Wiebe, W. J. (1998). Prokaryotes: The unseen majority. *Proc. Natl. Acad. Sci. USA*, *95*, 6578–6583.

WHO. (2018). *Arsenic*. https://www.who.int/news-room/fact-sheets/detail/arsenic

Wu, K., Wu, C., Jiang, X., Xue, R., Pan, W., Li, W. C., Luo, X., & Xue, S. (2022). Remediation of arsenic-contaminated paddy field by a new iron oxidizing strain (Ochrobactrum sp.) and iron-modified biochar. *Journal of Environmental Sciences (China)*, *115*, 411–421. https://doi.org/10.1016/j.jes.2021.08.009

Wu, Z., Ren, H., Mcgrath, S. P., Wu, P., & Zhao, F. J. (2011). Investigating the contribution of the phosphate transport pathway to arsenic accumulation in rice. *Plant Physiology*, *157*(1), 498–508. https://doi.org/10.1104/pp.111.178921

Wyszkowska, J., Tomkiel, M., Baćmaga, M., Borowik, A., & Kucharski, J. (2016). Response of microorganisms and enzymes to soil contamination with a mixture of pethoxamid terbuthylazine. *Environmental Earth Sciences*, *75*(18). https://doi.org/10.1007/s12665-016-6092-5

Xiao, E., Cui, J., Sun, W., Jiang, S., Huang, M., Kong, D., Wu, Q., Xiao, T., Sun, X., & Ning, Z. (2021). Root microbiome assembly of As-hyperaccumulator Pteris vittata and its efficacy in arsenic requisition. *Environmental Microbiology*, *23*(4), 1959–1971. https://doi.org/10.1111/1462-2920.15299

Xiong, J., He, Z., van Nostrand, J. D., Luo, G., Tu, S., Zhou, J., & Wang, G. (2012). Assessing the Microbial Community and Functional Genes in a Vertical Soil Profile with Long-Term Arsenic Contamination. *PLoS ONE*, *7*(11), 1–9. https://doi.org/10.1371/journal.pone.0050507

Xu, X. Y., McGrath, S. P., & Zhao, F. J. (2007). Rapid reduction of arsenate in the medium mediated by plant roots. *New Phytologist*, *176*(3), 590–599. https://doi.org/10.1111/j.1469-8137.2007.02195.x

Yamaji, N., Sakurai, G., Mitani-Ueno, N., & Ma, J. F. (2015). Orchestration of three transporters and distinct vascular structures in node for intervascular transfer of silicon in rice. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(36), 11401–11406. https://doi.org/10.1073/pnas.1508987112

Yu, Z., Liu, X., Zeng, X., Yin, H., Yu, R., & Zeng, W. (2020). Effect of Arsenic Pollution Extent on Microbial Community in Shimen Long-Term Arsenic-Contaminated Soil. *Water, Air, and Soil Pollution*, *231*(7), 1–11. https://doi.org/10.1007/s11270-020-04716-6

Zabaloy, M. C., Gómez, E., Garland, J. L., & Gómez, M. A. (2012). Assessment of microbial community function and structure in soil microcosms exposed to glyphosate. *Applied Soil Ecology*, *61*, 333–339. https://doi.org/10.1016/j.apsoil.2011.12.004

Zaller, J. G., Heigl, F., Ruess, L., & Grabmaier, A. (2014). *belowground interactions between fungi in a model ecosystem*. 1–8. https://doi.org/10.1038/srep05634

Zama, E. F., Reid, B. J., Sun, G. X., Yuan, H. Y., Li, X. M., & Zhu, Y. G. (2018). Silicon (Si) biochar for the mitigation of arsenic (As) bioaccumulation in spinach (Spinacia oleracean) and improvement in the plant growth. *Journal of Cleaner Production*, *189*, 386–395. https://doi.org/10.1016/j.jclepro.2018.04.056

Zemanová, V., Pavlíková, D., Hniličkа, F., & Pavlík, M. (2021). Arsenic toxicity-induced physiological and metabolic changes in the shoots of pteris cretica and spinacia oleracea. *Plants*, *10*(10). https://doi.org/10.3390/plants10102009

Zhang, J., Hamza, A., Xie, Z., Hussain, S., Brestic, M., Tahir, M. A., Ulhassan, Z., Yu, M., Allakhverdiev, S. I., & Shabala, S. (2021). Arsenic transport and interaction with plant metabolism: Clues for improving agricultural productivity and food safety. *Environmental Pollution*, *290*(August), 117987. https://doi.org/10.1016/j.envpol.2021.117987

Zhang, X., Van Doan, C., Arce, C. C. M., Hu, L., Gruenig, S., Parisod, C., Hibbard, B. E., Hervé, M. R., Nielson, C., Robert, C. A. M., Machado, R. A. R., & Erb, M. (2019). Plant defense resistance in natural enemies of a specialist insect herbivore. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(46), 23174–23181. https://doi.org/10.1073/pnas.1912599116

Zhao, F. J., McGrath, S. P., & Meharg, A. A. (2010). Arsenic as a food chain contaminant: Mechanisms of plant uptake and metabolism and mitigation strategies. *Annual Review of Plant Biology*, *61*, 535–559. https://doi.org/10.1146/annurev-arplant-042809-112152

Zhao, Z., Gao, X., Ke, Y., Chang, M., Xie, L., Li, X., Gu, M., Liu, J., & Tang, X. (2019). A unique aluminum resistance mechanism conferred by aluminum and salicylic-acid-activated root efflux of benzoxazinoids in maize. *Plant and Soil*, *437*(1–2), 273–289. https://doi.org/10.1007/s11104-019-03971-9

Zheng, D., Liwinski, T., & Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Research*, *30*(6), 492–506. https://doi.org/10.1038/s41422-020-0332-7

Zheng, L., McMullen, M. D., Bauer, E., Schön, C. C., Gierl, A., & Frey, M. (2015). Prolonged expression of the BX1 signature enzyme is associated with a recombination hotspot in the benzoxazinoid gene cluster in Zea mays. *Journal of Experimental Botany*, *66*(13), 3917–3930. https://doi.org/10.1093/jxb/erv192

Zhou, S., Richter, A., & Jander, G. (2018). Beyond defense: Multiple functions of benzoxazinoids in maize metabolism. *Plant and Cell Physiology*, *59*(8), 1528–1533. https://doi.org/10.1093/pcp/pcy064

Zinsstag, J., Schelling, E., Waltner-Toews, D., & Tanner, M. (2011). From "one medicine" to "one health" and systemic approaches to health and well-being. *Preventive Veterinary Medicine*, *101*(3–4), 148–156. https://doi.org/10.1016/j.prevetmed.2010.07.003

Zobiole, Luiz H.S., Kremer, R. J., Oliveira, R. S., & Constantin, J. (2011). Glyphosate affects chlorophyll, nodulation and nutrient accumulation of " second generation" glyphosate-resistant soybean (Glycine max L.). *Pesticide Biochemistry and Physiology*, *99*(1), 53–60. https://doi.org/10.1016/j.pestbp.2010.10.005

Zobiole, Luiz Henrique Saes, de Oliveira, R. S., Huber, D. M., Constantin, J., de Castro, C., de Oliveira, F. A., & de Oliveira, A. (2010). Glyphosate reduces shoot concentrations of mineral nutrients in glyphosate-resistant soybeans. *Plant and Soil*, *328*(1), 57–69. https://doi.org/10.1007/s11104-009-0081-3

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Annexes

Chapter I

1. ASVs sensitive to modes of application

experiment	kingdom	phylum	class	ASV	logFC weed:soil	rAbu_weed	rAbu_soil
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV16	0.268	0.56%	0.50%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV503	-7.301	0.05%	0.04%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV863	6.004	0.03%	0.00%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV889	5.046	0.03%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV718	4.402	0.02%	0.02%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV874	-5.834	0.02%	0.02%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV870	5.924	0.02%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1018	5.359	0.02%	0.01%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1226	2.798	0.02%	0.01%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1094	4.545	0.01%	0.01%
1	Bacteria	Actinobacteria	Thermoleophilia	ASV1217	4.926	0.01%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1015	4.882	0.01%	0.01%
1	Bacteria	Actinobacteria	Thermoleophilia	ASV1188	4.545	0.01%	0.01%
1	Bacteria	Actinobacteria	Actinobacteria	ASV1250	5.367	0.01%	0.01%
1	Bacteria	Acidobacteria	Acidobacteriia	ASV1353	4.945	0.01%	0.01%
1	Bacteria	Acidobacteria	Subgroup_6	ASV1286	5.014	0.01%	0.01%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1489	4.723	0.01%	0.00%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1155	4.775	0.01%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1322	5.501	0.01%	0.01%
1	Bacteria	Latescibacteria	unassigned	ASV1152	5.839	0.01%	0.01%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1145	5.176	0.01%	0.00%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1464	5.342	0.01%	0.00%
1	Bacteria	Firmicutes	Bacilli	ASV1336	5.077	0.01%	0.01%
1	Bacteria	Firmicutes	Bacilli	ASV909	-5.311	0.01%	0.03%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1330	4.976	0.01%	0.01%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1266	4.126	0.01%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1150	5.396	0.01%	0.01%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1521	4.873	0.01%	0.01%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV1567	4.9	0.01%	0.00%
1	Bacteria	Actinobacteria	Thermoleophilia	ASV1309	4.844	0.01%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1380	4.486	0.01%	0.00%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1163	-3.871	0.01%	0.01%
1	Bacteria	Actinobacteria	Acidimicrobiia	ASV1268	4.703	0.01%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1654	5.123	0.01%	0.00%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV982	4.84	0.01%	0.02%
1	Bacteria	Chloroflexi	Anaerolineae	ASV1159	-2.381	0.01%	0.01%
1	Bacteria	Acidobacteria	Holophagae	ASV1581	4.681	0.01%	0.01%
1	Bacteria	Actinobacteria	Actinobacteria	ASV1484	4.627	0.01%	0.01%
1	Bacteria	Acidobacteria	Subgroup_6	ASV1311	5.191	0.01%	0.00%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1197	4.539	0.01%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1084	-5.134	0.01%	0.02%
1	Bacteria	Actinobacteria	Actinobacteria	ASV1356	-4.255	0.01%	0.01%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV1712	4.696	0.01%	0.00%
1	Bacteria	Acidobacteria	Subgroup_6	ASV1609	4.354	0.01%	0.01%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1212	-5.015	0.01%	0.01%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1424	5.495	0.01%	0.00%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1313	-4.491	0.01%	0.01%

