Impact of plant growth promoting rhizobacteria (PGPR) on stress resistance of winter wheat (*Triticum aestivum* L.)

Von der Fakultät für Lebenswissenschaften

der Universität Leipzig

genehmigte

D I S S E R T A T I O N

zur Erlangung des akademischen Grades

Doctor rerum naturalium

(Dr. rer. nat.)

vorgelegt

von Master of Science (Biologie), Claudia Breitkreuz

geboren am 15. November 1989 in Borna

Dekan: Prof. Dr. Marc Schönwiesner

Gutachter: Prof. Dr. François Buscot

Prof. Dr. Jörg Matschullat

Tag der Verteidigung, 16. Dezember 2022

Durchgeführt am Helmholtz-Zentrum für Umweltforschung GmbH – UFZ,

Abteilung für Bodenökologie in Halle (Saale)

Gefördert durch die Deutsche Bundesstiftung Umwelt (DBU) mit einem Promotionsstipendium (AZ 20015/391)

CONTENTS

PREFACE

This thesis is written as a cumulative PhD thesis. After a general introduction, Chapter 1-3 are independent papers that were accepted for publication in international peer-reviewed journals. Thereby, the differential formats of the chapters meet the requirements of the respective journals, where the manuscripts have been published. The thesis is finalized by a synpopsis including an integrating discussion over all Chapters and critical review of limitations and derived possible perspectives for future studies. A CV, list of publications and presentations, as well as my contribution to each are provided at the end of this thesis.

BIBLIOGRAPHIC DESCRIPTION

Claudia Breitkreuz

Impact of plant growth promoting rhizobacteria (PGPR) on stress resistance of winter wheat

Fakultät für Lebenswissenschaften

Universität Leipzig

Dissertation

180 pages, 434 references, 29 figures, 29 tables

The here presented cumulative thesis provides important insigths into the complex interplay of plant-soil-interactions in agricultural systems under future expected drought as a consequence of climate change. Thereby, not only drought-induced impacts, but also the single and interacting effects of different abiotic (soil type and land use management) and biotic (cultivar type and plant development stages) factors on functional and structural changes of wheat rhizosphere bacterial communities, but also individual bacterial species, were evaluated by cultivation independent and dependent approaches, respectively. The experiments were conducted either as a pot experiment in the cold greenhouse or under field conditions using the platform of the Global Change Experimental Facility (GCEF). The GCEF is a long-term experiment investigating the effect of climate change on different land-use managements.

In *Chapter 1* a pot experiment was conducted to study the impact of multiple factors, i.e. soil type, different agricultural systems and weat cultivar, on wheat rhizosphere bacterial communities under extreme drought. The results indicated a strong soil-type specific response of the rhizosphere bacterial communities under drought. The manuscript highlight the importance of studying multiple factors to reveal common or specific adaptation processes in the rhizosphere of wheat under drought.

Chapter 2 aimed to study wheat rhizosphere bacterial communities' adaptation to different climate scenarios, in different agricultural management systems and at two different plant development stages under field conditions using the GCEF. In addition, the study was used to evaluate the accuracy of computational tools to predict functional profiles based on gene abundances by comparing these to real measured activities. We observed an overall strong effect of plant development stage, followed by agricultural management system and climate treatment on community compostion and function, which was also qualitatively confirmed by the computational approach.

Chapter 3 is a follow-up study of Chapter 2, using the same experimental system, but focusing on individual bacterial species in the rhizospere of wheat. Specifically screening for phosphate-solubilizing bacteria, we identified three dominant genera in the rhizosphere of wheat, i.e. Streptomyces, Pseudomonas and Phyllobacterium, whereby dominance of Pseudomonas and Phyllobacterium species shifted according to plant development stage. Since Phyllobacterium species have not been reported as effective P-solubilizer in the rhizosphere of wheat before and further indicated a strong drought-tolerance in vitro, we introduced Phyllobacterium species as promising candidate for use as biofertilizers.

ZUSAMMENFASSUNG

Weizen ist eine der weltweit am häufigsten angebauten Kulturpflanzen und trägt zur Sicherung der Nahrungsmittelproduktion in verschiedenen Regionen der Welt bei. Obwohl er fast überall angebaut werden kann, ist die Produktion durch Trockenheit limitiert. Daher rücken mehr und mehr die mikrobiellen Gemeinschaften im Boden und in der Rhizosphäre in den Mittelpunkt der modernen agrarbiologischen Forschung, um die Produktivität bei Trockenheit aufrechtzuerhalten und eine nachhaltige Produktion zu fördern. Während bereits zahlreiche Studien über die Weizenproduktion und den positiven Einfluss des Bodenmikrobioms in ariden und semiariden Regionen der Welt durchgeführt wurden, sind vergleichbare Studien in Mitteleuropa selten. Dies könnte sich aufgrund der anhaltenden Klimakrise und der zu erwartenden ausbleibenden Sommerniederschläge ändern. Dabei haben die meisten Studien, die sich mit der Akklimatisierung des Weizenrhizobioms an Wasserdefizite befasst haben, bestenfalls den Einfluss von Trockenheit und ein oder zwei weiteren biotischen oder abiotischen Einflussfaktoren, die zudem miteinander interagieren können, auf die Struktur und Funktion der mikrobiellen Gemeinschaften in der Rhizosphäre untersucht. Ziel dieser Arbeit war es daher, verschiedene komplementäre Analysemethoden zu kombinieren, um trockenheitsbedingte strukturelle und funktionelle Veränderungen in den bakteriellen Gemeinschaften und auch einzelner Arten in der Weizenrhizosphäre, in Abhängigkeit von Bodentyp, Landnutzungssystem, Weizensorte und Pflanzenentwicklungsstadium zu untersuchen, und zu ermitteln, wie sich diese Veränderungen auf die Produktivität des Weizens als Folge möglicher Szenarien des Klimawandels in Mitteldeutschland auswirken.

Die vorliegende Arbeit leitet mit einer allgemeinen Einführung und Vorstellung des Projekts ein, gefolgt von drei aufeinanderfolgenden Kapiteln, die die wichtigsten Ergebnisse enthalten, die in von Fachleuten begutachteten Artikeln veröffentlicht wurden. Beginnend mit einem Experiment im Gewächshaus (Kapitel 1) und weiterführend zu einem realistischen Klimaszenario unter Feldbedingungen (Kapitel 2 und 3), beschreiben die drei Kapitel die

alleinigen und interagierenden Auswirkungen von Trockenheit und Anbausystem (Kapitel 1-3), Bodentyp und Weizensorte (Kapitel 1), sowie Pflanzenwachstumsstadien (Kapitel 2 und 3) auf Bakteriengemeinschaften und einzelne Taxa des Weizenrhizobioms. Die verwendeten Methoden reichen dabei von der traditionellen Kultivierung und In-vitro-Bioassays (Kapitel 3), über extrazelluläre Enzymaktivitätspotenziale (Kapitel 1 und 2), bis hin zu fortschrittlicheren Technologien, wie Metabarcoding (Kapitel 1 und 2) und computergestützten Vorhersagen (Kapitel 1 und 2). Zum Abschluss der Arbeit werden in einer abschließenden Synopsis die gewonnenen Ergebnisse zusammengetragen und kritisch betrachtet, sowie Ideen für zukünftige Studien formuliert.

In Kapitel 1 untersuchten wir die Auswirkungen des Bodentyps (lehmig vs. sandig), der Bewirtschaftung (konventionell vs. ökologisch), der Weizensorte (anspruchslos vs. anspruchsvoll) und die Wechselwirkungen zwischen diesen Faktoren auf die Zusammensetzung und Funktion der Bakteriengemeinschaft in der Rhizosphäre von Weizen unter extremen Trockenheitsbedingungen. Das Wasserdefizit übte einen starken Druck auf die Rhizosphärenbakteriengemeinschaften aus und stand in Wechselwirkung mit dem Bodentyp und der Bewirtschaftung, nicht aber mit den Weizensorten. In den Sandböden beobachteten wir eine starke trockenheitsbedingte Veränderung der Zusammensetzung der Gemeinschaft mit einem Rückgang der Artenvielfalt und der extrazellulären Enzymproduktion, während die Veränderungen durch die Trockenheit in den fruchtbaren Lehmböden weniger stark ausgeprägt waren. Eine besondere Ausnahme von diesem Muster wurde für Enzymaktivitäten gefunden, die am Kohlenstoffkreislauf im Sandboden beteiligt sind, was auf eine positive Rückkopplung zwischen Pflanze und Bodengemeinschaften unter Trockenheit hindeutet.

In Kapitel 2 wurden zwei einzelne, jedoch miteinander verknüpfte Ziele verfolgt. Erstens nutzten wir die Plattform der Global Change Experimental Facility (GCEF), um die Auswirkungen von zwei Anbaupraktiken (konventionell vs. ökologisch) und zwei Klimabehandlungen (ambient vs. zukünftig) auf die Zusammensetzung der Bakteriengemeinschaft und die Aktivitätsprofile extrazellulärer Enzyme, die an den C-, N- und P-Zyklen in der Rhizosphäre von Weizen beteiligt sind, in zwei verschiedenen

Pflanzenwachstumsstadien zu untersuchen. Die Klimabehandlung in der GCEF hatte keinen Einfluss auf die Rhizosphärenbakteriengemeinschaften. Die Zusammensetzung und die Funktionen der Rhizosphärenbakteriengemeinschaften unterschieden sich signifikant zwischen dem vegetativen und dem generativen Wachstumsstadium der Pflanzen, sowohl im konventionellen als auch im ökologischen Landbau. In einem zweiten Schritt nutzten wir die gewonnenen Daten, um die Genauigkeit rechnerischer Ansätze wie Tax4Fun und PanFP zur Vorhersage funktioneller Profile von Bakteriengemeinschaften auf der Grundlage von 16S rDNA-Daten zu überprüfen. Zu diesem Zweck verglichen wir die gemessenen Enzymaktivitäten mit den jeweiligen Genhäufigkeiten in der Gemeinschaft unter verschiedenen Klima- und Anbaubedingungen und in den beiden Entwicklungsstadien der Pflanzen. Diese Analyse ergab qualitative, aber nicht unbedingt quantitative Übereinstimmungen, d. h. wir fanden Auswirkungen der verschiedenen Behandlungen auf die gemessenen Enzymaktivitäten, die sich auch in den Genhäufigkeiten widerspiegeln.

Kapitel 3 stellt einen ergänzenden Ansatz zu Kapitel 2 dar, wobei der Schwerpunkt auf einzelnen Bakterienarten liegt. Mit kulturabhängigen Methoden wurden gezielt stark Phosphat-solubilisierende Bakterien aus der Rhizosphäre von Weizen isoliert und auf ihre In-vitro-Trockenheitstoleranz getestet. Unter den mehr als 800 isolierten Arten dominierten *Phyllobacterium*-, *Pseudomonas*- und *Streptomyces*-Arten. Während Anbaumanagement und Klimabehandlung nur geringe Auswirkungen hatten, wirkten sich die Wachstumsstadien des Weizens signifikant auf die Zusammensetzung und Funktionen der Isolate aus, wobei eine Dominanz von *Pseudomonas*-Arten in der vegetativen Wachstumsphase durch eine Dominanz von *Phyllobacterium*-Arten in der generativen Wachstumsphase ersetzt wurde. Da das Potenzial zur P-Solubilisierung mit einer hohen in vitro-Trockenheitstoleranz einherging, wurden *Phyllobacterium*-Arten als vielversprechende pflanzenwachstumsfördernde Rhizobakterien (PGPR) für Weizen unter zukünftigen Trockenheitsbedingungen charakterisiert.

Zusammenfassung

In der Synopsis dieser Arbeit bewerteten wir die multifaktoriellen und multidisziplinären Ansätze, und untersuchten, inwieweit die Anpassungen der Bakteriengemeinschaften in Feld- und Topfversuchen übereinstimmen oder sich unterscheiden.

Insgesamt fanden wir allgemeine, aber auch differenzielle Anpassungsprozesse von Bakteriengemeinschaften und einzelnen Arten in der Rhizosphäre von Weizen an die Trockenheit, wobei einzelne Faktoren, aber auch interagierende Effekte einen starken Einfluss auf diese Prozesse ausübten. Diese Studie unterstreicht damit die Bedeutung multifaktorieller Ansätze, um gemeinschafts- oder artspezifische Rückkopplungen zwischen Pflanze und Boden zu untersuchen.

SUMMARY

Wheat is one of the worldwide most cultivated crop and highly contribute to secure food production in different world regions. Although, it grows almost ubiquitous, its production is severely vulnerable to drought. Soil and rhizosphere microbial communities associated to plants come more and more into the focus of modern agrobiology research, as a solution to maintain productivity under drought, and reinforce sustainable production. Whereas numerous studies on wheat production and the beneficial influence of the soil microbiome under drought have been performed in arid and semiarid regions of the world, comparable studies in Central Europe are rare. This might change due to the ongoing climate crisis and expected less frequent precipitations during the vegetation season. So far, most studies that focus on acclimatization of the wheat rhizobiome to water deficit mostly consider, at best, two interacting factors, and lack to consider other biotic or abiotic drivers of rhizosphere microbial communities structure and function. Therefore, the aim of this thesis was to combine complementary analytical approaches to investigate drought-induced structural and functional changes in wheat rhizosphere bacterial communities and individual species in dependency of soil type, farming system, wheat cultivar and plant development stage, and to determine how these changes affect wheat performance as a consequence of possible climate change scenarios in Central Germany.

The presented thesis starts with a general introduction and presentation of the project, followed by three consecutive chapters containing the main findings published in peer-reviewed articles. Starting with an experiment performed in the greenhouse (Chapter 1) and then moving to a realistic climate scenario under field conditions (Chapter 2 and 3), the three chapters demonstrate the sole and interacting effects of drought and farming system (Chapter 1-3), soil type and wheat cultivar (Chapter 1), as well as plant growth stages (Chapter 2 and 3) on bacterial communities and individual taxa of the wheat rhizobiome. The methods used reach from traditional cultivation and *in-vitro* bioassays (Chapter 3), over extracellular enzyme activity

potentials (Chapter 1 and 2) to more advanced technologies such as metabarcoding (Chapter 1 and 2) and computational tools (Chapter 1 and 2), addressing single bacterial taxa as well as community level. Finalizing the thesis, a concluding synopsis compiles and critically reviews the gained results and formulates future study perspectives.

In Chapter 1, we evaluated the impact of soil type (loamy *vs.* sandy), farming management (conventional *vs.* organic), wheat cultivar (non-demanding *vs.* demanding), and the interacting effects of these factors on wheat rhizobacterial community composition and function under extreme drought conditions. Water deficit exerted a strong pressure on rhizobacterial communities, and interacted with soil type and farming management, but not with the wheat cultivar types. In the sandy soil, we observed a strong drought-induced shift in community composition, with a decrease in species diversity and extracellulare enzyme production, while changes by drought were less prominent in the fertile loamy soil. A particular exception from this pattern was found for enzyme activities involved in carbon cycling in the sandy soil suggesting a positive plant-soil-feedback on enzyme activities by drought conditioning.

In Chapter 2, two individual, but interrelated aims were pursued. First, we used the platform of the Global Change Experimental Facility (GCEF) to explore the impact of two farming practices (conventional vs. organic) and two climate treatments (ambient vs. future) on bacterial community composition and activity profiles of extracellulare enzymes involved in C,N and P cycles in the wheat rhizosphere at two different plant growth stages. The climate treatment in the GCEF had no effect on the rhizobacterial communities. Rhizobacterial community composition and functions significantly differed between vegetative and mature growth stages of the plants, in both conventional and organic farming. In a second step, we reused the data to explore further the accuracy of computational approaches, like Tax4Fun and PanFP, to predict functional profiles of bacterial communities based on 16S rDNA abundance data. To this end, we compared the measured enzyme activities with respective gene abundances in the community under different climate and farming treatments, and at the two plant development stages. This analysis revealed qualitative, but not necessarily quantitative

concordances, i.e. we found effects of the different treatments on the measured enzyme activities reflected in the gene abundances.

Chapter 3 is a complementary approach to Chapter 2 with a focus on individual bacterial species level. Culture-dependent methods were used to specifically isolate strong P-solubilizing bacteria from the rhizosphere of wheat, which were tested for their *in-vitro* drought tolerance. Among the more than 800 isolated species, *Phyllobacterium*, *Pseudomonas* and *Streptomyces* species dominated. While farming management and climate treatment had only minor effects on composition and functions of the isolates, the wheat growth stages had an impact, whereby a dominance of *Pseudomonas* species at the vegetative growth phase was replaced by dominance of *Phyllobacterium* species at the mature growth phase. Since P-solubilizing potential was paralleled by a high *in-vitro* drought tolerance, *Phyllobacterium* species were characterized as promising plant growth promoting rhizobacteria (PGPR) of wheat under future drought conditions.

In the synopsis part, we evaluated the multifactorial and multidisciplinary approaches and investigated to what extent the adaptations of bacterial communities in field and pot experiments coincided or differed.

Overall, we found common and distinct adaptation processes of bacterial communities and individual species in the rhizosphere of wheat to drought, whereby single factors, but also interacting effects exerted a strong impact on these processes. This study underlines the importance of multifactorial approaches to reveal community- or species-specific plant-soil-feedbacks.

INTRODUCTION

When extreme events become the new normal

The greenhouse effect is a natural process that stabilizes temperature on Earth and makes life possible. Under clear skies, circa 60 % of this greenhouse effect arises from water vapor in the atmosphere, 26 % from carbon dioxide, 8 % from ozone and ca. 6 % from trace gases (Ch_4, N_2O and others), and these values correspond to a balance between their contribution to heat absorption and re-radiation from the sun and the Earth's surface, respectively (Kiehl and Trenberth 1997). In the last century, anthropogenic activities, e.g. the burning of fossil fuels to produce energy, caused a misbalance of these gases in the atmosphere. For instance, since the industrial revolution the atmospheric concentration of carbon dioxide has increased from 280 parts per million (ppm) to 370 ppm in 2003 (increase of 31 %, CDIAC) and 409.8 ppm in 2019 (increase of 42 %, CDIAC) (Figure 1). The consequences of increasing and accumulating anthropogenic produced greenhouses gases in the atmosphere over decades as well as the effects of land use changes on the global climate have long been underestimated.

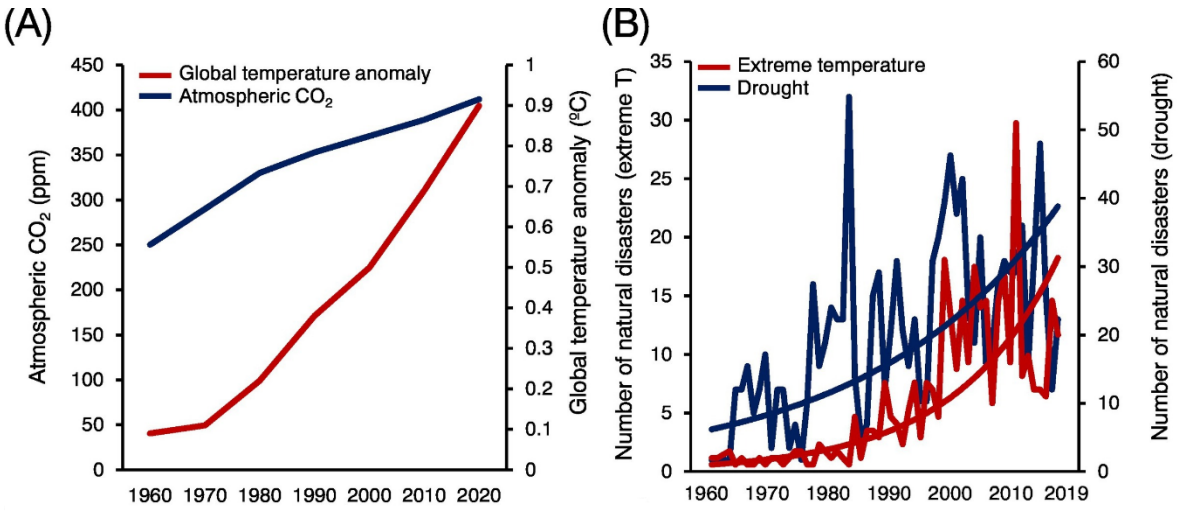


Figure 1 Exceeding bounds of natural variability in (A) CO_2 concentrations and global temperature, which led to (B) higher number of natural disasters in drought and extreme temperatures between 1960 and 2019 (Zandalinas et al. 2021)

Figure 1 shows that since 1960, atmospheric CO_2 concentrations steadily increased, which caused overall changes in global mean temperatures exceeding the bounds of natural

variability (Zandalinas et al. 2021). Long-term changes in climate are accompanied by an increasing number of extreme and disastrous events in local climates around the globe, e.g. floods (Pall et al. 2011, Min et al. 2011), extended periods of drought (Ciais et al 2003, Hari et al 2020, Zandalinas et al. 2021) and storms (Demski et al 2017), which severely endangers human life standards and food production. While extreme events like floods and storms are highly destructive, they occur more occasionally, and their spatial and temporal influence is restricted to certain regions, e.g. coastal regions and islands. In contrast, heat events and related rainfall deficits put extreme pressure on vegetation over larger areas and for longer periods of time, especially during active growing seasons of plants (Hari et al. 2020, Chung et al 2014). Becoming more frequent in the last decades, heat events and related drought periods are regarded as the most challenging outcome of global warming.

Figure 2 shows the distribution of weather anomalies and the vegetation health index (VHI) across Europe in the summers (June-August) 2003, 2018 and 2019 (Hari et al. 2020), which were extremely hot and dry. Especially Central Europe experienced high mean summer temperature and precipitation anomalies pressing the VHI below 30, which is the critical threshold for a healthy vegetation (Figure 2). Investigating the origin of 2003 heat event, Liu et al. (2020) identified anomalies in the atmospheric circulation and the strong humidity-temperature coupling as main reason for the extreme drought in Central Europe. The heatwaves of 2018 and 2019 differed from the one in 2003, not only regarding the geographical extension, which included Northern Scandinavian countries (Figure 2, Hari et al 2020), and the occurrence in two consecutive year, but mainly due to their origin. Changes in land cover with higher percentage of agricultural sites compared to forests caused a higher net surface radiation, which was mainly responsible for the heat event in 2018 (Liu et al. 2020), and possibly 2019. Since land cover defines the amount of soil water retention and carbon sequestration - a closed vegetation cover can take more carbon dioxide from the atmosphere and reduce evaporation - agricultural sites are highly vulnerable to future effects of climate change.

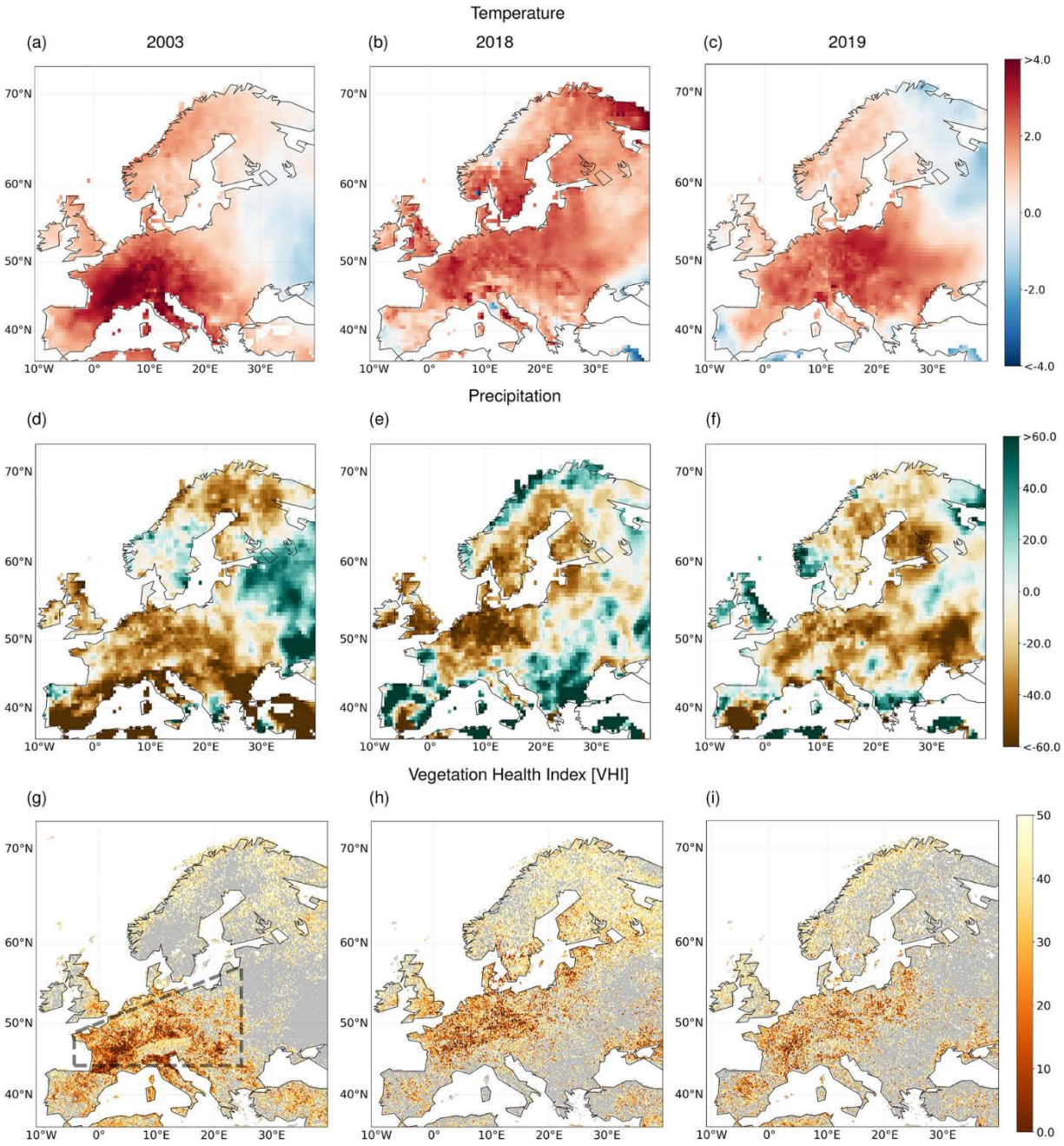


Figure 2 Weather anomalies and vegetation health index (VHI) across Europe during extreme events in 2003, 2018 and 2019. For each year (a-c) mean summer (June-August) temperature (°C), (d-f) precipitation anomalies (%) and (g-i) consequences for vegetation (poor vegetation health, VHI≤30) are presented (Hari et al. 2020).