1	Bacteria	Planctomycetes	Planctomycetacia	ASV1455	4.371	0.01%	0.01%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1112	-5.124	0.01%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1227	-5.128	0.01%	0.01%
1	Bacteria	Actinobacteria	Thermoleophilia	ASV1468	3.845	0.01%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1459	4.48	0.01%	0.01%
1	Bacteria	Chloroflexi	Dehalococcoidia	ASV1204	-5.467	0.01%	0.01%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV1632	4.598	0.01%	0.00%
1	Bacteria	Thaumarchaeota	Nitrososphaeria	ASV1538	4.253	0.01%	0.01%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1705	4.93	0.01%	0.00%
1	Bacteria	Firmicutes	Bacilli	ASV1805	5.109	0.01%	0.00%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1283	-3.748	0.01%	0.01%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1419	-4.39	0.01%	0.01%
1	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1976	4.253	0.01%	0.00%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1501	-3.267	0.01%	0.01%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1564	4.294	0.01%	0.00%
1	Bacteria	Acidobacteria	Subgroup_22	ASV1617	4.336	0.01%	0.01%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2061	4.346	0.01%	0.00%
1	Bacteria	Planctomycetes	Pla4_lineage	ASV2016	3.521	0.01%	0.00%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1187	-5.215	0.01%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1717	4.875	0.01%	0.00%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1595	-4.979	0.01%	0.01%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1870	4.069	0.01%	0.00%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1880	4.112	0.01%	0.00%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1616	4.016	0.01%	0.00%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1522	-4.793	0.01%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2003	4.091	0.01%	0.00%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV1671	-4.315	0.01%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1968	3.413	0.01%	0.00%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2055	4.066	0.01%	0.00%
1	Bacteria	Acidobacteria	Subgroup_22	ASV1792	4.022	0.01%	0.00%
1	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV2058	-3.237	0.01%	0.00%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1540	-5.25	0.01%	0.01%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1465	-5.075	0.01%	0.01%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1688	4.001	0.01%	0.01%
1	Bacteria	Acidobacteria	Subgroup_6	ASV2542	4.467	0.01%	0.00%
1	Bacteria	Chloroflexi	Ktedonobacteria	ASV1972	2.941	0.00%	0.00%
1	Bacteria	Actinobacteria	Thermoleophilia	ASV1416	-5.43	0.00%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1452	3.793	0.00%	0.00%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1990	4.117	0.00%	0.00%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1721	-3.479	0.00%	0.01%
1	Bacteria	Gemmatimonadetes	S0134_terrestrial_group	ASV1850	-4.162	0.00%	0.01%
1	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1019	-5.082	0.00%	0.02%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV2342	-3.495	0.00%	0.00%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1881	-2.619	0.00%	0.00%
1	Bacteria	Chloroflexi	Gitt-GS-136	ASV2379	3.19	0.00%	0.00%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2117	3.645	0.00%	0.00%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1756	-4.38	0.00%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2394	3.837	0.00%	0.00%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1814	-4.536	0.00%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2503	3.929	0.00%	0.00%
1	Bacteria	Chloroflexi	TK10	ASV1804	-4.576	0.00%	0.01%
1	Bacteria	unassigned	unassigned	ASV2292	3.833	0.00%	0.00%
1	Bacteria	Planctomycetes	OM190	ASV2400	3.966	0.00%	0.00%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1366	-4.698	0.00%	0.01%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV2013	-2.907	0.00%	0.00%
1	Bacteria	Chloroflexi	TK10	ASV2216	-3.731	0.00%	0.00%

1	Bacteria	Planctomycetes	Planctomycetacia	ASV1539	-5.222	0.00%	0.01%
1	Bacteria	Gemmatimonadetes	S0134_terrestrial_group	ASV2217	3.688	0.00%	0.00%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2211	3.806	0.00%	0.00%
1	Bacteria	Chloroflexi	TK10	ASV2214	3.699	0.00%	0.00%
1	Bacteria	Chloroflexi	Ktedonobacteria	ASV1259	-4.649	0.00%	0.01%
1	Bacteria	Planctomycetes	Phycisphaerae	ASV2756	-3.377	0.00%	0.00%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2751	3.084	0.00%	0.00%
1	Bacteria	Actinobacteria	Actinobacteria	ASV2018	-3.635	0.00%	0.01%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV2024	-4.768	0.00%	0.01%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV2260	3.314	0.00%	0.00%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV1752	-4.177	0.00%	0.01%
1	Bacteria	Planctomycetes	Phycisphaerae	ASV1715	-4.86	0.00%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1719	-4.107	0.00%	0.01%
1	Bacteria	Chloroflexi	TK10	ASV2946	3.748	0.00%	0.00%
1	Bacteria	Chloroflexi	Chloroflexia	ASV2002	-3.592	0.00%	0.00%
1	Bacteria	Planctomycetes	Phycisphaerae	ASV1974	-4.672	0.00%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2403	2.953	0.00%	0.00%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2406	-3.091	0.00%	0.00%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV2418	2.788	0.00%	0.00%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2470	-3.464	0.00%	0.00%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV2635	3.711	0.00%	0.00%
1	Bacteria	Acidobacteria	Thermoanaerobaculia	ASV1763	-5.199	0.00%	0.01%
1	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1765	-3.735	0.00%	0.01%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV2272	-3.978	0.00%	0.00%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2355	-3.696	0.00%	0.00%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2915	3.123	0.00%	0.00%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV2213	-3.967	0.00%	0.00%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1555	-4.579	0.00%	0.01%
1	Bacteria	Actinobacteria	Thermoleophilia	ASV2188	3.065	0.00%	0.00%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV2425	-2.964	0.00%	0.00%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV1969	-3.522	0.00%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2450	-3.94	0.00%	0.00%
1	Bacteria	Actinobacteria	Actinobacteria	ASV1919	-3.716	0.00%	0.01%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2496	-3.576	0.00%	0.00%
1	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2193	-4.025	0.00%	0.01%
1	Bacteria	Planctomycetes	Phycisphaerae	ASV1936	-4.181	0.00%	0.01%
1	Bacteria	Chloroflexi	Anaerolineae	ASV2287	-3.567	0.00%	0.00%
1	Bacteria	Actinobacteria	Actinobacteria	ASV2156	-3.718	0.00%	0.01%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2209	-3.542	0.00%	0.00%
1	Bacteria	Planctomycetes	Phycisphaerae	ASV2221	-3.533	0.00%	0.00%
1	Bacteria	Acidobacteria	Subgroup_22	ASV1826	-4.625	0.00%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2246	-3.466	0.00%	0.00%
1	Bacteria	Chloroflexi	Anaerolineae	ASV2339	-3.671	0.00%	0.00%
1	Bacteria	Planctomycetes	Pla3_lineage	ASV3492	3.16	0.00%	0.00%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV1915	-4.844	0.00%	0.01%
1	Bacteria	Actinobacteria	Actinobacteria	ASV2565	-3.136	0.00%	0.00%
1	Bacteria	Bacteroidetes	Ignavibacteria	ASV2081	-2.803	0.00%	0.00%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2386	-3.027	0.00%	0.00%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2169	-3.423	0.00%	0.01%
1	Bacteria	Latescibacteria	unassigned	ASV2442	-4.561	0.00%	0.00%
1	Bacteria	Armatimonadetes	Chthonomonadetes	ASV3090	-2.518	0.00%	0.00%
1	Bacteria	Latescibacteria	unassigned	ASV2274	-3.569	0.00%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2696	-2.338	0.00%	0.00%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV2524	-3.16	0.00%	0.00%
1	Bacteria	Bacteroidetes	Bacteroidia	ASV2815	-3.034	0.00%	0.00%
1	Bacteria	Chloroflexi	Anaerolineae	ASV1634	-5.064	0.00%	0.01%