The temperate zone of Central Germany where this study was conducted is characterized by such an agriculture-dominated landscape. Established climate models predict for this region long-term increases in temperatures and a reduction in summer precipitation up to 20 % by 2080 (mean values of: COSMO-CLM (Rockel et al. 2008), REMO (Jacob and Podzun 1997), and RCAO (Doscher et al. 2002)). Additionally, extreme events, such as the two consecutive dry years in 2018 and 2019, which were exceptional so far, but exerted extreme pressure on

vegetation health in Central Germany (Figure 2), are expected to be more frequent and severe in the future (Hari et al. 2020). Mitigating and adapting to climate change and derived weather anomalies has thus become a central focus for food production (Berrang-Ford et al 2011).

Feedback to agricultural production and need for management adaptation

Between 1979 and1989, worldwide areas for cropland and permanent pastures increased about 2.2 % and 0.1 % respectively, while forest area decreased by 1.8 % (Dale 1997). Today, half of the habitable land is used for agricultural production, and 37 % are covered by forests (Ritchie 2019). From the anthropogenic point of view, the increasing need for agricultural products to the feed growing world population justifies intensive agricultural production. By 2050, the world's population is expecting to grow to 9.8 billion people (UN 2017), which requires an increase in agricultural production by 70 % compared to 2005 (ELD, 2015). Intensification of agricultural production has become a major constraint to fight global warming, but concurrently the agricultural lands are highly sensitive to climate variations limiting their productivity. For instance, global yield variation between 15% and 35% for wheat, oilseeds and coarse grains can be explained by El Nino Southern Oscillation phenomenon that causes alternating flood and drought events (Ferris 1999). For comparison, extreme drought events, such as in 2003 caused a 30% reduction in European gross primary production (Ciais et al. 2005), and the one in 2018, a drop of 17 % in wheat production compared to previous five year average (FAOSTAT).

The "intensive agriculture" paradigm states that increased input of mineral fertilizers and pesticides combined with intensive mechanical management leads to an increased yield. Under conditions of water scarcity this paradigm is however challenged, as the applied fertilizers cannot be taken up by the plant, accumulate in the soil and may create a toxic environment (Magid et al. 2020), or, in case of nitrogen fertilizers, be leached to deeper soil layers and groundwater (Wang and Li 2019). To minimize the ecological footprint of agriculture, following changes in agricultural management have been proposed to reach sustainability (summarized in Bodner et al. 2015 and Howden et al. 2007):

- The choice of appropriate plant varieties/species with increased resistance to heat shock, drought and pests, as well as adaptation of fertilization, irrigation and pest control regimes according to prevailing climate.
- Efficient water management to retain water under water deficit and prevent water logging, erosion, and nutrient leaching during extreme rainfalls by e.g. crop residue retention.
- Adapting cropping activities to precipitation patterns in timing and location.
- Broader spectrum of activities, e.g. agriculture and livestock raising.

Nevertheless, these improvements are still based on traditional approaches of high input systems, which induce changes in biogeochemical and hydrological cycles of the soils, threaten soil organisms and functions (State of knowledge of soil biodiversity, 2020), and often leave agricultural lands vulnerable and with limited self-regulating properties to be able to cope with disturbances (Orgiazzi et al. 2016). Therefore, there is an urgent need to bioremediate the degraded areas of long-term agricultural sites while maintaining productivity. In line with this objective, the concept of biofertilization has raised increased attention (Al Abboud et al. 2014, Bhat et al. 2015, Hernández-Fernández et al. 2021). Biofertilizers are beneficial microbes often mixed with a stabilizing carrier material, such as organic manure or clay. It was shown that the use of biofertilizers stimulates various soil processes, i.e. fixation of nutrients, soil stability, biological control of diseases or bioremediation of contaminated soils, and thus indirectly or directly promotes plant growth (reviewed in Sneha et al. 2018). However, biofertilizer application does not necessarily guarantee better plant performance (Mitter et al, 2021), since site-specific abiotic parameters, and especially the on-site soil microbial communities can restrict or even eliminate biofertilizer efficiency under changing environmental conditions (Debnath et al. 2019).

Difficulties in exploring the soil microbiome and identification of plant beneficial microbial taxa

The soil microbiome is a highly diverse community, which comprises microbial taxa from all three domains of life, *Bacteria*, *Archaea* and *Eukarya*. Bacteria and fungi contribute 10^2-10^4 times more to soil biomass than other groups of microorganisms (Fierer, 2017). As such, most studies on soil functioning relate to bacteria- and fungi-mediated mechanisms (Kuzyakov and Blagodatskaya, 2015; Verstraete and Mertens, 2004, Fierer, 2017). Through their activities, both groups provide important ecosystem services and, thus, are crucial for soil health and productivity. Ecosystem services include the production and consumption of atmospheric trace gases, the regulation of soil acidity, carbon dynamics and nutrient cycles balancing the pools of available and unavailable nutrients in the soil matrix (Fierer, 2017, reviewed in Verstraete and Mertens, 2004). Plants take advantage and even foster these services on the narrow root-soil interface, i.e. the rhizosphere, to promote their growth (reviewed in Morgan et al. 2005). Releasing carbon-rich root exudates into the rhizosphere, plants influence the rhizosphere microbiome and seek to selectively attract beneficial microbial taxa from the surrounding pool of microorganisms (Yuan et al 2018). Depending on the involved microorganisms such mutual symbioses are termed either mycorrhizal associations, plant growth-promoting fungi (plant-fungal symbiosis, Rayner 1927, Hossain et al. 2017) or plant growth promoting rhizobacteria (PGPR) (plant-bacterial symbiosis, Kloepper and Schroth 1978).

While mycorrhizal and other plant-fungal associations dominate in undisturbed forest and grassland ecosystems (Banerjee et al. 2019), in intensively managed agricultural systems beneficial plant-microbe interactions are mainly attributed to bacteria (Miller and Lodge, 1997, de Vries et al. 2013). Thereby, the pool of soil bacterial taxa from which beneficial ones can be recruited by plants is highly diverse, and their community composition strongly depends on surrounding conditions. The characteristics of different soil types, which vary in terms of edaphic parameters such as water storage and transport capacities, nutrient status and degree of mineral complexation, in combination with changes in climatic conditions, are main drivers for shifts in the presence and abundance of certain bacterial taxa (Singh et al 2007, Ramirez

et al 2020). Furthermore, plant identity, plant diversity and developmental stage exert a controlling influence on the selection process in the rhizosphere microbiome based on the changes in the quantity and quality of rhizodeposits (Garbeva et al. 2008, Francioli et al. 2018). The magnitude of oscillations and changes of all these influential factors makes it difficult to identify key species or beneficial species-interactions that drive ecosystem functions (reviewed in Schloter et al. 2018). Of note, the functional redundancy, a measure of the number of different species that contribute to one and the same function (Lawton and Brown, 1994), is high among bacteria (Allison et al. 2008). Microbial taxa, which might be functionally redundant in one system or at a certain time point, might become of greater unique importance in another system with different environmental conditions and species composition (Schloter et al. 2018). In recent years, community composition in particular has become the focus of research, and techniques in this area such as next-generation amplicon sequencing have greatly improved (reviewed in Slatko et al. 2018). However, changes in composition are not necessarily associated with functional changes. Standardized protocols to measure community functionality, as e.g. extracellular enzyme measurements, fail to trace back activities to single organisms (German et al. 2011). Appropriate methods of metatranscriptomics and metabolomics are still cost-intensive, require strong experience in sample preparation and data analysis, plus, sequence data interpretation is limited by existing numbers of sequenced genomes (Aguiar-Pulido et al. 2016, Sandhu et al. 2019). All this makes broad analyses of rhizosphere microbiota challenging. Computational approaches, which are based on functional analyses of barcode data, may be a more cost-saving alternative, but their accuracy strongly depends on the completeness of the used databases (Aßhauer et al. 2015: Tax4Fun, Jun et al. 2015). Consequently, a much more meaningful characterization of microbial diversity might be achieved by not only combining the different analytical methods to resolve structural and functional diversity on community and individual species level, but also by implementing environmental data as important drivers of diversity.

Our approach with wheat

To be able to detect key species and sub-communities of soil microorganisms enriched in response to certain environmental conditions, at least one common factor that exerts a measurable impact on the microbial community composition has to be selected. In agricultural systems, the reduction of biodiversity to a low number of cultivated plants grown in monocultures predestines the plant itself as such a factor. Winter wheat is one of the five most widely grown crops in the world and the most widely grown crop in Germany (FAOSTAT). Winter wheat requires favorable conditions in terms of nutrient supply and precipitation, and just these conditions are susceptible to changing climate endangering productivity and yield of wheat monocultures. Numerous studies have so far described the positive effect of PGPR on wheat growth under conditions of salinity (Naili et al. 2018, Desoky et al. 2020) and drought (Zafar-ul-Hye et al. 2019, Ansari et al. 2021), and also more direct effects for nutrient acquisition (Liu et al. 2019, Wang et al. 2020). While these studies indicate that taxonomically different PGPR act on wheat, Kavamura et al (2021) suggest the existence of a wheat core microbiome with a full set of beneficial functional properties. This core microbiome may persist even under changing climatic and edaphic conditions (Kavamura et al. 2021, Simonin et al. 2020). For instance, Schlatter et al. (2020) described a core rhizosphere microbiome with bacterial taxa from the genera *Bradyrhizobium, Sphingomonadaceae, Massilia, Variovorax, Oxalobacteraceae,* and *Caulobacteraceae* for dryland wheat. The identification of such a wheat core microbiome or possible key species may not only be of ecological interest, but also a crucial element toward a sustainable wheat production under future climate change.

Although several studies already investigated the impact of drought on the wheat microbiome, the combination of multifactorial and/or multidisciplinary approaches are still underrepresented. Therefore, the central aim of this study was to determine how the response of wheat rhizosphere bacterial community to water deficit is influenced by further biotic and abiotic factors, and how these factor interactions drive changes in rhizosphere microbiome that influence plant drought tolerance. For this purpose, state of the art Illumina MiSeq amplicon sequencing, computational tools and classic cultivation techniques were combined to examine

the impact of drought on wheat rhizobacterial communities and individual species in dependency of soil type, farming system, wheat cultivar and plant growth stage. This integrative pathway was realized in pot and field experiments co-manipulating different numbers of environmental factors, whereby some overlapped (Figure 3).

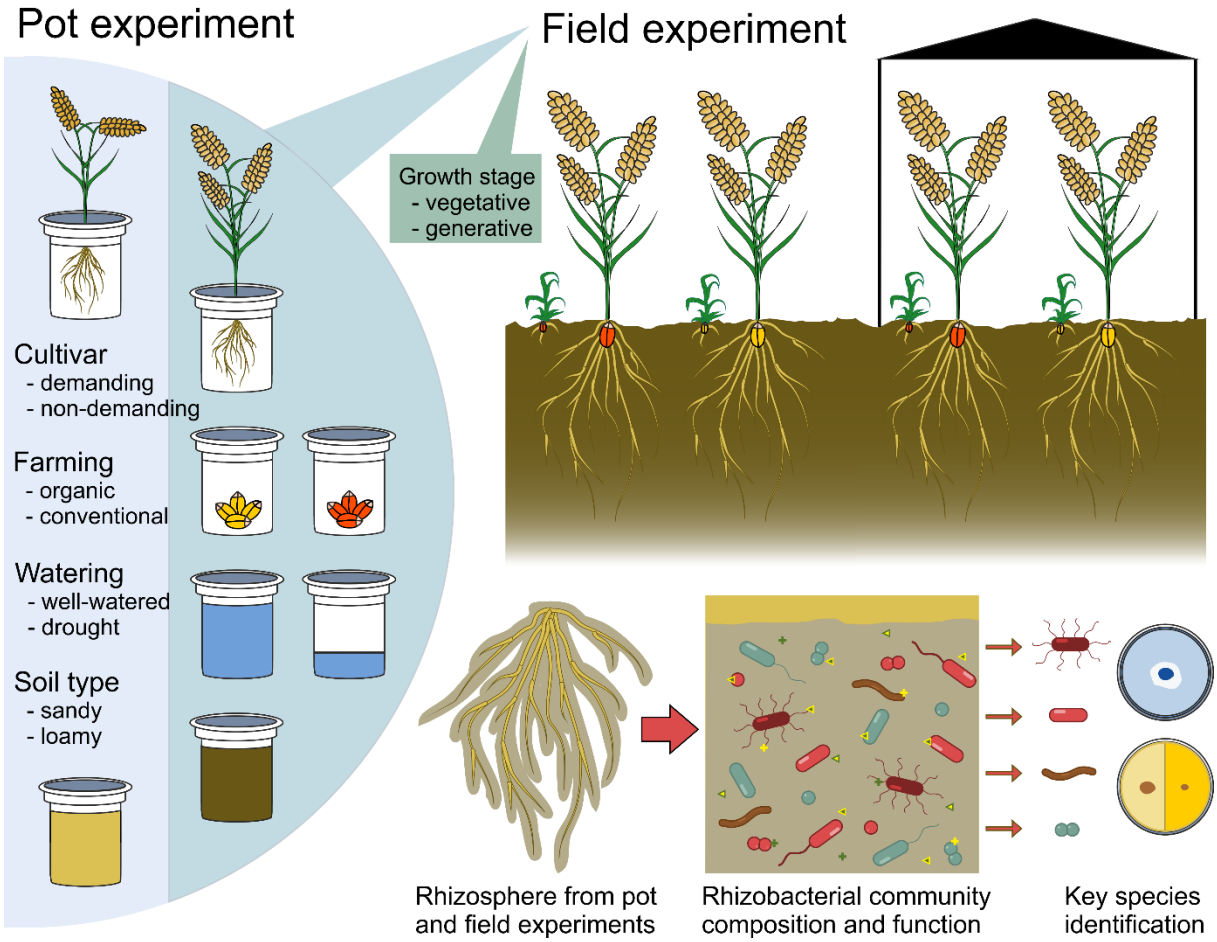


Figure 3 Conceptual overview of the thesis. The objective was to reveal the relative impacts of multiple interacting factors on wheat rhizosphere microbial community composition and function, both at the prokaryote DNA and bacterial isolate levels. Pot experiment examined how the factors wheat cultivar, farming system, watering and soil type affected community composition and function (presented in Chapter 1 of the thesis). Field experiment surveyed how the factors farming system, watering, and the plant growth stage affected rhizosphere community composition and function (presented in Chapter 2), as well as the functional traits of bacterial isolates (presented in Chapter 3).

Chapter 1 evaluates how rhizosphere prokaryote community responses to drought are modulated by treatments of farming system (conventional *vs.* organic), soil type (sandy *vs.* loamy soil), wheat cultivar (demanding *vs.* non-demanding) and water availability (drought *vs.* well-watered). The multifactorial experiment was performed as pot experiment in the green house. In order to trace back changes due to applied treatments over a whole growing season, rhizosphere samples were collected at harvest and compared to those of the starting

conditions. We expected a decrease in diversity under drought with particular enrichment of drought-tolerant taxa. The effect of drought was further expected to be stronger in the sandy soil, than in the fertile loamy soil, as well as in conventional than in organic farming.

Chapter 2 follows two goals. First, the impacts of three experimental factors, farming system (CF *vs.* OF), climate manipulation (ambient *vs.* future) and plant growth stage (vegetative *vs.* mature) on wheat rhizosphere prokaryote community composition and function were evaluated under field conditions in the Global Change Experimental Facility (GCEF, 20 plots with a size of 24 x 16 m, detailed study design in Schädler et al. 2019). And second, the performances of the 16S sequencing datasets based, functional annotation tools Tax4Fun and PanFP were assessed by evaluating their accuracy to predict the results obtained in relation to the effects of the three tested experimental factors land use, climate and plant development stage. For this, the functional tool predicted extracellular enzyme gene numbers were cross-compared with the corresponding measured enzyme activities in the rhizosphere of wheat. Expectations in this chapter were mainly related to the method comparison part assuming concordances and deviations between predicted and measured functions to be a consequence of community' response to the applied treatments. Thereby, we expected that the deviations should be more pronounced in treatments that induce a strong dynamic in the bacteria community.

Chapter 3 aims to identify plant beneficial taxa in wheat rhizosphere. In contrast to the other two studies, cultivation-based approaches were applied. They focused on two important traits, phosphate cycling and drought tolerance, using the same experimental system as described in Chapter 2. In response to the demand of plants for nutrients at different growth stages, we expected the abundance and activity of P-solubilizing bacteria to be increased at the active, vegetative growth stage. We further expected the climate manipulation to trigger a less diverse community, which however maintains activity under future climate conditions, while activity potentials should be increased under organic farming with lower nutrient availability.

Overall, the three studies represent two directions of scaling. Upscaling from pot (Chapter 1) to field experiment (Chapters 2 and 3), and down-scaling from the community level (Chapters 1 and 2) to the functions of individual bacterial isolates (Chapter 3). The relative impacts of

drought and interactions with the other experimental factors, as well as possible implementations for future work are discussed in the synopsis part of the thesis.

Bibliography

Aguiar-Pulido, V., Huang, W., Suarez-Ulloa, V., Cickovski, T., Mathee, K., & Narasimhan, G. (2016). Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis: supplementary issue: bioinformatics methods and applications for big metagenomics data. Evolutionary Bioinformatics, 12, EBO-S36436.

Al Abboud, M. A., Ghany, T. A., & Alawlaqi, M. M. (2014). Role of biofertilizers in agriculture: a brief review. Mycopath, 11(2).

Allison, S. D., & Martiny, J. B. (2008). Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences, 105(Supplement 1), 11512-11519.

Ansari, F. A., Jabeen, M., & Ahmad, I. (2021). Pseudomonas azotoformans FAP5, a novel biofilm-forming PGPR strain, alleviates drought stress in wheat plant. International Journal of Environmental Science and Technology, 18(12), 3855-3870.

Aßhauer, K. P., Wemheuer, B., Daniel, R., & Meinicke, P. (2015). Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics, 31(17), 2882-2884.

Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A. Y., Gattinger, A., ... & van der Heijden, M. G. (2019). Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. The ISME journal, 13(7), 1722-1736.

Berrang-Ford, L., Ford, J. D., & Paterson, J. (2011). Are we adapting to climate change?. Global environmental change, 21(1), 25-33.

Bhat, T. A., Ahmad, L., Ganai, M. A., & Khan, O. A. (2015). Nitrogen fixing biofertilizers; mechanism and growth promotion: a review. J Pure Appl Microbiol, 9(2), 1675-1690.

Bodner, G., Nakhforoosh, A., & Kaul, H. P. (2015). Management of crop water under drought: a review. Agronomy for Sustainable Development, 35(2), 401-442.

CDIAC: Carbon Dioxide Information Analysis Center. https://cdiac.ess-dive.lbl.gov/. last access: 27th of April 2022.

Chung, U., Gbegbelegbe, S., Shiferaw, B., Robertson, R., Yun, J. I., Tesfaye, K., ... & Sonder, K. (2014). Modeling the effect of a heat wave on maize production in the USA and its implications on food security in the developing world. Weather and Climate Extremes, 5, 67-77.

Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., ... & Valentini, R. (2005). Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature, 437(7058), 529-533.

Dale (1997) The relationship between land-use change and climate change. Ecological applications, 7(3), 753-769

Debnath, S., Rawat, D., Kumar Mukherjee, A., Adhikary, S., and Kundu, R. (2019). “Applications and constraints of plant beneficial microorganisms in agriculture, biostimulants,” in Plant Science, eds S. M. Mirmajlessi and R. Radhakrishnan (IntechOpen), 1–25. doi: 10.5772/intechopen.89190

Demski, C., Capstick, S., Pidgeon, N., Sposato, R. G., & Spence, A. (2017). Experience of extreme weather affects climate change mitigation and adaptation responses. Climatic Change, 140(2), 149-164.

Desoky, E. S. M., Saad, A. M., El-Saadony, M. T., Merwad, A. R. M., & Rady, M. M. (2020). Plant growth-promoting rhizobacteria: Potential improvement in antioxidant defense system and suppression of oxidative stress for alleviating salinity stress in Triticum aestivum (L.) plants. Biocatalysis and Agricultural Biotechnology, 30, 101878.

De Vries, F. T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M. A., Bjørnlund, L., ... & Bardgett, R. D. (2013). Soil food web properties explain ecosystem services across European land use systems. Proceedings of the National Academy of Sciences, 110(35), 14296-14301.

Doscher, R., Willén, U., Jones, C., Rutgersson, A., Meier, H. M., Hansson, U., & Graham, L. P. (2002). The development of the regional coupled ocean-atmosphere model RCAO. Boreal environment research, 7(3), 183-192..

ELD: Report for policy and decision makers: Reaping Economic and Environmental Benefits from Sustainable Land Management. Economics of Land Degradation (ELD) Initiative, Bonn, Germany (2015)

FAOSTAT: Food and Agriculture Organization oft he United Nations. https://www.fao.org/faostat/en/#data/QCL. Last access: 27th of April 2022.

Ferris, J. N. (1999). An analysis of the impact of ENSO (El Nino/Southern Oscillation) on global crop yields (No. 1099-2016-89147).

Howden, S. M., Soussana, J. F., Tubiello, F. N., Chhetri, N., Dunlop, M., & Meinke, H. (2007). Adapting agriculture to climate change. Proceedings of the national academy of sciences, 104(50), 19691-19696

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology, 15(10), 579-590.

Francioli, D., Schulz, E., Buscot, F., & Reitz, T. (2018). Dynamics of soil bacterial communities over a vegetation season relate to both soil nutrient status and plant growth phenology. Microbial ecology, 75(1), 216-227.

Garbeva, P., Van Elsas, J. D., & Van Veen, J. A. (2008). Rhizosphere microbial community and its response to plant species and soil history. Plant and soil, 302(1), 19-32.

GSBI, S. (2020). State of knowledge of soil biodiversity–Status, challenges and potentialities. Summary for policy makers.

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.

Hari, V., Rakovec, O., Markonis, Y., Hanel, M., & Kumar, R. (2020). Increased future occurrences of the exceptional 2018–2019 Central European drought under global warming. Scientific reports, 10(1), 1-10.

Hernández-Fernández, M., Cordero-Bueso, G., Ruiz-Muñoz, M., & Cantoral, J. M. (2021). Culturable yeasts as biofertilizers and biopesticides for a sustainable agriculture: A comprehensive review. Plants, 10(5), 822.

Hossain, M., Sultana, F., & Islam, S. (2017). Plant growth-promoting fungi (PGPF): phytostimulation and induced systemic resistance. Plant-microbe interactions in agro-ecological perspectives, 135-191.

Jun, S. R., Robeson, M. S., Hauser, L. J., Schadt, C. W., & Gorin, A. A. (2015). PanFP: pangenome-based functional profiles for microbial communities. BMC research notes, 8(1), 1-7.

Jacob, D., & Podzun, R. (1997). Sensitivity studies with the regional climate model REMO. Meteorology and Atmospheric Physics, 63(1), 119-129.

Kavamura, V. N., Mendes, R., Bargaz, A., & Mauchline, T. H. (2021). Defining the wheat microbiome: Towards microbiome-facilitated crop production. Computational and structural biotechnology journal, 19, 1200-1213.

Kiehl, J. T., & Trenberth, K. E. (1997). Earth's annual global mean energy budget. Bulletin of the American meteorological society, 78(2), 197-208.

Kloepper, J. W., & Schroth, M. N. (1981). Plant growth-promoting rhizobacteria and plant growth under gnotobiotic conditions. Phytopathology, 71(6), 642-644.

Kuzyakov, Y., & Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: concept & review. Soil Biology and Biochemistry, 83, 184-199.

Lawton, J. H., & Brown, V. K. (1994). Redundancy in ecosystems. In Biodiversity and ecosystem function (pp. 255-270). Springer, Berlin, Heidelberg.

Liu, X., Jiang, X., He, X., Zhao, W., Cao, Y., Guo, T., ... & Tang, X. (2019). Phosphate-solubilizing Pseudomonas sp. strain P34-L promotes wheat growth by colonizing the wheat rhizosphere and improving the wheat root system and soil phosphorus nutritional status. Journal of Plant Growth Regulation, 38(4), 1314-1324.

Liu, X., He, B., Guo, L., Huang, L., & Chen, D. (2020). Similarities and differences in the mechanisms causing the European summer heatwaves in 2003, 2010, and 2018. Earth's Future, 8(4), e2019EF001386.

Magid, J., Pedersen, K. E., Hansen, M., Cedergreen, N., & Brandt, K. K. (2020). Comparative assessment of the risks associated with use of manure and sewage sludge in Danish agriculture. In Advances in Agronomy (Vol. 164, pp. 289-334). Academic Press.

Miller, R. M., & Lodge, D. J. (1997). Fungal responses to disturbance: agriculture and forestry. The mycota, 4, 65-84.

Min, S. K., Zhang, X., Zwiers, F. W., & Hegerl, G. C. (2011). Human contribution to more-intense precipitation extremes. Nature, 470(7334), 378-381.

Mitter, E. K., Tosi, M., Obregón, D., Dunfield, K. E., & Germida, J. J. (2021). Rethinking crop nutrition in times of modern microbiology: innovative biofertilizer technologies. Frontiers in Sustainable Food Systems, 5, 606815.

Morgan, J. A. W., Bending, G. D., & White, P. J. (2005). Biological costs and benefits to plant–microbe interactions in the rhizosphere. Journal of experimental botany, 56(417), 1729-1739.

Naili, F., Neifar, M., Elhidri, D., Cherif, H., Bejaoui, B., Aroua, M., ... & Cherif, A. (2018). Optimization of the effect of PGPR–based biofertlizer on wheat growth and yield. Biom. Biostat. Int. J, 7, 226-232.

Orgiazzi, A., Panagos, P., Yigini, Y., Dunbar, M. B., Gardi, C., Montanarella, L., & Ballabio, C. (2016). A knowledge-based approach to estimating the magnitude and spatial patterns of potential threats to soil biodiversity. Science of the Total Environment, 545, 11-20.

Pall, P., Aina, T., Stone, D. A., Stott, P. A., Nozawa, T., Hilberts, A. G., ... & Allen, M. R. (2011). Anthropogenic greenhouse gas contribution to flood risk in England and Wales in autumn 2000. Nature, 470(7334), 382-385.

Ramírez, P. B., Fuentes-Alburquenque, S., Díez, B., Vargas, I., & Bonilla, C. A. (2020). Soil microbial community responses to labile organic carbon fractions in relation to soil type and land use along a climate gradient. Soil Biology and Biochemistry, 141, 107692.

Rayner, M. C. (1927). Mycorrhiza (continued). New phytologist, 26(1), 22-45.

Ritchie, H. (2019). Half of the world's habitable land is used for agriculture. Our World in Data, November, 11.

Rockel, B., Will, A., & Hense, A. (2008). The regional climate model COSMO-CLM (CCLM). Meteorologische Zeitschrift, 17(4), 347-348.

Sandhu, S. S., Pourang, A., & Sivamani, R. K. (2019). A review of next generation sequencing technologies used in the evaluation of the skin microbiome: what a time to be alive. Dermatology online journal, 25(7).