1	Bacteria	Bacteroidetes	Bacteroidia	ASV2241	-3.126	0.00%	0.01%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1528	-3.89	0.00%	0.01%
1	Bacteria	Acidobacteria	Subgroup_6	ASV2108	-4.451	0.00%	0.00%
1	Bacteria	Acidobacteria	Acidobacteriia	ASV3062	-2.984	0.00%	0.00%
1	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2473	-4.235	0.00%	0.01%
1	Bacteria	Planctomycetes	Planctomycetacia	ASV3012	-4.014	0.00%	0.00%
1	Bacteria	Chloroflexi	Anaerolineae	ASV3447	-2.617	0.00%	0.00%
1	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2142	-4.669	0.00%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV497	5.876	0.05%	0.01%
2	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4	ASV719	-4.284	0.03%	0.02%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV602	-5.755	0.02%	0.04%
2	Bacteria	Actinobacteria	Thermoleophilia	ASV930	4.895	0.02%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV874	-4.691	0.02%	0.02%
2	Bacteria	Acidobacteria	Acidobacteriia	ASV677	-4.97	0.02%	0.03%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1100	3.851	0.02%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV873	-5.284	0.02%	0.02%
2	Bacteria	Actinobacteria	Actinobacteria	ASV1011	5.377	0.02%	0.01%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV794	5.593	0.02%	0.02%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV781	-5.852	0.02%	0.02%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV937	5.08	0.02%	0.01%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV898	5.573	0.02%	0.01%
2	Bacteria	Actinobacteria	Actinobacteria	ASV1189	3.604	0.01%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV825	3.98	0.01%	0.02%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1111	3.995	0.01%	0.01%
2	Bacteria	Acidobacteria	Subgroup_6	ASV1246	4.113	0.01%	0.01%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1020	4.829	0.01%	0.01%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1015	-4.781	0.01%	0.01%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1301	4.301	0.01%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV952	-4.079	0.01%	0.02%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1392	4.132	0.01%	0.01%
2	Bacteria	Bacteroidetes	Ignavibacteria	ASV1171	3.13	0.01%	0.01%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1340	5.184	0.01%	0.01%
2	Bacteria	Acidobacteria	Subgroup_17	ASV1107	-4.941	0.01%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1513	3.305	0.01%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1586	5.438	0.01%	0.01%
2	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1628	4.166	0.01%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1147	4.95	0.01%	0.00%
2	Bacteria	Actinobacteria	Acidimicrobiia	ASV1268	-4.891	0.01%	0.01%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV982	-5.936	0.01%	0.02%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1473	4.745	0.01%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1329	5.382	0.01%	0.01%
2	Bacteria	Bacteroidetes	Ignavibacteria	ASV1376	4.784	0.01%	0.01%
2	Bacteria	Planctomycetes	Planctomycetacia	ASV1517	6.039	0.01%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1593	4.701	0.01%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1737	3.801	0.01%	0.00%
2	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1028	-4.884	0.01%	0.02%
2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV969	-5.02	0.01%	0.02%
2	Bacteria	Actinobacteria	Actinobacteria	ASV1356	4.215	0.01%	0.01%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1194	-5.007	0.01%	0.01%
2	Bacteria	Firmicutes	Bacilli	ASV784	-4.652	0.01%	0.04%
2	Bacteria	Acidobacteria	Acidobacteriia	ASV1276	-3.905	0.01%	0.01%
2	Bacteria	Planctomycetes	Phycisphaerae	ASV1142	-5.523	0.01%	0.01%
2	Bacteria	Acidobacteria	Holophagae	ASV1869	4.648	0.01%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1898	3.326	0.01%	0.00%
2	Bacteria	Planctomycetes	Phycisphaerae	ASV1386	-2.964	0.01%	0.01%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1557	4.273	0.01%	0.01%

2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1370	-4.787	0.01%	0.01%
2	Bacteria	Acidobacteria	Subgroup_6	ASV1508	-3.979	0.01%	0.01%
2	Bacteria	Firmicutes	Bacilli	ASV1875	4.005	0.01%	0.00%
2	Bacteria	Chloroflexi	Anaerolineae	ASV1121	-5.37	0.01%	0.01%
2	Bacteria	Chloroflexi	Anaerolineae	ASV1789	4.062	0.01%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1773	3.71	0.01%	0.00%
2	Bacteria	Actinobacteria	Acidimicrobiia	ASV1839	3.471	0.01%	0.00%
2	Bacteria	Actinobacteria	Acidimicrobiia	ASV2019	3.664	0.01%	0.00%
2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1545	-4.151	0.01%	0.01%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1643	3.914	0.01%	0.01%
2	Bacteria	Latescibacteria	unassigned	ASV1251	-4.171	0.01%	0.01%
2	Bacteria	Acidobacteria	Subgroup_22	ASV1617	-4.057	0.01%	0.01%
2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2076	3.893	0.01%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1187	-4.927	0.01%	0.01%
2	Bacteria	Bacteroidetes	Ignavibacteria	ASV1565	-4.219	0.01%	0.01%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1847	4.601	0.01%	0.00%
2	Bacteria	Planctomycetes	Planctomycetacia	ASV2194	3.341	0.01%	0.00%
2	Bacteria	Actinobacteria	Thermoleophilia	ASV2363	4.479	0.01%	0.00%
2	Bacteria	Planctomycetes	Planctomycetacia	ASV1734	-2.936	0.01%	0.01%
2	Bacteria	Chloroflexi	Anaerolineae	ASV2103	3.082	0.01%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1038	-4.836	0.01%	0.02%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1911	3.694	0.01%	0.00%
2	Bacteria	Bacteroidetes	Ignavibacteria	ASV1398	-5.312	0.01%	0.01%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV963	-5.421	0.01%	0.03%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1965	3.474	0.01%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1583	-3.3	0.01%	0.01%
2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1871	3.467	0.01%	0.00%
2	Bacteria	Actinobacteria	Acidimicrobiia	ASV2187	3.955	0.01%	0.00%
2	Bacteria	Gemmatimonadetes	Longimicrobia	ASV1809	-3.742	0.01%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1252	-4.492	0.01%	0.01%
2	Bacteria	Chloroflexi	Anaerolineae	ASV2027	-3.011	0.01%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2297	4.166	0.00%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1691	-3.869	0.00%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2124	-3.162	0.00%	0.00%
2	Bacteria	Chloroflexi	Anaerolineae	ASV1694	-3.542	0.00%	0.01%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2032	4	0.00%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2132	2.847	0.00%	0.00%
2	Bacteria	Actinobacteria	Actinobacteria	ASV2258	3.316	0.00%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1990	2.941	0.00%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1967	3.288	0.00%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1203	-5.1	0.00%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2048	3.817	0.00%	0.01%
2	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV2252	3.181	0.00%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1667	-4.183	0.00%	0.01%
2	Bacteria	Chloroflexi	Gitt-GS-136	ASV2379	3.894	0.00%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2515	3.891	0.00%	0.00%
2	Bacteria	Acidobacteria	Thermoanaerobaculia	ASV1899	-4.492	0.00%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2042	3.636	0.00%	0.00%
2	Bacteria	Chloroflexi	Chloroflexia	ASV2174	3.482	0.00%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2146	3.239	0.00%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2340	3.318	0.00%	0.00%
2	Bacteria	Chloroflexi	Anaerolineae	ASV2163	-3.884	0.00%	0.00%
2	Bacteria	Rokubacteria	NC10	ASV1324	-5.677	0.00%	0.01%
2	Bacteria	unassigned	unassigned	ASV1708	-3.423	0.00%	0.01%
2	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4	ASV1781	-4.028	0.00%	0.01%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1961	-4.514	0.00%	0.01%

2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2290	3.359	0.00%	0.00%
2	Bacteria	Acidobacteria	Subgroup_25	ASV2459	3.837	0.00%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1366	-5.061	0.00%	0.01%
2	Bacteria	Chloroflexi	Anaerolineae	ASV2139	3.35	0.00%	0.00%
2	Bacteria	Chloroflexi	TK10	ASV2158	2.909	0.00%	0.00%
2	Bacteria	Chloroflexi	TK10	ASV2216	3.623	0.00%	0.00%
2	Bacteria	Planctomycetes	Planctomycetacia	ASV1687	-4.252	0.00%	0.01%
2	Bacteria	Gemmatimonadetes	S0134_terrestrial_group	ASV2217	-4.009	0.00%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2728	3.542	0.00%	0.00%
2	Bacteria	Chloroflexi	KD4-96	ASV1901	-3.441	0.00%	0.00%
2	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1957	-3.178	0.00%	0.01%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2211	-3.769	0.00%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1683	-4.119	0.00%	0.01%
2	Bacteria	Chloroflexi	Ktedonobacteria	ASV2338	-3.369	0.00%	0.00%
2	Bacteria	Planctomycetes	Phycisphaerae	ASV2756	3.108	0.00%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2335	3.838	0.00%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1391	-5.167	0.00%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2360	3.534	0.00%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1866	-4.593	0.00%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2260	-4.175	0.00%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1779	-4.162	0.00%	0.01%
2	Bacteria	Firmicutes	Bacilli	ASV1446	-5.312	0.00%	0.02%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1698	-4.377	0.00%	0.01%
2	Bacteria	Planctomycetes	Planctomycetacia	ASV1714	-4.64	0.00%	0.01%
2	Bacteria	Planctomycetes	Phycisphaerae	ASV1997	-3.611	0.00%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2074	-4.232	0.00%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2115	-3.751	0.00%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2265	-3.33	0.00%	0.00%
2	Bacteria	Actinobacteria	Acidimicrobiia	ASV2738	3.246	0.00%	0.00%
2	Bacteria	Acidobacteria	Subgroup_6	ASV1647	-5.118	0.00%	0.01%
2	Bacteria	Chloroflexi	Anaerolineae	ASV1895	-3.998	0.00%	0.01%
2	Bacteria	Thaumarchaeota	Nitrososphaeria	ASV2052	-3.404	0.00%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2655	3.144	0.00%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2286	2.709	0.00%	0.00%
2	Bacteria	Planctomycetes	Planctomycetacia	ASV2521	3.127	0.00%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV1307	-4.686	0.00%	0.02%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1711	-4.038	0.00%	0.01%
2	Bacteria	Chloroflexi	Anaerolineae	ASV1815	-3.902	0.00%	0.01%
2	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV2006	-3.598	0.00%	0.01%
2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2403	-3.445	0.00%	0.00%
2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1903	-4.481	0.00%	0.01%
2	Bacteria	Planctomycetes	Pla3_lineage	ASV2053	-4.299	0.00%	0.01%
2	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV2419	3.759	0.00%	0.00%
2	Bacteria	Actinobacteria	Acidimicrobiia	ASV2618	3.965	0.00%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2889	3.888	0.00%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2364	-3.526	0.00%	0.00%
2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1555	-5.199	0.00%	0.01%
2	Bacteria	Armatimonadetes	unassigned	ASV2140	-4.491	0.00%	0.01%
2	Bacteria	Actinobacteria	Thermoleophilia	ASV2774	3.08	0.00%	0.00%
2	Bacteria	Planctomycetes	Planctomycetacia	ASV1969	-4.248	0.00%	0.01%
2	Bacteria	Firmicutes	Bacilli	ASV2122	-3.294	0.00%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2113	-3.426	0.00%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1882	-5.257	0.00%	0.01%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2191	-3.528	0.00%	0.00%
2	Bacteria	Planctomycetes	Phycisphaerae	ASV1936	-3.59	0.00%	0.01%
2	Bacteria	Chloroflexi	Anaerolineae	ASV2287	-3.374	0.00%	0.00%