Schädler, M., Buscot, F., Klotz, S., Reitz, T., Durka, W., Bumberger, J., ... & Auge, H. (2019). Investigating the consequences of climate change under different land-use regimes: a novel experimental infrastructure. Ecosphere, 10(3), e02635.

Schlatter, D. C., Yin, C., Hulbert, S., & Paulitz, T. C. (2020). Core rhizosphere microbiomes of dryland wheat are influenced by location and land use history. Applied and environmental microbiology, 86(5), e02135-19.

Schloter, M., Nannipieri, P., Sørensen, S. J., & van Elsas, J. D. (2018). Microbial indicators for soil quality. Biology and Fertility of Soils, 54(1), 1-10.

Singh, B. K., Munro, S., Potts, J. M., & Millard, P. (2007). Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Applied Soil Ecology, 36(2-3), 147-155.

Simonin, M., Dasilva, C., Terzi, V., Ngonkeu, E. L., Diouf, D., Kane, A., ... & Moulin, L. (2020). Influence of plant genotype and soil on the wheat rhizosphere microbiome: evidences for a core microbiome across eight African and European soils. FEMS Microbiology Ecology, 96(6), fiaa067.

Slatko, B. E., Gardner, A. F., & Ausubel, F. M. (2018). Overview of next-generation sequencing technologies. Current protocols in molecular biology, 122(1), e59.

Sneha, S., Anitha, B., Sahair, R. A., Raghu, N., Gopenath, T. S., Chandrashekrappa, G. K., & Basalingappa, M. K. (2018). Biofertilizer for crop production and soil fertility. Academia Journal of Agricultural Research, 6(8), 299-306.

UN: World Population Prospects: The 2017 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP/248. United Nations, New York, NY (2017)

Verstraete, W., & Mertens, B. (2004). The key role of soil microbes. In Developments in soil science (Vol. 29, pp. 127-157). Elsevier.

Wang, Z. H., & Li, S. X. (2019). Nitrate N loss by leaching and surface runoff in agricultural land: A global issue (a review). Advances in agronomy, 156, 159-217.

Wang, J., Li, R., Zhang, H., Wei, G., & Li, Z. (2020). Beneficial bacteria activate nutrients and promote wheat growth under conditions of reduced fertilizer application. BMC microbiology, 20(1), 1-12.

Yuan, J., Zhao, J., Wen, T., Zhao, M., Li, R., Goossens, P., ... & Shen, Q. (2018). Root exudates drive the soil-borne legacy of aboveground pathogen infection. Microbiome, 6(1), 1-12.

Zafar-ul-Hye, M., Danish, S., Abbas, M., Ahmad, M., & Munir, T. M. (2019). ACC deaminase producing PGPR Bacillus amyloliquefaciens and Agrobacterium fabrum along with biochar improve wheat productivity under drought stress. Agronomy, 9(7), 343.

Zandalinas, S. I., Fritschi, F. B., & Mittler, R. (2021). Global warming, climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. Trends in Plant Science, 26(6), 588-599.

.

CHAPTER 1

Interactions Between Soil Properties, Agricultural Management and Cultivar Type Drive Structural and Functional Adaptations of the Wheat Rhizosphere Microbiome To Drought

Claudia Breitkreuz, Laura Herzig, François Buscot, Thomas Reitz, Mika Tarkka

Environmental Microbiology (2021), published

Accepted: May 19th 2021

environmental microbiology

Environmental Microbiology (2021) 23(10), 5866–5882 doi:10.1111/1462-2920.15607

Special Issue Article

Interactions between soil properties, agricultural management and cultivar type drive structural and functional adaptations of the wheat rhizosphere microbiome to drought

Claudia Breitkreuz [1]*, Laura Herzig,[1] François Buscot,[1,2] Thomas Reitz[1,2] and Mika Tarkka[1,2]

[1]*Soil Ecology Department, UFZ - Helmholtz-Centre for Environmental Research, Theodor-Lieser-Straße 4, Halle, 06120, Germany.*

[2]*German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, Leipzig, 04103, Germany.*

Summary

Rhizosphere microbial communities adapt their structural and functional compositions to water scarcity and have the potential to substantially mitigate drought stress of crops. To unlock this potential, it is crucial to understand community responses to drought in the complex interplay between soil properties, agricultural management and crop species. Two winter wheat cultivars, demanding and non-demanding, were exposed to drought stress in loamy Chernozem and sandy Luvisol soils under conventional or organic farming management. Structural and functional adaptations of the rhizosphere bacteria were assessed by 16S amplicon sequencing, the predicted abundance of drought-related functional genes in the bacterial community based on 16S amplicon sequences (Tax4Fun) and the activity potentials of extracellular enzymes involved in the carbon cycle. Bacterial community composition was strongly driven by drought and soil type. Under drought conditions, Gram-positive phyla became relatively more abundant, but either less or more diverse in Luvisol and Chernozem soil respectively. Enzyme activities and functional gene abundances related to carbon degradation were increased under drought in the rhizosphere of the demanding wheat cultivar in organic farming. We demonstrate that soil type, farming system and wheat cultivar each constitute important factors during the structural and/or functional adaptation of rhizobacterial communities in response to drought.

Received 8 February, 2021; revised 17 May, 2021; accepted 19 May, 2021. *For correspondence. E-mail claudia.breitkreuz@ufz.de; Tel. +49 345 558 5416. Fax: +49 (0)345 - 558 5449

Introduction

Mitigating negative impacts of climate change on crop production is one of the most challenging tasks of modern agriculture. In temperate ecosystems of Central Germany, climate change has been characterized by an increase in temperatures and a changed intra-annual precipitation pattern moving main rain events out of the growing season (Schädler *et al*., 2019). Winter wheat is the second most-produced crop worldwide (FAO, 2018) and covers more than half of the cereal production area in Germany (DESTATIS, 2021). Significant yield losses in the first decade of the 21st century were strongly correlated with heat and drought-related weather indices (Lüttger and Feike, 2018). Also during the extreme drought in 2018, wheat production in Germany significantly dropped on average by 12.6% (DESTATIS, 2021). Thereby, a strong gradient from the Northeastern to the Southwestern part of Germany emerged, ranging from -21.2% in Brandenburg, -20.8% in Saxony-Anhalt to almost no losses in Baden-Württemberg (-2.6%) (DESTATIS, 2021). Since drought events are going to further aggravate in the future, sharp declines in agricultural productivity are expected (Hari *et al*., 2020). Thus, if novel cultivars and agricultural systems are not applied, immense losses in crop yields can be expected.

Besides management options, the negative impacts of drought on agricultural yields can be mitigated by certain bacterial taxa colonizing the crop rhizosphere (Marasco *et al*., 2012; Coleman-Derr and Tringe, 2014; Azarbad *et al*., 2020). These plant-beneficial bacteria express a wide range of functional traits to cope with drought

themselves and promote crop adaptation to drought conditions. To maintain their functionality soil bacteria are able to produce biofilms and protect themselves from dehydration under water scarcity (Kim *et al*., 2012). Mechanisms for plant adaptation include biofertilization and reduction of stress hormone production, as well as induction of systemic resistance to various biotic and abiotic stress factors (reviewed in Goswami *et al*., 2016). The most commonly investigated marker related to drought-adaptation of plants is the bacterially produced 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which suppresses production of the stress-related phytohormone ethylene (Glick, 2005). Bacterial enzymes that are secreted into the rhizosphere can also reduce plants' drought stress responses aboveground by affecting glutathione, butanediol and spermidine metabolisms (Cho *et al*., 2008; Kasim *et al*., 2013; Zhou *et al*., 2016). However, the essential factor by which plant growth is impaired is the drought-related reduction in available soil carbon and nutrients (reviewed in van der Molen *et al*., 2011). To cope with the imbalance of need and availability of C sources, microbes degrade more complex carbohydrates in dry soils. This is indicated by the enrichment of microbial functional genes and enzyme activities involved in degradation of celluloses and hemicelluloses (Bouskill *et al*., 2016; Martiny *et al*., 2017).

The extent of plant growth support by rhizobacteria is strongly dependent on their trait composition, which is in turn linked to community structure and diversity. Less diverse communities with single functional key players are considered to be more vulnerable to disturbances than more diverse communities due to the high functional redundancy potential of these (Nautiyal and Dion, 2008; Wagg *et al*., 2021). Both community structure and its trait composition are steered by various environmental factors. Vegetation cover (Francioli *et al*., 2018) and plant growth stage (Wang *et al*., 2016; Breitkreuz *et al*., 2020), as well as soil moisture dynamics, alter composition and traits of soil and plant rhizosphere communities prominently (De Vries *et al*., 2012; Santos-Medellín *et al*., 2017; Ochoa-Hueso *et al*., 2018). Moreover, Lauber *et al*. (2008) found that the microbial community structure is strongly affected by soil type, a finding that was further supported by a study comparing soil bacteria as well as fungi-dominated food webs across various European field sites (de Vries *et al*., 2013). Besides, intensive cropland management decreases soil biodiversity and favours a bacteria-dominated food web due to the application of agrochemicals, high nutrient inputs and mechanical treatments as tillage (reviewed by Thiele-Bruhn *et al*., 2012, de Vries *et al*., 2013). Kavamura *et al*. (2021) recently reviewed the findings of over a decade of research on the wheat microbiome and outlined the intricate complexity of interactions under different abiotic and biotic conditions. So far, dependence of drought-induced community adaptation of rhizobacterial communities in dependency on soil type, agricultural management and crop cultivar has been poorly investigated, despite its critical importance for both, soil health and maintenance of agricultural yields.

For this purpose, we conducted a pot experiment to address this knowledge gap. It builds upon recent findings made in the Global Change Experimental Facility (GCEF, Schädler *et al*., 2019) that bacterial community adaptations in the rhizosphere of winter wheat to climate change are modified by agricultural management intensity (Breitkreuz *et al*., unpublished). For the pot experiment, we used loamy Haplic Chernozem and sandy Albic Luvisol, which strongly differ in their physical and chemical properties. The nutrient and humus-rich Chernozem (Altermann *et al*., 2005) offers favourable conditions for wheat growth. Contrary, the nutrient and humus-poor sandy Luvisol is characterized by a much lower water-holding capacity and thus higher vulnerability to drought (Schweitzer, 2010). To consider agricultural management as a driver of microbial community response, we collected topsoil from organic (OF) and conventional (CF) farming plots of well-established experimental field platforms. Two winter wheat cultivars with different site suitability, i.e. different tolerance to drought, were sown in October 2016 and exposed to drought and control conditions from tillering stage until harvest in July 2017. The composition of rhizosphere bacterial communities was determined using next-generation Illumina amplicon sequencing. Community traits were estimated by prediction of functional gene abundances, based on the relative abundances of amplicon sequence variants using Tax4Fun (Aßhauer *et al*., 2015), as well as by measuring the potential of cellulose and hemicellulose decomposing enzyme activities.

Our work was directed by three working hypotheses. We hypothesized (i) that drought reduces diversity of bac- teria and leads to an increased relative abundance of - drought-tolerant bacteria and (ii) that these drought impacts are more pronounced in the sandy Luvisol than in the loamy Chernozem. We further expected, that (iii) the communities in OF soils are more resistant to drought than in CF soils, since the lower management intensity in OF provides a more diverse community with a broader functional repertoire that can cope with drought stress.

Results

Soil type and farming system-specific initial soil parameters and watering treatment effects

Chernozem and Luvisol soils collected from conventional and organic farming field plots were analysed for their chemical properties (Table 1). As expected, total nitrogen (N) and carbon (C) contents, as well as available N

Environmental Microbiology, 23, 5866–5882

Table 1. Initial properties of the used soils.

	Haplic Chernozem				Albic Luvisol			
	Conventional farming		Organic farming		Conventional farming		Organic farming	
Total organic carbon in %	1.95	(0.04)a	1.95	(0.10)a	0.81	(0.02)b	0.69	(0.04)
Total nitrogen in %	0.16	(0.01)a	0.15	(0.01)b	0.06	(0.01)c	0.06	(0.01)
C/N ratio	12.2	(0.70)ab	12.9	(0.50)a	12.6	(0.40)ab	11.9	(0.50)
Available mineral nitrogen (mg kg^{-1})	14.8	(1.10)a	13.5	(0.90)b	9.22	(0.43)c	8.16	(0.21)
NO_3-N (mg kg^{-1})	12.7	(0.60)a	10.8	(0.60)b	7.22	(0.27)c	6.82	(0.09)
NH_4-N (mg kg^{-1})	2.11	(0.50)b	2.68	(0.27)a	2.00	(0.16)b	1.34	(0.12)
Available phosphorus (mg/100 g dry soil)	3.42	(0.18)c	3.49	(0.28)c	5.74	(0.32)a	4.75	(0.21)
Available potassium (mg/100 g dry soil)	14.29	(0.35)b	14.19	(1.25)b	17.26	(0.58)a	7.42	(0.37)
pH	6.38	(0.05)a	6.37	(0.03)a	5.87	(0.05)c	6.08	(0.05)
Maximum water holding capacity (WHC_{max})	35%				28%			

Standard deviations are provided in brackets. Different letters within each line indicate significant differences between treatments according to ANOVA and Tukey's HSD test.

concentration and water holding capacities were higher for Chernozem than for Luvisol. However, we measured higher available phosphorus concentrations in the Luvisol (Table 1). The effect of farming system was more pronounced in the Luvisol, with higher concentrations of all measured parameters, except for total N, and higher pH in the conventional compared to the organic farming system. In contrast, only in Chernozem mineral N concentrations were higher in conventional than in organic farming system (Table 1).

At the beginning of the experiment, all pots were adjusted to 60% of the soil type-specific maximum water holding capacity (WHC_{max}). Due to the lower water holding capacity, the gravimetric soil moisture content of Luvisol (12.4 ± 0.7%) was lower compared to that of Chernozem (16.4 ± 0.4%). At wheat harvest, well-watered pots showed comparable soil moisture contents with averages of 16.2 ± 0.7% for the Chernozem and 11.1 ± 0.7% for the Luvisol. The drought treatment caused strong decreases in moisture in both soil types, with averages of 9.1 ± 1.2% for Chernozem and 5.8 ± 0.7% for the Luvisol (Table S1).

Wheat performance strongly affected by experimental factors

Wheat biomass production was strongly impaired by drought (analysis of variance (ANOVA), $p < 0.001$, $F = 512.36$). The reduction of wheat dry biomass by drought was comparable between the treatments ranging from 37% to 45% (ANOVA, $p > 0.05$ for soil, farming system and wheat cultivar). Exclusively for organic farming treatment on Chernozem, the reduction of wheat dry biomass was larger, 56% for 'RGT-Reform' and 57% for 'Dichter'.

Besides the expected reduction in plant biomass, watering regime significantly influenced the C:N ratio of grain (ANOVA, $p < 0.001$, $F = 31.99$) and straw (ANOVA, $p < 0.001$, $F = 26.51$), with higher ratios in control than under drought conditions (Fig. S1). While the farming system significantly influenced grain C:N ratios (CF > OF, ANOVA, $p < 0.001$, $F = 68.68$), wheat cultivars differed in their straw C:N ratios ('RGT-Reform' > 'Dichter', ANOVA, $p = 0.02$, $F = 5.62$) (Fig. S1). Interestingly, the soil type had no effect on grain and straw C:N ratios. In contrast, the water use per gram biomass (ANOVA, $p < 0.001$, $F = 172.87$, Fig. S1) significantly differed between the soil types and were, in line with the biomass itself, strongly influenced by the watering regime (ANOVA, $p < 0.001$, $F = 786.56$). Thereby, higher water use (Fig. S1) was observed in Luvisol than in Chernozem, and under well-watered conditions compared to drought conditions for both soil types.

Treatment effects on bacterial community composition

Among the set of experimental factors, soil type had by far the largest impact on bacterial community assemblage (PERMANOVA, $p < 0.001$, $R^2 = 0.41$). In contrast, the bacterial community compositions were comparable between the two wheat cultivars (Table 2). We therefore subsequently focused on the wheat cultivar 'Dichter', while corresponding results for 'RGT-Reform' are provided in the Supplementary Material (Tables S2 and S3; Fig. S2).

Non-metric multidimensional scaling (NMDS) revealed for both soil types a separation of the original ('start') bacterial communities from the ones present at wheat harvest in July 2017 (Fig. 1A and C). At harvest, the bacterial communities were additionally clearly separated by farming system and watering regime, as well as by compartment (rhizosphere vs. bulk soil) (Fig. 1A and C; Table 2). In the Chernozem (Fig. 1A and B), the bulk soil communities were similarly affected by watering (PERMANOVA, $R^2 = 0.15$) and farming system ($R^2 = 0.13$).

In the rhizosphere of the same soil type the effect of watering was stronger ($R^2 = 0.21$) than that of the

Table 2. Effect of experimental factors on bacterial community composition in bulk soil and wheat rhizosphere for Chernozem and Luvisol soil at wheat harvest.

	Haplic Chernozem				Albic Luvisol			
	Bulk		Rhizosphere		Bulk		Rhizosphere	
	p-value	R^2	*p*-value	R^2	*p*-value	R^2	*p*-value	R^2
Watering	<0.001	0.15	<0.001	0.21	0.002	0.07	<0.001	0.25
Farming system	<0.001	0.13	<0.001	0.11	<0.001	0.48	<0.001	0.38
Wheat cultivar	0.43	0.02	0.11	0.02	0.37	0.01	0.08	0.02
Watering: Farming	0.19	0.02	0.02	0.03	0.003	0.05	<0.001	0.09

PERMANOVA was applied to test for significant differences (*p*-value) in community composition and the respective explanatory value of each treatment (R^2). Bold values highlight significant impacts of applied treatments on community composition, *p*-values <0.05.

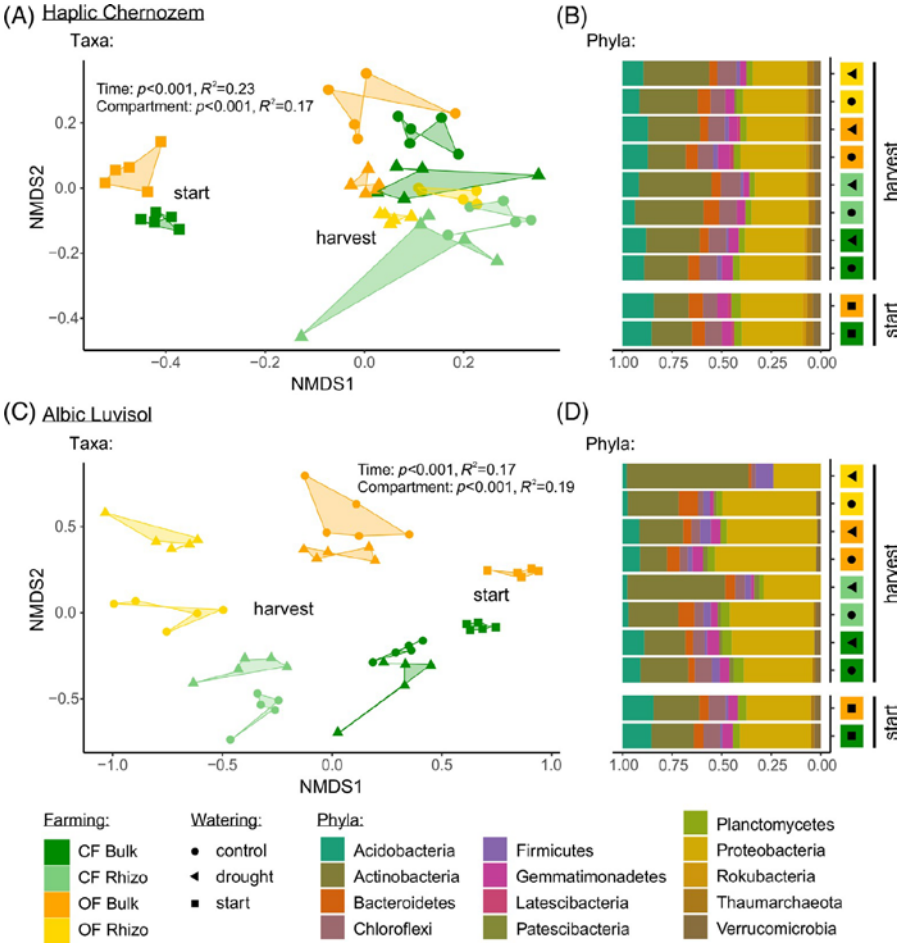


Fig 1. Impact of experimental factors on bacterial community composition in Chernozem and Luvisol for wheat cultivar 'Dichter'. NMDS for (A) Chernozem and for (C) Luvisol represent separation of communities from start conditions to harvest. Significance (*p*) and explanatory values (R^2) for time point and compartment according to PERMANOVA analyses are provided for each soil (refer to Table 2 for further PERMANOVA results). Relative abundances of predominant phyla within (B) Chernozem and (D) Luvisol are given for start conditions and at harvest.

farming system (R^2 = 0.12). For Luvisol, a different pattern was found (Fig. 1C and D), since the farming system was identified as the main driving factor of bacterial community composition (Table 2). In bulk soil, the effect of the farming system (R^2 = 0.47) explained almost half of the variance, but only little explanation was provided

by watering regime (R^2 = 0.07). In the rhizosphere, the impact of the water regime on community composition increased (R^2 = 0.25), but that of the farming system remained larger (R^2 = 0.38). The effects of watering and farming interacted for bulk soil and rhizosphere in Luvisol but only for rhizosphere in Chernozem. However, these interactions explained only little of the total variance (Table 2).

Changes in the distribution of main bacterial phyla across the treatments

In the Chernozem soil in all treatments and with both wheat varieties, the abundance of Acidobacteria, Gemmatimonadetes, Rokubacteria and Verrucomicrobia decreased, while Actinobacteria and Thaumarchaeota increased in rhizosphere compared to bulk soil (Table S2). Moreover, relative abundances of rhizosphere Bacteroidetes, Gemmatimonadetes, Patescibacteria and Proteobacteria decreased (Table S2), while Acidobacteria, Chloroflexi and Firmicutes for 'Dichter' (Fig. 2), and Rokubacteria and Thaumarchaeota for 'RGT-Reform' (Fig. S2) were increased under drought. The effect of farming system was weak for Chernozem soil and differently affected phyla abundances in the rhizospheres of the two wheat varieties (Fig. 2 and Fig. S2).

In Luvisol, farming system and watering-related shifts in relative abundances of phyla were more pronounced than in

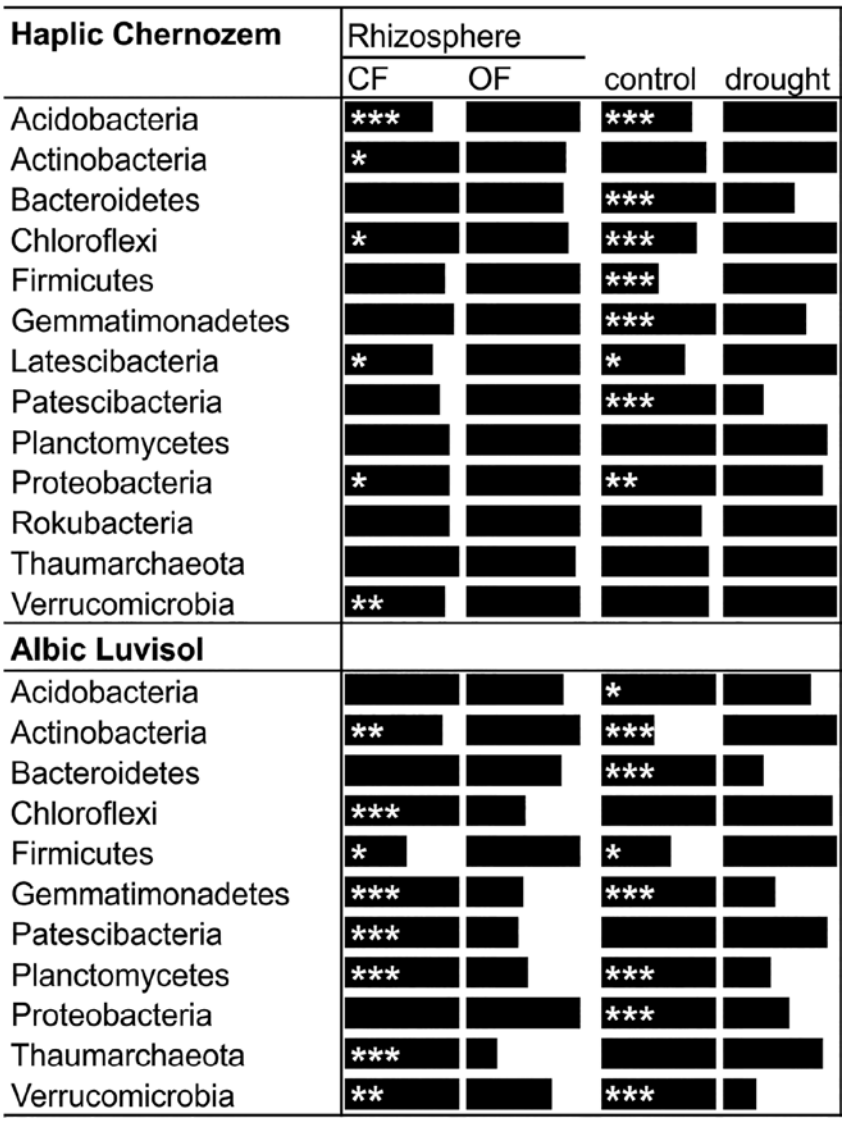


Fig 2. Bars showing the relative abundances of phyla in the rhizosphere of 'Dichter' at the harvest (separated by the two soil types). ANOVA was applied to test for differences between farming systems – conventional farming (CF) versus organic farming (OF) – and watering regimes – control versus drought – in the rhizosphere: ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$.

Chernozem (Fig. 1D; Table S3). Thereby, relative abundances of Acidobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Proteobacteria and Verrucomicrobia declined, but Actinobacteria and Firmicutes increased under drought. Interestingly, some of the phyla whose abundance increased – Actinobacteria and Firmicutes – or decreased – Gemmatimonadetes, Planctomycetes and Verrucomicrobia – under drought, also increased or decreased in the same manner in organic compared to conventional farming (Figs 2 and S2). Moreover, the relative abundances of drought nonresponsive phyla Chloroflexi, Patescibacteria and Thaumarchaeota were lower in the organic than in the conventional treatment (Fig. 2). Independent of watering regime and farming system, relative abundances of phyla Acidobacteria, Gemmatimonadetes and partly of Chloroflexi were lower in rhizosphere samples compared to bulk soil (Table S3). We further compared treatment effects on diversity indices of the two most dominant phyla, Proteobacteria and Actinobacteria in the rhizosphere (Figs 3 and S3). Overall, we observed a decline of their relative Shannon diversity and evenness values under drought, as well as from start to harvest, in both soil types. These effects were more pronounced in Luvisol than Chernozem. One exception of this pattern was found for Actinobacteria diversity in the Chernozem, which increased by trend under drought in the rhizospheres of both wheat cultivars (Figs 3 and S3). The effect of farming on diversity indices was prominent in Luvisol (ANOVA, $p < 0.001$, CF > OF) but not in Chernozem.