2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2017	-4.233	0.00%	0.01%
2	Bacteria	Planctomycetes	Planctomycetacia	ASV2050	-3.176	0.00%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2632	-3.516	0.00%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2456	-3.61	0.00%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2096	-4.003	0.00%	0.00%
2	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV2422	-3.846	0.00%	0.00%
2	Bacteria	Chloroflexi	Gitt-GS-136	ASV2445	-3.259	0.00%	0.00%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2327	-4.3	0.00%	0.00%
2	Bacteria	Armatimonadetes	Fimbriimonadia	ASV2509	3.48	0.00%	0.00%
2	Bacteria	Planctomycetes	Planctomycetacia	ASV1915	-4.035	0.00%	0.01%
2	Bacteria	Chloroflexi	Anaerolineae	ASV2183	-3.615	0.00%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2305	-3.578	0.00%	0.00%
2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2386	-3.333	0.00%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2448	-2.939	0.00%	0.00%
2	Bacteria	Actinobacteria	MB-A2-108	ASV3170	-2.799	0.00%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2133	-3.364	0.00%	0.00%
2	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2169	-4.241	0.00%	0.01%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2228	-4.028	0.00%	0.01%
2	Bacteria	Latescibacteria	unassigned	ASV2274	-3.705	0.00%	0.01%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2421	-4.312	0.00%	0.00%
2	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2567	-3.517	0.00%	0.00%
2	Bacteria	Chloroflexi	Anaerolineae	ASV1634	-4.952	0.00%	0.01%
2	Bacteria	Bacteroidetes	Bacteroidia	ASV2241	-4.132	0.00%	0.01%
2	Bacteria	Planctomycetes	OM190	ASV2839	-2.761	0.00%	0.00%
2	Bacteria	Bacteroidetes	Ignavibacteria	ASV2291	-4.088	0.00%	0.00%
2	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2842	-3.709	0.00%	0.00%
2	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2543	-4.084	0.00%	0.01%
1	Fungi	Basidiomycota	Agaricomycetes	ASV33	-4.642	0.26%	0.82%
1	Fungi	Ascomycota	Sordariomycetes	ASV63	4.283	0.23%	0.18%
1	Fungi	Ascomycota	Sordariomycetes	ASV76	5.316	0.20%	0.08%
1	Fungi	Ascomycota	unassigned	ASV60	-2.813	0.18%	0.32%
1	Fungi	Ascomycota	Sordariomycetes	ASV86	3.901	0.17%	0.05%
1	Fungi	Ascomycota	Leotiomycetes	ASV95	4.554	0.17%	0.08%
1	Fungi	Ascomycota	Leotiomycetes	ASV88	3.751	0.15%	0.07%
1	Fungi	Ascomycota	Sordariomycetes	ASV80	3.621	0.15%	0.12%
1	Fungi	Ascomycota	Eurotiomycetes	ASV92	4.742	0.12%	0.07%
1	Fungi	Ascomycota	Pezizomycetes	ASV91	-2.564	0.11%	0.16%
1	Fungi	Ascomycota	Eurotiomycetes	ASV141	3.61	0.09%	0.06%
1	Fungi	Ascomycota	Leotiomycetes	ASV130	2.921	0.08%	0.04%
1	Fungi	Ascomycota	Sordariomycetes	ASV205	3.282	0.06%	0.01%
1	Fungi	unassigned	unassigned	ASV186	-2.99	0.03%	0.07%
2	Fungi	Basidiomycota	Agaricomycetes	ASV33	-6.012	0.26%	0.82%
2	Fungi	Ascomycota	Sordariomycetes	ASV86	4.885	0.17%	0.05%
2	Fungi	Ascomycota	Leotiomycetes	ASV95	4.023	0.17%	0.08%
2	Fungi	Ascomycota	Sordariomycetes	ASV79	-4.024	0.16%	0.11%
2	Fungi	Ascomycota	Leotiomycetes	ASV94	2.72	0.13%	0.09%
2	Fungi	Ascomycota	Dothideomycetes	ASV116	-3.385	0.09%	0.10%
2	Fungi	Ascomycota	Eurotiomycetes	ASV141	-3.531	0.09%	0.06%
2	Fungi	Ascomycota	Dothideomycetes	ASV119	-3.02	0.07%	0.10%
2	Fungi	Basidiomycota	Microbotryomycetes	ASV122	-3.134	0.05%	0.09%
2	Fungi	Ascomycota	unassigned	ASV215	2.775	0.03%	0.03%
2	Fungi	Ascomycota	Sordariomycetes	ASV188	-2.627	0.03%	0.05%
2	Fungi	unassigned	unassigned	ASV193	-2.276	0.03%	0.06%
2	Fungi	Basidiomycota	Microbotryomycetes	ASV194	-2.861	0.03%	0.05%
2	Fungi	Ascomycota	Leotiomycetes	ASV149	-2.765	0.02%	0.09%
2	Fungi	Basidiomycota	unassigned	ASV211	-3.297	0.01%	0.05%

2. ASVs sensitive to herbicides

experiment	herbicide	kingdom	phylum	class	ASV	logFC ctr:hc	rAbu_ctr	rAbu_hc
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2	0.252	3.86%	3.55%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV7	0.451	0.82%	0.72%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV18	0.276	0.55%	0.51%
1	gly	Bacteria	Actinobacteria	Actinobacteria	ASV32	-0.733	0.25%	0.33%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV651	6.508	0.06%	0.04%
1	gly	Bacteria	Acidobacteria	Subgroup_5	ASV711	4.571	0.05%	0.04%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV794	6.769	0.04%	0.03%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV724	6.367	0.04%	0.03%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV781	6.747	0.04%	0.03%
1	gly	Bacteria	Acidobacteria	Holophagae	ASV860	6.533	0.03%	0.02%
1	gly	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV948	6.324	0.03%	0.02%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1021	6.099	0.03%	0.02%
1	gly	Bacteria	Chloroflexi	KD4-96	ASV894	6.478	0.03%	0.02%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1051	5.759	0.02%	0.01%
1	gly	Bacteria	Firmicutes	Bacilli	ASV784	5.163	0.02%	0.01%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV914	5.947	0.02%	0.02%
1	gly	Bacteria	Acidobacteria	Acidobacteriia	ASV967	6.389	0.02%	0.01%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1273	5.409	0.02%	0.01%
1	gly	Bacteria	Acidobacteria	Subgroup_6	ASV906	5.532	0.02%	0.02%
1	gly	Bacteria	Acidobacteria	Acidobacteriia	ASV1001	5.71	0.02%	0.01%
1	gly	Bacteria	Actinobacteria	Actinobacteria	ASV1263	4.705	0.02%	0.01%
1	gly	Bacteria	Firmicutes	Bacilli	ASV1067	4.757	0.02%	0.01%
1	gly	Bacteria	Actinobacteria	Actinobacteria	ASV461	-4.167	0.02%	0.04%
1	gly	Bacteria	Actinobacteria	Thermoleophilia	ASV1309	5.566	0.02%	0.01%
1	gly	Bacteria	Chloroflexi	Ktedonobacteria	ASV1184	5.374	0.02%	0.01%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1103	5.629	0.02%	0.01%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1270	5.811	0.02%	0.01%
1	gly	Bacteria	Chloroflexi	Gitt-GS-136	ASV1167	4.83	0.02%	0.01%
1	gly	Bacteria	Bacteroidetes	Ignavibacteria	ASV1405	4.431	0.02%	0.01%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1430	4.342	0.02%	0.01%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1128	4.764	0.02%	0.01%
1	gly	Bacteria	Latescibacteria	unassigned	ASV1403	5.363	0.02%	0.01%
1	gly	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	ASV1144	4.888	0.02%	0.01%
1	gly	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV470	-3.509	0.01%	0.03%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1140	4.961	0.01%	0.01%
1	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV1598	4.237	0.01%	0.01%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1701	4.317	0.01%	0.01%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1077	5.785	0.01%	0.01%
1	gly	Bacteria	Acidobacteria	Subgroup_6	ASV1350	5.847	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1370	5.535	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1557	5.314	0.01%	0.01%
1	gly	Bacteria	Acidobacteria	Subgroup_11	ASV1702	5.233	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1600	4.766	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1110	5.177	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1190	4.469	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV691	-4.209	0.01%	0.02%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1465	4.878	0.01%	0.01%
1	gly	Bacteria	Thaumarchaeota	Nitrososphaeria	ASV1768	5.315	0.01%	0.01%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1953	5.239	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2132	5.323	0.01%	0.01%
1	gly	Bacteria	Chloroflexi	KD4-96	ASV1396	4.924	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1667	4.473	0.01%	0.01%
1	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV1671	4.757	0.01%	0.01%
1	gly	Bacteria	Latescibacteria	unassigned	ASV1480	4.517	0.01%	0.01%

1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1880	4.38	0.01%	0.01%
1	gly	Bacteria	Bacteroidetes	Ignavibacteria	ASV1534	4.06	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1698	4.757	0.01%	0.01%
1	gly	Bacteria	Latescibacteria	unassigned	ASV2067	4.429	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1873	4.754	0.01%	0.01%
1	gly	Bacteria	Firmicutes	Bacilli	ASV1208	4.863	0.01%	0.01%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1681	4.95	0.01%	0.01%
1	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV2345	4.527	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1452	4.808	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1605	4.203	0.01%	0.01%
1	gly	Bacteria	Actinobacteria	Actinobacteria	ASV1800	3.399	0.01%	0.01%
1	gly	Bacteria	Planctomycetes	Phycisphaerae	ASV2620	3.897	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1590	4.063	0.01%	0.01%
1	gly	Bacteria	Acidobacteria	Acidobacteriia	ASV1928	4.453	0.01%	0.00%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2055	4.453	0.01%	0.01%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1349	4.283	0.01%	0.01%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1513	3.85	0.01%	0.01%
1	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV2077	4.165	0.01%	0.00%
1	gly	Bacteria	Planctomycetes	Pla3_lineage	ASV2062	3.583	0.01%	0.01%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1961	4.566	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2640	4.128	0.01%	0.01%
1	gly	Bacteria	Planctomycetes	OM190	ASV1941	4.473	0.01%	0.00%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1488	4.73	0.01%	0.00%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1879	3.817	0.01%	0.00%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1423	4.448	0.01%	0.00%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2076	4.265	0.01%	0.00%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1752	4.166	0.01%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2236	3.741	0.01%	0.00%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2412	4.116	0.01%	0.00%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1503	-5.307	0.01%	0.01%
1	gly	Bacteria	Dependentiae	Babeliae	ASV2627	3.87	0.01%	0.00%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1150	-5.504	0.01%	0.01%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV2241	3.732	0.01%	0.00%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV2211	3.69	0.00%	0.00%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV926	-6.193	0.00%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1686	3.534	0.00%	0.00%
1	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV2438	3.377	0.00%	0.00%
1	gly	Bacteria	Acidobacteria	Thermoanaerobaculia	ASV1095	-4.966	0.00%	0.01%
1	gly	Bacteria	Acidobacteria	Subgroup_6	ASV869	-6.047	0.00%	0.01%
1	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1102	-5.453	0.00%	0.01%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1112	-5.677	0.00%	0.01%
1	gly	Bacteria	Chloroflexi	Dehalococcoidia	ASV1656	-4.617	0.00%	0.01%
1	gly	Bacteria	Acidobacteria	Acidobacteriia	ASV3062	3.488	0.00%	0.00%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1624	-5	0.00%	0.01%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1588	-4.606	0.00%	0.01%
1	gly	Bacteria	Actinobacteria	Acidimicrobiia	ASV2413	-3.208	0.00%	0.00%
1	gly	Bacteria	Gemmatimonadetes	S0134_terrestrial_group	ASV1200	-5.08	0.00%	0.01%
1	gly	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	ASV1556	-4.446	0.00%	0.01%
1	gly	Bacteria	Fibrobacteres	Fibrobacteria	ASV1621	-4.323	0.00%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1705	-5.098	0.00%	0.01%
1	gly	Bacteria	Actinobacteria	Acidimicrobiia	ASV2187	-3.993	0.00%	0.01%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1741	-4.122	0.00%	0.00%
1	gly	Bacteria	Thaumarchaeota	Nitrososphaeria	ASV1205	-5.091	0.00%	0.01%
1	gly	Bacteria	Actinobacteria	Actinobacteria	ASV1484	-4.643	0.00%	0.01%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1585	-4.411	0.00%	0.01%
1	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV2020	-4.625	0.00%	0.01%
1	gly	Bacteria	Acidobacteria	Holophagae	ASV1581	-5.674	0.00%	0.01%

1	gly	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1856	-4.125	0.00%	0.01%
1	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2751	-4.216	0.00%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1561	-4.402	0.00%	0.01%
1	gly	Bacteria	Planctomycetes	Pla3_lineage	ASV2031	-4.474	0.00%	0.00%
1	gly	Bacteria	Armatimonadetes	unassigned	ASV1295	-4.961	0.00%	0.01%
1	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV3001	-4.581	0.00%	0.01%
1	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2242	-4.295	0.00%	0.00%
1	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV477	3.77	0.08%	0.05%
1	tb	Bacteria	Acidobacteria	Subgroup_17	ASV569	3.857	0.06%	0.04%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV651	5.444	0.06%	0.04%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV794	7.11	0.04%	0.03%
1	tb	Bacteria	Acidobacteria	Holophagae	ASV763	6.536	0.04%	0.03%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV654	4.627	0.04%	0.03%
1	tb	Bacteria	Acidobacteria	Subgroup_6	ASV649	3.858	0.04%	0.03%
1	tb	Bacteria	Latescibacteria	unassigned	ASV803	5.38	0.04%	0.02%
1	tb	Bacteria	Acidobacteria	Subgroup_5	ASV790	5.896	0.03%	0.02%
1	tb	Bacteria	Acidobacteria	Holophagae	ASV860	6.23	0.03%	0.02%
1	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV948	6.715	0.03%	0.02%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV987	6.396	0.03%	0.02%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV905	6.46	0.03%	0.02%
1	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV901	4.99	0.03%	0.02%
1	tb	Bacteria	Acidobacteria	Acidobacteriia	ASV962	4.942	0.03%	0.02%
1	tb	Bacteria	Latescibacteria	unassigned	ASV1000	6.633	0.03%	0.01%
1	tb	Bacteria	Acidobacteria	Holophagae	ASV1027	5.996	0.03%	0.02%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV170	-5.184	0.03%	0.12%
1	tb	Bacteria	Chloroflexi	KD4-96	ASV894	5.976	0.03%	0.02%
1	tb	Bacteria	Acidobacteria	Subgroup_17	ASV1107	5.703	0.02%	0.02%
1	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV952	3.901	0.02%	0.02%
1	tb	Bacteria	Planctomycetes	Phycisphaerae	ASV892	4.21	0.02%	0.02%
1	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV891	4.048	0.02%	0.02%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV991	6.274	0.02%	0.01%
1	tb	Bacteria	Actinobacteria	Thermoleophilia	ASV983	4.818	0.02%	0.01%
1	tb	Bacteria	Actinobacteria	Actinobacteria	ASV1120	6.13	0.02%	0.01%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1273	6.009	0.02%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV972	4.957	0.02%	0.01%
1	tb	Bacteria	Chloroflexi	Anaerolineae	ASV1121	5.985	0.02%	0.01%
1	tb	Bacteria	Acidobacteria	Subgroup_6	ASV1186	6.014	0.02%	0.01%
1	tb	Bacteria	Acidobacteria	Acidobacteriia	ASV1659	5.483	0.02%	0.01%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV856	5.274	0.02%	0.01%
1	tb	Bacteria	Actinobacteria	Actinobacteria	ASV1263	5.444	0.02%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1292	5.646	0.02%	0.01%
1	tb	Bacteria	Actinobacteria	Thermoleophilia	ASV1309	5.025	0.02%	0.01%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1525	5.033	0.02%	0.01%
1	tb	Bacteria	Acidobacteria	Subgroup_6	ASV1073	5.64	0.02%	0.01%
1	tb	Bacteria	Actinobacteria	Thermoleophilia	ASV1199	5.071	0.02%	0.01%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1270	5.759	0.02%	0.01%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1430	4.999	0.02%	0.01%
1	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV884	-4.467	0.02%	0.02%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1089	5.656	0.02%	0.01%
1	tb	Bacteria	Dependentiae	Babeliae	ASV1174	3.964	0.02%	0.01%
1	tb	Bacteria	Acidobacteria	Subgroup_6	ASV1056	4.955	0.02%	0.01%
1	tb	Bacteria	Acidobacteria	Subgroup_5	ASV1291	5.438	0.02%	0.01%
1	tb	Bacteria	Acidobacteria	Subgroup_6	ASV990	4.872	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1140	5.428	0.01%	0.01%
1	tb	Bacteria	Planctomycetes	Planctomycetacia	ASV1598	4.77	0.01%	0.01%
1	tb	Bacteria	Euryarchaeota	Thermoplasmata	ASV1055	4.87	0.01%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1203	5.547	0.01%	0.01%