Functional adaptation of the bacterial communities to drought

Functional adaptation of the microbial community was estimated by measuring activity potentials of microbial enzymes involved in carbon cycling, and the abundances of drought stress-responsive functional genes in the rhizosphere using functional predictions by Tax4Fun.

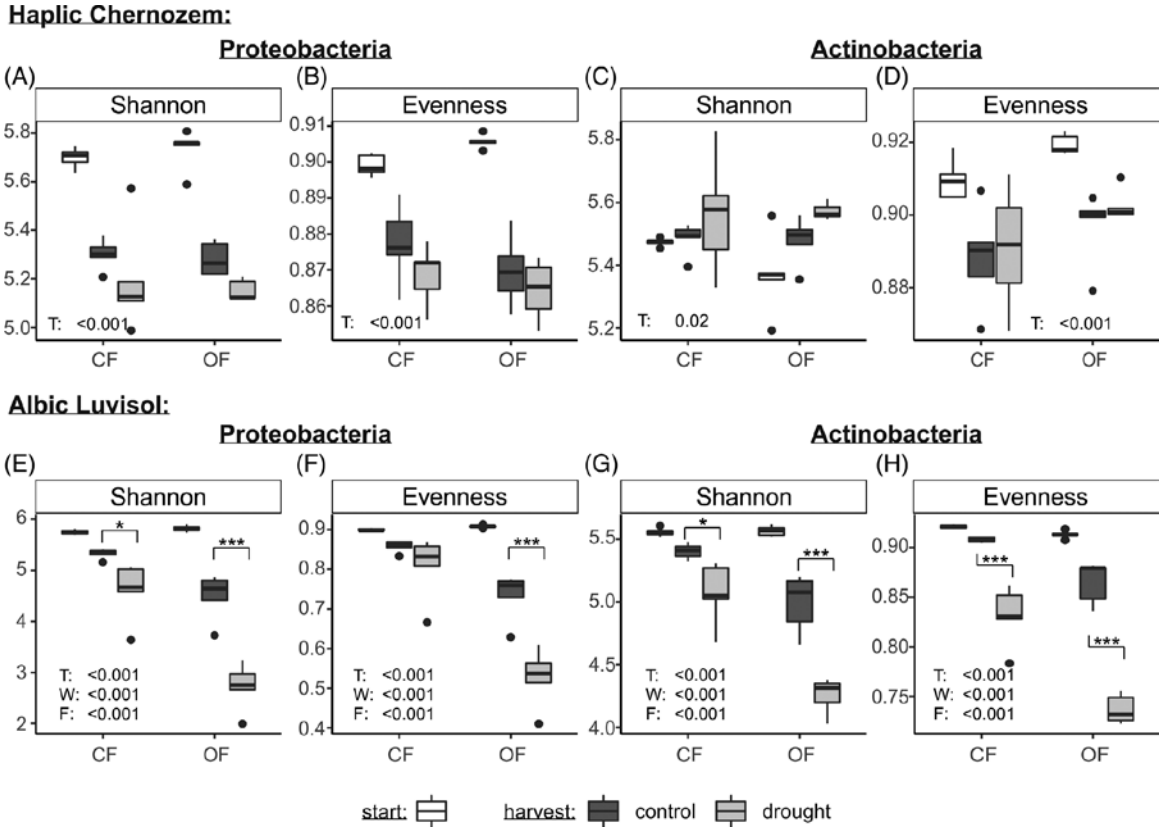


Fig 3. Alpha-diversity indices for Proteobacteria (subfigures A, B, E, F) and Actinobacteria (subfigures C, D, G, H) in the rhizosphere of 'Dichter' in dependency of sampling time (T), watering regime (W) and farming system (F) (each treatment, $n = 5$). Panels A–D and E–H illustrate differences in Chernozem and Luvisol respectively. Significant differences induced by the treatments are given in each panel as *p*-values (ANOVA). Significant differences within treatments are marked according to TukeyHSD as following: ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$.

Environmental Microbiology, 23, 5866–5882

Enzyme activities of cellulases, glucosidases and xylosidases were higher in Chernozem than in Luvisol at harvest (ANOVA, $p < 0.001$ for all three enzymes). Besides, we determined higher enzyme activities at the harvest compared to starting conditions in Chernozem soil, while the opposite pattern was observed in Luvisol soil (Figs 4 and S4). At harvest, glucosidase and cellulase activities were significantly higher in conventional farming treatments than in organic farming treatments for Chernozem (Figs 4 and S4). In the Luvisol, we detected the same farming system effect on all three measured enzyme activities (Figs 4 and S4). The effect of drought on enzyme activities was stronger for 'Dichter' (Fig. 4) than for 'RGT-Reform' (Fig. S4). For 'Dichter', enzyme activities were lower under drought conditions in Chernozem and for cellulase activity in Luvisol. In contrast glucosidase and xylosidase activities were increased by drought in the latter soil type (Fig. 4). Interaction effects between watering and farming system were significant for glucosidase, cellulase and xylosidase activities in the Luvisol. Thereby, enzyme activities were increased under drought conditions in the organic farming soil and did not differ between watering treatments in the conventional soil (Fig. 4). In pots with 'RGT-Reform', drought negatively affected xylosidase and glucosidase activities in the Chernozem but promoted glucosidase activities in Luvisol (Fig. S4). Instead of a general drought effect on cellulase activity in Chernozem, a significant interaction between watering and farming system occurred: cellulase activity reduction was confined to organic farming soil (Fig. S4B).

The Tax4Fun-predicted abundances of drought-related functional genes in the rhizobacterial community at harvest were related to the abundances of genes in the starting community. Increases and decreases showed a soil type and farming-specific pattern and were comparable for both wheat cultivars (Table 3). Among the functional genes that are supposed to be more abundant under drought, only genes of dextransucrase and fructan beta-fructosidase showed increased abundances in the communities of both soil types. The increase of these genes was most prominent in the Luvisol with organic farming management. In contrast, genes related to biofilm production, as well as ACC-deaminase production were surprisingly more abundant in communities of well-watered soils, irrespective of soil type and farming system (Table 3). Functional genes related to glutathione, spermidine and butanediol degradation were more abundant under control conditions, which was in line with our expectations (Table 3).

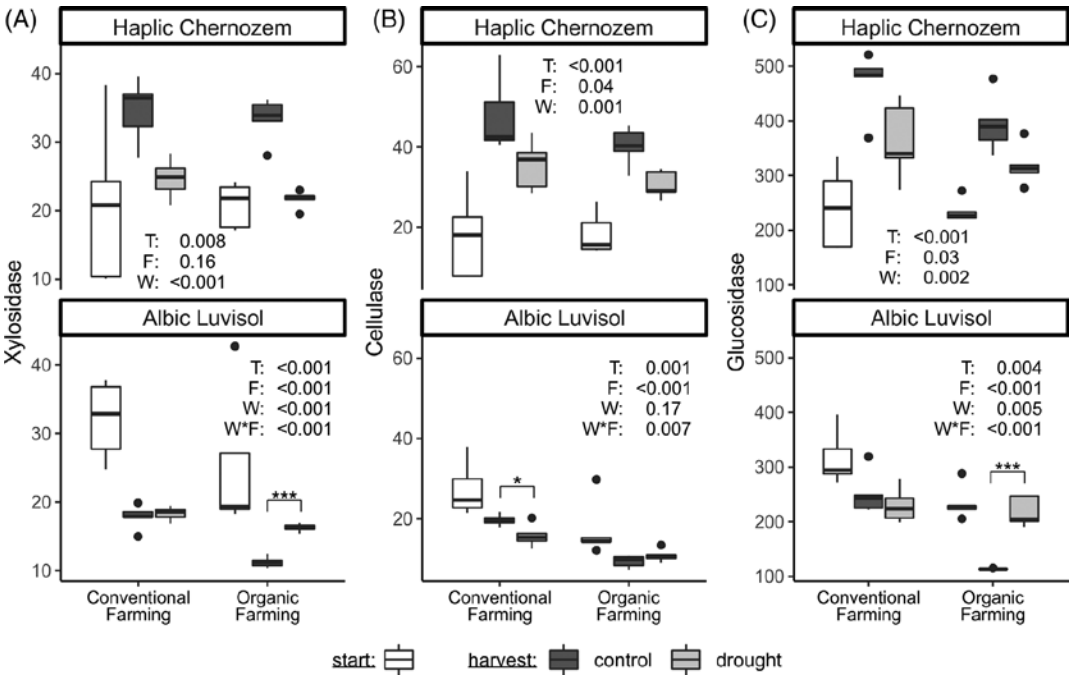


Fig 4. Activity potentials of C-cycling enzymes in the rhizosphere of wheat compared to starting conditions without plants.
(A) Xylosidase, (B) cellulase and (C) glucosidase activities are presented for the wheat cultivar 'Dichter'. Significant impacts of experimental factors were calculated using ANOVA and are given as *p*-values for each enzyme and soil type. Thereby, sampling time effect (T) was tested between start and harvest, while watering (W) and farming (F) effects, as well as interaction of the two factors, were evaluated at harvest. Significant interaction effects are given as follows: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

Environmental Microbiology, 23, 5866–5882

Table 3. Abundances of bacterial functional genes related to drought stress response of plants.

		Haplic Chernozem				Albic Luvisol			
		Conventional		Organic		Conventional		Organic	
		control	drought	control	drought	control	drought	control	drought
"Dichter"	Dext[1]	b	ab	ab	ab	ab	ab	ab	a
	Fruct[1]	b	ab	ab	ab	b	b	ab	a
	PGA[1]	c	bc	ab	bc	a	c	bc	c
	ACC[1]	b	b	a	b	b	b	b	b
	Buta[2]	b	b	a	b	ab	b	ab	b
	Sper[2]	b	ab	a	ab	ab	ab	ab	ab
	Glut[2]	bc	bc	a	bc	b	c	bc	c
"RGT-Reform"	Dext[1]	ab	ab	ab	ab	b	a	ab	a
	Fruct[1]	ab	a	ab	a	b	a	ab	a
	PGA[1]	a	ab	ab	ab	a	ab	ab	b
	ACC[1]	a	ab	ab	ab	ab	a	ab	b
	Buta[2]	a	ab	ab	ab	ab	ab	a	b
	Sper[2]	ab	ab	ab	ab	ab	a	ab	b
	Glut[2]	a	a	a	ab	ab	a	ab	b

Blue bars indicate an increase and red bars a decrease of functional genes at harvest compared to starting community. Significant differences of gene abundances between treatments (i.e. in each row) are indicated by different small letters (Tukey's HSD). Dext: dextransucrase, Fruc: fructan beta-fructosidase, PGA: biofilm PGA synthesis lipoprotein, ACC: 1-aminocyclopropane-1-carboxylate deaminase, Buta: (R,R)-butanediol dehydrogenase, Sper: spermidine dehydrogenase, Glut: glutathione reductase (NADPH).
X[1] supposed to be enriched under drought conditions.
X[2] supposed to be decreased under drought conditions.

Discussion

Performance of wheat is impaired by drought

Even though we selected wheat cultivars with different site requirements, growth performance was equally impaired by drought for both cultivars. This may be related to comparable cultivar properties that are relevant for the specific treatments that were used in our study. In contrast, Shao *et al*. (2005) found strong differences in the capability of 10 different wheat cultivars to cope with drought, which were related to the origin of cultivation and individual water stress thresholds of the wheat cultivars. We observed a decrease in water use per biomass production under drought conditions. It was shown that the reduction in transpiration and also turgor leads to a decrease in nitrate uptake and induces N deficiency (Ferrario-Méry *et al*., 1998; Xu and Zhou, 2006). In parallel, sugars accumulate in leaves to avoid dehydration-induced tissue damage (Nio *et al*., 2011; Reguera *et al*., 2013). In line with this, we found significantly higher C to N ratios in wheat straw under drought conditions. Interestingly, we detected the same pattern for the C to N ratio of grain. In wheat grain, carbon is stored in form of starch making up to 75% of the final dry weight (Hurkman *et al*., 2003). Severe drought conditions were found to decrease not only grain weight but also starch accumulation in wheat endosperm (Yu *et al*., 2016; Zhang *et al*., 2017), which would suggest lower grain C to N values under drought conditions. The contrasting pattern, observed in our study, may be related to impaired protein storage in grains under drought. The grain C to N ratio was at its lowest in OF wheat, suggesting severe N limitation. Overall, our results indicate that lower fertility of organic farming soil enhances negative drought effects on wheat performance. However, since most of the currently available wheat cultivars were bred with the focus on high yields, these cultivars have a lack of functional traits to perform well in low-input agroecosystems (Van Bueren *et al*., 2011).

The lower water use per gram biomass may suggest a higher water use efficiency of wheat plants in Chernozem soil than in Luvisol soil. Under field conditions, the high water retention capacity of the Chernozem soil offers a more favourable environment for wheat growth in the field (Altermann *et al*., 2005) than sandy soils that allow a fast run-off and transpiration of water (Schweitzer, 2010). However, by using pots watered from below, we

prevented the loss of water and nutrients and stimulated the growth of winter wheat in Luvisol. Higher water use per gram biomass in Luvisol than Chernozem might be related to faster root growth (Iannucci *et al*., 2017), enabling wheat to reach water and mobile nutrients in Luvisol sooner (Liu *et al*., 2017). Moreover, low clay content of Luvisol (3%) leads to less frequent bonding of nutrients to soil matrix than in Chernozem (20%) (Altermann *et al*., 2005; Schweitzer, 2010). The exorbitant use of water to produce biomass indicates a much higher vulnerability to drought in Luvisol compared to Chernozem.