1	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1557	5.009	0.01%	0.01%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1872	5.452	0.01%	0.01%
1	tb	Bacteria	Actinobacteria	Thermoleophilia	ASV1506	5.184	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1110	5.392	0.01%	0.01%
1	tb	Bacteria	Acidobacteria	Subgroup_6	ASV1496	4.806	0.01%	0.01%
1	tb	Bacteria	Rokubacteria	NC10	ASV1458	5.147	0.01%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1486	4.55	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2132	4.901	0.01%	0.01%
1	tb	Bacteria	Acidobacteria	Subgroup_22	ASV1113	4.888	0.01%	0.01%
1	tb	Bacteria	Gemmatimonadetes	BD2-11_terrestrial_group	ASV1526	4.699	0.01%	0.01%
1	tb	Bacteria	Latescibacteria	unassigned	ASV1251	4.77	0.01%	0.01%
1	tb	Bacteria	Chloroflexi	KD4-96	ASV1396	5.38	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1419	5.042	0.01%	0.01%
1	tb	Bacteria	Acidobacteria	Subgroup_6	ASV1609	4.397	0.01%	0.01%
1	tb	Bacteria	Latescibacteria	unassigned	ASV1480	4.845	0.01%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1500	4.389	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1233	3.84	0.01%	0.01%
1	tb	Bacteria	Bacteroidetes	Ignavibacteria	ASV1534	5.014	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1698	4.933	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1020	-5.404	0.01%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1541	4.227	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1873	4.709	0.01%	0.01%
1	tb	Bacteria	Acidobacteria	Acidobacteriia	ASV1527	4.984	0.01%	0.01%
1	tb	Bacteria	Latescibacteria	unassigned	ASV1761	4.333	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1452	4.765	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1613	4.073	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1721	4.534	0.01%	0.01%
1	tb	Bacteria	Actinobacteria	Actinobacteria	ASV1800	3.805	0.01%	0.01%
1	tb	Bacteria	Planctomycetes	Planctomycetacia	ASV1730	4.314	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1889	5.025	0.01%	0.01%
1	tb	Bacteria	Planctomycetes	Phycisphaerae	ASV2620	4.794	0.01%	0.01%
1	tb	Bacteria	Chloroflexi	Chloroflexia	ASV1529	4.682	0.01%	0.00%
1	tb	Bacteria	Actinobacteria	Actinobacteria	ASV1877	4.505	0.01%	0.01%
1	tb	Bacteria	Actinobacteria	Thermoleophilia	ASV1481	4.516	0.01%	0.00%
1	tb	Bacteria	Planctomycetes	Planctomycetacia	ASV1162	-5.586	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1507	3.613	0.01%	0.00%
1	tb	Bacteria	Bacteroidetes	Ignavibacteria	ASV1910	4.016	0.01%	0.00%
1	tb	Bacteria	Acidobacteria	Acidobacteriia	ASV1994	4.442	0.01%	0.01%
1	tb	Bacteria	Rokubacteria	NC10	ASV2200	4.315	0.01%	0.00%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV2322	4.274	0.01%	0.01%
1	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1603	4.477	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1764	3.8	0.01%	0.01%
1	tb	Bacteria	Chloroflexi	KD4-96	ASV1901	4.11	0.01%	0.00%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1980	3.935	0.01%	0.00%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1888	3.6	0.01%	0.01%
1	tb	Bacteria	Planctomycetes	Planctomycetacia	ASV1823	4.633	0.01%	0.00%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1582	4.429	0.01%	0.00%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1860	3.903	0.01%	0.00%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1927	4.409	0.01%	0.00%
1	tb	Bacteria	Chloroflexi	Dehalococcoidia	ASV2437	3.982	0.01%	0.00%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1366	4.577	0.01%	0.01%
1	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV2006	4.241	0.01%	0.00%
1	tb	Bacteria	Planctomycetes	Planctomycetacia	ASV995	-4.485	0.01%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV2227	3.861	0.01%	0.00%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1553	-5.284	0.01%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1060	-5.737	0.01%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV979	-5.834	0.01%	0.02%

1	tb	Bacteria	Actinobacteria	Actinobacteria	ASV2127	4.105	0.01%	0.00%
1	tb	Bacteria	Planctomycetes	Planctomycetacia	ASV1289	-3.164	0.01%	0.01%
1	tb	Bacteria	Planctomycetes	Phycisphaerae	ASV1387	4.305	0.01%	0.00%
1	tb	Bacteria	Dependentiae	Babeliae	ASV2627	4.07	0.01%	0.00%
1	tb	Bacteria	Actinobacteria	Thermoleophilia	ASV2177	4.21	0.01%	0.00%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV2241	4.144	0.01%	0.00%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV926	-5.935	0.00%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV2065	3.907	0.00%	0.00%
1	tb	Bacteria	Planctomycetes	Planctomycetacia	ASV2438	3.836	0.00%	0.00%
1	tb	Bacteria	Planctomycetes	Phycisphaerae	ASV2084	4.004	0.00%	0.00%
1	tb	Bacteria	Acidobacteria	Thermoanaerobaculia	ASV1095	-5.692	0.00%	0.01%
1	tb	Bacteria	Acidobacteria	Subgroup_6	ASV869	-6.792	0.00%	0.02%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1102	-4.987	0.00%	0.01%
1	tb	Bacteria	Planctomycetes	Phycisphaerae	ASV2508	3.557	0.00%	0.00%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1210	-4.808	0.00%	0.01%
1	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1367	-3.164	0.00%	0.01%
1	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1440	-5.422	0.00%	0.01%
1	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1029	-6.535	0.00%	0.01%
1	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2503	-4.687	0.00%	0.01%
1	tb	Bacteria	Latescibacteria	unassigned	ASV2520	3.673	0.00%	0.00%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1588	-4.551	0.00%	0.01%
1	tb	Bacteria	Gemmatimonadetes	S0134_terrestrial_group	ASV1200	-5.299	0.00%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV2213	-3.806	0.00%	0.00%
1	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1360	-5.463	0.00%	0.01%
1	tb	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	ASV1556	-5.282	0.00%	0.01%
1	tb	Bacteria	Acidobacteria	Acidobacteriia	ASV1766	-4.311	0.00%	0.01%
1	tb	Bacteria	Thaumarchaeota	Nitrososphaeria	ASV1205	-5.361	0.00%	0.01%
1	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1585	-3.761	0.00%	0.00%
1	tb	Bacteria	Planctomycetes	Planctomycetacia	ASV2425	-3.589	0.00%	0.00%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV2342	-5.057	0.00%	0.01%
1	tb	Bacteria	Armatimonadetes	unassigned	ASV1295	-5.242	0.00%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1296	-5.603	0.00%	0.01%
1	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1718	-4.616	0.00%	0.00%
1	tb	Bacteria	Actinobacteria	Actinobacteria	ASV2598	-5.01	0.00%	0.00%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV19	0.549	0.62%	0.53%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV39	0.681	0.48%	0.39%
2	gly	Bacteria	Actinobacteria	Actinobacteria	ASV32	-0.645	0.22%	0.33%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV842	7.292	0.06%	0.03%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV683	6.734	0.04%	0.02%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV816	6.542	0.03%	0.02%
2	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV654	6.403	0.03%	0.02%
2	gly	Bacteria	Acidobacteria	Subgroup_6	ASV946	4.695	0.03%	0.02%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1071	5.948	0.03%	0.02%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV929	5.678	0.03%	0.02%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV933	6.242	0.02%	0.01%
2	gly	Bacteria	Actinobacteria	Thermoleophilia	ASV947	5.639	0.02%	0.02%
2	gly	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	ASV1241	5.39	0.02%	0.01%
2	gly	Bacteria	Actinobacteria	Thermoleophilia	ASV1078	5.476	0.02%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1080	5.823	0.02%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1916	5.224	0.02%	0.01%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1732	5.608	0.02%	0.01%
2	gly	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	ASV1785	5.248	0.02%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1593	5.138	0.02%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1950	5.156	0.02%	0.01%
2	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1103	5.05	0.02%	0.01%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2543	4.992	0.02%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV882	5.83	0.02%	0.01%

2	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV1278	5.43	0.02%	0.01%
2	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1418	5.264	0.02%	0.01%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1111	5.188	0.01%	0.01%
2	gly	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	ASV1934	5.293	0.01%	0.01%
2	gly	Bacteria	Actinobacteria	Actinobacteria	ASV1120	5.142	0.01%	0.01%
2	gly	Bacteria	Actinobacteria	Thermoleophilia	ASV1267	5.078	0.01%	0.01%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV629	-5.72	0.01%	0.02%
2	gly	Bacteria	Acidobacteria	Subgroup_6	ASV1499	5.158	0.01%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1524	5.427	0.01%	0.01%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1483	4.401	0.01%	0.01%
2	gly	Bacteria	Firmicutes	Bacilli	ASV464	-4.601	0.01%	0.04%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1640	4.789	0.01%	0.01%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1646	3.086	0.01%	0.01%
2	gly	Bacteria	Chloroflexi	Anaerolineae	ASV1642	4.981	0.01%	0.01%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1327	4.689	0.01%	0.01%
2	gly	Bacteria	Euryarchaeota	Thermoplasmata	ASV1532	5.004	0.01%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1811	4.879	0.01%	0.01%
2	gly	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1976	4.551	0.01%	0.01%
2	gly	Bacteria	Bacteroidetes	Ignavibacteria	ASV1937	3.671	0.01%	0.01%
2	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV2013	4.93	0.01%	0.01%
2	gly	Bacteria	Chloroflexi	Ktedonobacteria	ASV1657	3.93	0.01%	0.01%
2	gly	Bacteria	Nitrospirae	Nitrospira	ASV1975	4.655	0.01%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1777	4.261	0.01%	0.01%
2	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV1597	4.415	0.01%	0.01%
2	gly	Bacteria	Chloroflexi	Ktedonobacteria	ASV1259	4.652	0.01%	0.01%
2	gly	Bacteria	Thaumarchaeota	Nitrososphaeria	ASV1538	4.975	0.01%	0.01%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV2632	4.097	0.01%	0.01%
2	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV1787	4.472	0.01%	0.01%
2	gly	Bacteria	Actinobacteria	Actinobacteria	ASV1800	4.483	0.01%	0.01%
2	gly	Bacteria	Planctomycetes	OM190	ASV1443	4.514	0.01%	0.00%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1718	4.262	0.01%	0.01%
2	gly	Bacteria	Chloroflexi	Anaerolineae	ASV1815	4.259	0.01%	0.01%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1924	4.576	0.01%	0.00%
2	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2290	3.765	0.01%	0.00%
2	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV2521	4.341	0.01%	0.00%
2	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1050	-4.851	0.01%	0.01%
2	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1102	-5.306	0.01%	0.01%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1155	-5.506	0.01%	0.01%
2	gly	Bacteria	Planctomycetes	Phycisphaerae	ASV2233	4.146	0.01%	0.00%
2	gly	Bacteria	Planctomycetes	Phycisphaerae	ASV3276	3.879	0.01%	0.00%
2	gly	Bacteria	Rokubacteria	NC10	ASV824	-5.509	0.01%	0.01%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1031	-5.308	0.01%	0.01%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1112	-5.243	0.01%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1531	3.565	0.01%	0.00%
2	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1824	3.924	0.01%	0.00%
2	gly	Bacteria	Actinobacteria	Thermoleophilia	ASV2774	3.953	0.01%	0.00%
2	gly	Bacteria	Acidobacteria	Subgroup_6	ASV1088	-5.063	0.00%	0.01%
2	gly	Bacteria	Actinobacteria	Actinobacteria	ASV956	-5.195	0.00%	0.02%
2	gly	Bacteria	Latescibacteria	unassigned	ASV1101	-4.224	0.00%	0.01%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1332	-4.839	0.00%	0.01%
2	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1430	-4.701	0.00%	0.01%
2	gly	Bacteria	Firmicutes	Bacilli	ASV1067	-5.028	0.00%	0.01%
2	gly	Bacteria	Planctomycetes	Planctomycetacia	ASV1196	-4.993	0.00%	0.01%
2	gly	Bacteria	Chloroflexi	Gitt-GS-136	ASV1572	-4.17	0.00%	0.01%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1419	-5.096	0.00%	0.01%
2	gly	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1595	-4.585	0.00%	0.01%
2	gly	Bacteria	Bacteroidetes	Bacteroidia	ASV1560	-3.862	0.00%	0.00%

2	gly	Bacteria	unassigned	unassigned	ASV1736	-4.241	0.00%	0.01%
2	gly	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	ASV2354	-4.578	0.00%	0.01%
2	gly	Bacteria	Chloroflexi	Dehalococcoidia	ASV1536	-4.912	0.00%	0.01%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2191	-4.029	0.00%	0.00%
2	gly	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV2006	-4.142	0.00%	0.00%
2	gly	Bacteria	Actinobacteria	Actinobacteria	ASV1919	-4.236	0.00%	0.00%
2	gly	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1261	-5.424	0.00%	0.01%
2	gly	Bacteria	Actinobacteria	Actinobacteria	ASV1559	-4.338	0.00%	0.01%
2	gly	Bacteria	Chloroflexi	Anaerolineae	ASV1153	-6.303	0.00%	0.01%
2	gly	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1863	-4.26	0.00%	0.00%
2	gly	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2117	-4.384	0.00%	0.00%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV9	0.553	0.93%	0.81%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV14	0.574	0.76%	0.64%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV19	0.679	0.62%	0.55%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV39	0.685	0.48%	0.39%
2	tb	Bacteria	Actinobacteria	Actinobacteria	ASV32	-0.648	0.22%	0.36%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV125	-0.515	0.13%	0.15%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV318	7.785	0.12%	0.06%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV435	6.186	0.11%	0.06%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV842	7.389	0.06%	0.03%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV707	6.24	0.06%	0.04%
2	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV737	3.398	0.05%	0.03%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1307	6.736	0.05%	0.02%
2	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV655	3.588	0.05%	0.03%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV927	5.76	0.04%	0.02%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1038	6.837	0.04%	0.02%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV457	5.755	0.03%	0.03%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1401	6.664	0.03%	0.02%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1081	4.087	0.03%	0.02%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1586	6.505	0.03%	0.02%
2	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV1074	6.107	0.03%	0.02%
2	tb	Bacteria	Acidobacteria	Subgroup_6	ASV946	5.427	0.03%	0.02%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV849	5.122	0.03%	0.02%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1071	4.877	0.03%	0.02%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1114	5.524	0.02%	0.02%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1216	4.244	0.02%	0.02%
2	tb	Bacteria	Actinobacteria	Actinobacteria	ASV1189	5.075	0.02%	0.01%
2	tb	Bacteria	Acidobacteria	Subgroup_6	ASV844	5.158	0.02%	0.01%
2	tb	Bacteria	Gemmatimonadetes	Longimicrobia	ASV1149	4.186	0.02%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1060	5.949	0.02%	0.01%
2	tb	Bacteria	Latescibacteria	unassigned	ASV970	5.387	0.02%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1211	4.427	0.02%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1916	5.685	0.02%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1732	5.648	0.02%	0.01%
2	tb	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	ASV1785	5.391	0.02%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1593	5.232	0.02%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1042	4.984	0.02%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1950	5.625	0.02%	0.01%
2	tb	Bacteria	Acidobacteria	Blastocatellia_(Subgroup_4)	ASV1934	5.372	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1689	5.569	0.01%	0.01%
2	tb	Bacteria	Nitrospirae	Nitrospira	ASV580	-3.308	0.01%	0.03%
2	tb	Bacteria	Chloroflexi	TK10	ASV1092	5.167	0.01%	0.01%
2	tb	Bacteria	Gemmatimonadetes	Longimicrobia	ASV1707	5.109	0.01%	0.01%
2	tb	Bacteria	Gemmatimonadetes	S0134_terrestrial_group	ASV1334	3.938	0.01%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1501	3.701	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1576	4.756	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV629	-4.828	0.01%	0.02%

2	tb	Bacteria	Acidobacteria	Subgroup_6	ASV1499	5.204	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1524	5.177	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1590	4.45	0.01%	0.01%
2	tb	Bacteria	Acidobacteria	Subgroup_6	ASV601	-5.704	0.01%	0.02%
2	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1451	4.824	0.01%	0.01%
2	tb	Bacteria	Chloroflexi	Gitt-GS-136	ASV1778	5.141	0.01%	0.01%
2	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1967	4.73	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1986	4.481	0.01%	0.01%
2	tb	Bacteria	Chloroflexi	Anaerolineae	ASV1642	4.791	0.01%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1676	4.763	0.01%	0.01%
2	tb	Bacteria	Actinobacteria	Thermoleophilia	ASV1989	4.702	0.01%	0.01%
2	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1976	4.263	0.01%	0.01%
2	tb	Bacteria	Acidobacteria	Subgroup_6	ASV923	-5.703	0.01%	0.02%
2	tb	Bacteria	Bacteroidetes	Ignavibacteria	ASV1937	3.116	0.01%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1588	4.887	0.01%	0.01%
2	tb	Bacteria	Nitrospirae	Nitrospira	ASV1975	4.284	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV2115	4.568	0.01%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV2632	4.201	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1212	4.415	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV870	-6.305	0.01%	0.02%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV898	-6.385	0.01%	0.02%
2	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV815	-5.681	0.01%	0.02%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV920	-4.438	0.01%	0.01%
2	tb	Bacteria	Planctomycetes	Phycisphaerae	ASV1844	4.177	0.01%	0.01%
2	tb	Bacteria	Actinobacteria	Thermoleophilia	ASV1105	-4.802	0.01%	0.02%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV2728	4.181	0.01%	0.00%
2	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1102	-4.825	0.01%	0.01%
2	tb	Bacteria	Proteobacteria	Gammaproteobacteria	ASV1155	-5.07	0.01%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV2944	4.35	0.01%	0.00%
2	tb	Bacteria	Acidobacteria	Subgroup_6	ASV2961	3.935	0.01%	0.00%
2	tb	Bacteria	Rokubacteria	NC10	ASV824	-5.841	0.01%	0.02%
2	tb	Bacteria	Acidobacteria	Acidobacteriia	ASV1502	3.744	0.01%	0.00%
2	tb	Bacteria	Chloroflexi	Ktedonobacteria	ASV2338	3.92	0.01%	0.00%
2	tb	Bacteria	Chloroflexi	Dehalococcoidia	ASV1220	-5.621	0.01%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1031	-5.852	0.01%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1112	-5.398	0.01%	0.01%
2	tb	Bacteria	Actinobacteria	Thermoleophilia	ASV2774	3.787	0.01%	0.00%
2	tb	Bacteria	Armatimonadetes	Fimbriimonadia	ASV2509	3.714	0.01%	0.00%
2	tb	Bacteria	Planctomycetes	Phycisphaerae	ASV1362	-3.526	0.00%	0.01%
2	tb	Bacteria	Latescibacteria	unassigned	ASV1101	-4.869	0.00%	0.01%
2	tb	Bacteria	Latescibacteria	unassigned	ASV1480	-5.496	0.00%	0.01%
2	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1028	-5.242	0.00%	0.01%
2	tb	Bacteria	Actinobacteria	Acidimicrobiia	ASV1268	-5.316	0.00%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1898	-4.618	0.00%	0.01%
2	tb	Bacteria	Acidobacteria	Subgroup_6	ASV1076	-4.903	0.00%	0.01%
2	tb	Bacteria	Firmicutes	Bacilli	ASV1067	-4.945	0.00%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1139	-4.139	0.00%	0.01%
2	tb	Bacteria	Planctomycetes	Planctomycetacia	ASV1196	-5.288	0.00%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1419	-5.62	0.00%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1643	-4.447	0.00%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1560	-3.867	0.00%	0.00%
2	tb	Bacteria	Actinobacteria	Actinobacteria	ASV1491	-5.198	0.00%	0.01%
2	tb	Bacteria	Chloroflexi	Anaerolineae	ASV1789	-4.548	0.00%	0.01%
2	tb	Bacteria	Firmicutes	Bacilli	ASV1875	-4.387	0.00%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1261	-5.366	0.00%	0.01%
2	tb	Bacteria	Verrucomicrobia	Verrucomicrobiae	ASV1231	-4.591	0.00%	0.01%
2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1408	-4.624	0.00%	0.01%

2	tb	Bacteria	Proteobacteria	Alphaproteobacteria	ASV1686	-4.326	0.00%	0.01%
2	tb	Bacteria	Acidobacteria	Holophagae	ASV1027	-5.549	0.00%	0.01%
2	tb	Bacteria	Bacteroidetes	Bacteroidia	ASV1744	-5.172	0.00%	0.01%
2	tb	Bacteria	Gemmatimonadetes	Gemmatimonadetes	ASV1856	-4.218	0.00%	0.00%
2	tb	Bacteria	Proteobacteria	Deltaproteobacteria	ASV2169	-4.642	0.00%	0.00%
1	gly	Fungi	Mortierellomycota	Mortierellomycetes	ASV19	6.537	0.81%	0.49%
1	gly	Fungi	Ascomycota	Sordariomycetes	ASV62	5.655	0.42%	0.25%
1	gly	Fungi	Ascomycota	Sordariomycetes	ASV76	5.018	0.32%	0.19%
1	gly	Fungi	Mortierellomycota	Mortierellomycetes	ASV75	-4.801	0.05%	0.18%
1	gly	Fungi	Ascomycota	Leotiomycetes	ASV95	-5.319	0.00%	0.13%
1	tb	Fungi	Basidiomycota	Agaricomycetes	ASV33	-6.339	0.61%	0.84%
1	tb	Fungi	Ascomycota	Sordariomycetes	ASV55	3.097	0.34%	0.24%
1	tb	Fungi	Ascomycota	Leotiomycetes	ASV83	-5.134	0.19%	0.27%
1	tb	Fungi	Mortierellomycota	Mortierellomycetes	ASV54	-3.316	0.15%	0.23%
2	gly	Fungi	Ascomycota	Sordariomycetes	ASV69	4.58	0.25%	0.16%
2	gly	Fungi	Ascomycota	Dothideomycetes	ASV116	4.294	0.13%	0.07%
2	gly	Fungi	Ascomycota	Leotiomycetes	ASV95	-4.928	0.05%	0.13%
2	tb	Fungi	Ascomycota	Sordariomycetes	ASV73	5.05	0.24%	0.14%
2	tb	Fungi	Ascomycota	Dothideomycetes	ASV108	4.177	0.15%	0.09%

Chapter II

1. Chapter I of Hang Guan's thesis

Maize plants and soil microbes interact to reduce arsenic concentrations and influence arsenic speciation in the soil water

Hang Guan, Veronica Caggìa, Andrea Gómez-Chamorro, Daniela Fischer, Miquel Coll-Crespí, Xiaowen Liu, Teresa Chávez-Capilla, Klaus Schlaeppi, Alban Ramette, Adrien Mestrot and Moritz Bigalke

(submitted to Science of the Total Environment)

Abstract

Arsenic (As) in soils can harm soil organisms and plants, and it enters the human food chain *via* the dietary consumption of crops. The mobility, bioavailability and toxicity of As are determined not only by its total concentration but also by its speciation. We performed a greenhouse pot experiment with maize plants to study the interactions of soil microbes, plants and As on total concentration and speciation of As in the soil water and the soils. The experiment had three soil treatments: native soil (NS), reconditioned soil (RS, sterilized soils reconditioned with soil indigenous microbes), and disturbed soil (DS, sterilized soils before planting). The three soil treatments were intersected without maize (No-plant) and with maize (Plant) at three As treatments (uncontaminated soils (As_0) and contaminated soils (As_{100} and As_{200}, addition of 100 and 200 mg As kg^{-1} soil) in a full factorial design. Due to both microbial disturbance (difference between RS and DS) and abiotic sterilization effects (difference between NS and RS), As was more mobile in the soil water of RS and DS than of NS. The microbial disturbance effect was more pronounced for organic As species than for inorganic species, implying a more prominent influence from the soil microbes involved in As methylation. The abiotic sterilization effect induced an increase in dissolved organic carbon content and a decrease in soil pH. The microbial disturbance effect was observed only in No-plant pots and the abiotic sterilization effect was more evident in No-plant pots, indicating that both effects were mitigated by maize plants. We hypothesize that maize presumably directly reduced As levels in soil water while also indirectly helping soil microbes to recover from soil sterilization, such that maize plants and soil microbes interacted to minimize As concentrations in soil water for self-protections.

Highlights:

- Biotic and abiotic disturbance increased arsenic concentrations in the soil water
- Biotic disturbance increased organic arsenic species concentrations in the soil water
- Maize plants mitigated high arsenic concentrations
- Maize interacted with soil microbes to lower arsenic concentrations in the soil water

Keywords: Metalloid, soil sterilization, microbial disturbance, soil-plant system, plant-microbe interactions

2. Chapter II of Hang Guan's thesis

Maize (*Zea mays* L.) plants in high-arsenic soils interact with soil microbes to limit the translocation of inorganic arsenic species to maize upper tissues

Hang Guan, Veronica Caggìa, Andrea Gómez-Chamorro, Xiaowen Liu, Miquel Coll-Crespí, Teresa Chávez-Capilla, Adrien Mestrot, Alban Ramette, Klaus Schläppi and Moritz Bigalke

(manuscript in preparation)

Abstract

Arsenic (As) is a toxic metalloid that can enter the food chain through plant uptake from soils and plant consumption. We performed a greenhouse pot experiment with maize (*Zea mays* L.) plants to study their interactions with soil microbes at different As levels in soils on different As species in plants. We conducted the experiment with three soil treatments: native soil (NS), reconditioned soil (RS, sterilized soils and reconditioned with soil indigenous microbes), and disturbed soil (DS, sterilized soils before planting) × three As groups (uncontaminated soils and contaminated soils (moderate-As soils and high-As soils). Maize plants were harvested after six months and separated into roots, stems, leaves, and grains. The concentrations of total As, inorganic As species (inAs), and three organic As species (orgAs), i.e., methylarsonic acid (MMA^V), dimethylarsinic acid (DMA^V) and trimethylarsine oxide (TMAO) were analyzed. In contaminated soils, corn tended to accumulate more As in its stem compared to uncontaminated soils, and the bioaccumulation coefficient (BAC) and translocation factor (TF) of the upper maize tissue were lower. OrgAs were more readily transferred into plants compared to inAs. In contaminated soils, total dry biomass of maize grown in sterilized soils was reduced more than in unsterilized soils, and microbial disturbance led to an increase in inAs and orgAs levels in maize stems. The abiotic sterilization effects caused phosphorus deficiency, which however only resulted in a reduction of maize dry biomass at elevated As concentrations. Partial correlation analysis suggested that inAs and MMA^V were responsible for this reduction. In summary, we found that maize limited inAs translocation to its essential upper tissues by interacting with soil microbes, probably to adapt to an As-stressed environment.

Declaration of consent

on the basis of Article 30 of the RSL Phil.-nat 18

Name/First name:	Veronica Caggìa
Matriculation number:	102-209-185
Study program:	Dissertation – Ecology and Evolution
Title of the thesis:	Effects of environmental chemicals on soil and plant health
Supervisors:	Prof. Dr. Matthias Erb, Prof. Dr. Klaus Schlaeppi, Prof. Dr. Sandra Spielvogel

I declare herewith that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 36 paragraph 1 litera r of the University Act of 5 September, 1996 is authorized to revoke the title awarded on the basis of this thesis.

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