Changes in bacterial community assemblage in response to soil type and farming system under drought

In both soil types, drought-induced shifts in the bacterial composition, which were more pronounced in the rhizosphere than in bulk soil. So far, drought is considered to have only minor direct impacts on diversity but larger impacts on composition of the soil microbiome (Acosta-Martinez *et al*., 2014, discussed in Naylor and Coleman-Derr, 2018). However, it is well known that plants closely interact with microbes competing for water and nutrients in the rhizosphere (Schimel and Bennett, 2004; Mendes *et al*., 2013; Philippot *et al*., 2013) and, thereby, foster specific selection of bacteria from the surrounding diverse bulk soil communities (Bell *et al*., 2014; Fan *et al*., 2017). Moreover, drought induces both, direct changes of the soil microbial community and indirect changes that are caused by alterations in plant physiology and biochemistry (reviewed in Naylor and Coleman-Derr, 2018). The additive microbial response to direct and indirect drought effects may explain the stronger impact of drought on the rhizosphere community, compared to that of bulk soil in our study.

Generally, it is described that drought reduces the relative abundances of fast-growing Gram-negative bacterial phyla, such as Proteobacteria, Verrucomicrobia, Gemmatimonadetes and Bacteroidetes, which predominantly exhibit a copiotrophic lifestyle. Instead, relative abundances of slow-growing Gram-positive bacterial phyla, e.g. Actinobacteria and Firmicutes, will be fostered, which are predominantly related to an oligotrophic lifestyle (as discussed in Naylor and Coleman-Derr, 2018). Correspondingly, we found that the relative abundance of Gram-negative Proteobacteria in the wheat rhizosphere decreased upon drought, while Actinobacteria prevailed under drought conditions, especially in Luvisol soil. This supports our assumption made in hypothesis one, that the abundances of drought-tolerant bacteria will be promoted by water limitation. Actinobacteria account for a large proportion of bacteria identified in the rhizosphere of wheat (Yadav *et al*., 2018), and their drought tolerance, as well as their potential to mitigate drought stress, has already been proven (Yandigeri *et al*., 2012; Singh *et al*., 2018). In addition, we found an enrichment of the phylum Firmicutes under drought in the rhizosphere, which may support plant performance under these unfavourable conditions (discussed in Naylor and Coleman-Derr, 2018, Xu *et al*., 2018).

However, Gram-negative phyla were also shown to play a role in plant drought tolerance. Two studies investigated the effects of drought on the rice and cotton rhizobiome and revealed that Chloroflexi species dominated under drought conditions (Santos-Medellín *et al*., 2017; Ullah *et al*., 2019). We also observed a drought-induced increase of Chloroflexi in Chernozem and no effect of drought in Luvisol, suggesting a general drought response by this phylum. For Acidobacteria species, the response to drought was dependent on the soil type, with a relative enrichment of species in Chernozem and a decrease in Luvisol. Acidobacteria species have been described as oligotrophs, favoured in soils with lower carbon availability (Fierer *et al*., 2007). Extreme drought significantly increased the relative abundance of Acidobacteria in a subtropical evergreen forest soil (Bu *et al*., 2018) but reduced their abundance in rice rhizobiome (Santos-Medellín *et al*., 2017). Differences in drought responses between different studies could be related to different levels of drought stress, other plant species, whether bulk soil or rhizosphere was studied, and varying soil properties. In line with our study, the importance of soil-specific properties was supported by a study along a precipitation gradient of Mediterranean forest sites. It showed that the relative abundance of Acidobacteria correlates positively with soil ammonium and phosphorus and negatively with nitrate and magnesium levels (Bachar *et al*., 2010). These results underline the central role of environmental parameters for understanding the rhizobiome response to drought.

Effects of drought on bacterial community composition were more prominent in Luvisol than in Chernozem, which is in agreement with our second hypothesis. The relative abundance of rhizosphere Actinobacteria was only minor affected by drought in Chernozem, but their diversity and evenness increased under drought. In contrast, drought responses for Luvisol included a strong increase of the relative abundance of rhizosphere Actinobacteria accompanied by a significant decrease in diversity indices. Especially the decrease in evenness and Shannon diversity indicate a less diverse, actinobacterial community with only few dominating species. A higher diversity and species richness are assumed to be beneficial to cope with disturbances and stress due to higher metabolic capacities (Nautiyal and Dion, 2008). These biological indices are usually strongly associated

with soil organic matter content and composition (Fierer *et al*., 2003; Delgado-Baquerizo *et al*., 2017), which implicates that they can be steered by agricultural management. Indeed we found for Luvisol, that lower P, K and C contents in OF soil were accompanied by dramatically lower actino- and proteobacterial diversity. This finding contradicts our third hypothesis, which stated that lower nutrient availability fosters a more diverse community with a possibly broader functional repertoire. In Chernozem, C and nutrient contents were comparable between CF and OF soils, which may explain the comparable diversity patterns observed for these two predominant phyla.

Functional community adaptations to drought are influenced by soil properties and wheat cultivar

Functional community adaptations are important for the capacity of the microbiome to buffer drought stress for the wheat plants. Since the soil sampling was conducted at wheat harvest, the results do not necessarily reflect direct links between the microbial community and wheat performance under drought but provide indications for those.

In Chernozem, the enzyme activity potentials were reduced under drought. In general, prolonged drought stress inhibits microbial carbon turnover by suppressing microbial biomass, thereby reducing the availability of substrates and inhibiting microbial metabolism (Naylor and Coleman-Derr, 2018). To our surprise, drought increased xylosidase and beta-glucosidase activities in the rhizosphere of the cultivar 'Dichter' under organic farming treatment in Luvisol soil, which is against this expected pattern, but partly supports our third hypothesis. In line with this finding, Mganga *et al*. (2019) and Sanaullah *et al*. (2011) also observed increased beta-glucosidase and cellulase activity under drought in the rhizosphere of grasses and suggested that this might be related to higher root turnover or altered rhizodeposition patterns. Indeed it has been shown that under drought an increased proportion of plant assimilates are allocated to the roots (Palta and Gregory, 1997), which changes root exudation and limits biomass production. Since this altered C allocation is not desirable for agricultural production, great efforts have been made on breeding wheat cultivars that can cope with unfavourable conditions and exhibit higher drought stress resistance (Bodner *et al*., 2015). The cultivar 'RGT-Reform' thus may comprise properties that allow to buffer drought stress, while 'Dichter' may be more dependent on the support of the rhizobacterial community to cope with drought stress. Since the effect was exclusively observed in organic farming soil, the contrasting responses to drought may also be caused by the use of fungicide-treated seeds in conventional farming systems versus untreated seeds in organic farming systems. However, due to the general bacteria-dominated food webs in agricultural systems (de Vries *et al*., 2013), as well as the high differences in nutrient availability between the two farming systems and soil types, we consider fungicide treatment of seeds as a comparable minor impact.

We further applied Tax4Fun predictions to estimate functional adaptations of the rhizobacterial community. We predicted an enrichment of functional genes encoding for enzymes involved in complex carbohydrate degradation under drought, especially in that of dextran and fructan. Using metagenomic sequencing, Martiny *et al*. (2017) have shown that changes in precipitation altered the potential for bacterial carbohydrate degradation. This supports our findings and reveals possible adaptation mechanisms of the communities to cope with drought stress conditions. We further investigated a set of functional genes involved in bacterial-mediated drought stress tolerance of plants. Biofilm production in the rhizosphere is often associated with systemic resistance against plant pathogens (Timmusk and Nevo, 2011), but it has also been identified as a response to drought conditions (Kim *et al*., 2012). Besides, ACC deaminase is a well-known marker for direct drought stress response in the rhizosphere of plants, as it controls expression of the plant stress hor- mone ethylene (Glick, 2005). Contrary to our expectation, the predicted abundances of functional genes involved in biofilm and ACC deaminase production were reduced under drought. Genes encoding for spermidine and (R,R)-butanediol dehydrogenase, as well as glutathione reductase that lower the spermidine, butanediol and glutathione levels respectively, decreased, even though not significantly, under drought. This was in line with our expectations, since higher levels of these genes and thus enzyme production have been associated with plant drought tolerance (Cho *et al*., 2008; Kasim *et al*., 2013; Zhou *et al*., 2016). These results suggest that among the members of the rhizobacterial communities in our study we found specific functional adaptations as key for drought tolerance.

Interactions of factors drive microbial community responses to drought

Several studies highlight the overall negative effect of drought on soil microbial community structure and function. However, such studies often deny the interplay of different factors contributing to the community response pattern. For instance, Hueso *et al*. (2012) observed that the amendment of organic manure confers a higher resistance on the microbial community to deal with drought compared to communities in unamended soils. In our study, we revealed soil type-, farming system- and wheat

cultivar-specific responses of the microbial community to drought. Changes of the relative abundances of several phyla were highly treatment specific. Moreover, we observed under drought conditions a strong decrease of Actinobacteria diversity indices in Luvisol soil, while the same indices were not affected or by trend even increased in Chernozem soil. Besides structural parameters, we observed treatment-specific responses of soil enzyme activities upon drought. Only for the combination of Luvisol soil × organic farming system × cultivar 'Dichter' an increase of soil enzyme activities was detected. Song *et al*. (2012) investigated the response of soil enzymes and root exudates from corn hybrids with different tolerance to drought at different growth stages. Despite the overall higher enzyme activities for the drought-tolerant hybrid along all growth stages, the relative importance of growth stage and/or water stress differed between the hybrids and varied among the enzyme classes measured. Therefore, the interacting effects of different abiotic and biotic factors play a crucial role to understand drought-induced soil microbial community responses. Clearly, more factors and levels of factors would be desirable to catch the broad spectrum of interactions influencing rhizobacterial community responses. On the other hand, a high number of factors also aggravate data interpretation. Thus, our approach comprises a selection of relevant levels of each factor that mainly influence wheat performance in agricultural systems.

Conclusions and outlook

The various interacting effects of the different treatment levels in our multifactorial approach revealed context-dependent responses at structural and functional levels of the bacterial communities in the wheat rhizosphere. Structural and partly functional adaptations occurred within a vegetation period. While drought-induced shifts in community structure were strongly pronounced and differed mainly between different soil types and farming systems, the functional potential of the respective rhizobiomes to mitigate drought stress was more dependent on the wheat cultivar and the interacting effects of soil type and farming system. These results suggest that a specific pool of functional traits in the bacterial community under drought and with respect to different surrounding conditions may be more relevant, than the diversity and structure of the microbial community itself.

While Zia *et al*. (2021) provide general implications of rhizosphere management strategies to mitigate drought stress in agricultural systems, the review of Kavamura *et al*. (2021) even goes a step further being more specific on winter wheat. Using multifactorial and multidisciplinary approaches, the identification of a specific wheat core microbiome with a certain pool of functions may be the key towards the development of microbiome-facilitated sustainable wheat production. With our work, we contributed to these aims with a particular focus on drought selection of rhizosphere microbiota – an ever-increasing process in Central European ecosystems.

Material and methods

Design of pot experiment and sampling

We selected two winter wheat cultivars, which differed in their site suitability (Landesamt für Umwelt, Landwirtschaft und Geologie, Sachsen-Anhalt, Sortenempfehlung 2016, Winterweizen). Cultivar 'RGT-Reform' (RGT, A-quality, approved 13th of March 2014, Société RAGT 2n, France) is recommended for a wide variety of agricultural production sites, whereas cultivation of 'Dichter' (D, A-quality, approved 10th of March 2014, Saatzucht Josef Breun GmbH & CO. KG, Germany) is recommended only for sites with favourable soil properties and climatic conditions. We thus classified the two cultivars as either non-demanding ('RGT-Reform') or demanding ('Dichter'), also with respect to water availability. The cultivars were grown on two soil types, which fundamentally differed in their properties: a loamy Haplic Chernozem with high humus and nutrient contents (Altermann *et al*., 2005), and a sandy Albic Luvisol with low water-holding capacity and nutrient contents (Schweitzer, 2010). Chernozem and Luvisol soils were collected in September 2016 from experimental field research stations in Bad Lauchstädt (Saxony-Anhalt, Germany; $51^{o}23^{0}$ N $11^{o}52^{0}$ E, 118 m a.s.l.) and in Thyrow (Brandenburg, Germany, $52^{o}16^{0}$ N, $13^{o}12^{0}$ E, 40 m a.s.l.) respectively. At both sites, the upper 15 cm of topsoil were collected from conventional and organic farming plots of experimental field platforms (Bad Lauchstädt: GCEF, established in 2013, (Schädler *et al*., 2019); Thyrow: demonstration system for arable farming systems, established in 2005).

To define initial properties for the four soils (two soils × two farming managements, further referred as 'original soil' in the text or as 'start' in graphs and tables), pH as well as the contents of C, N, P and K were determined. Additionally, the maximum water holding capacities (WHC_{max}) were determined experimentally for both soil types. For this purpose, soil was soaked with water until saturation. The starting weight and the soil moisture at saturation were used to calculate the amount of water taken up by the specific soil type in percentage.

For the pot experiment, 9.3 kg for Chernozem or 11.3 kg of Luvisol were weighed in bags, mixed with fertilizers and water (60% of soil type–specific WHC_{max}) and filled in 7 L Kick–Brauckmann pots (STOMA GmbH, Siegburg, Germany). The applied fertilizers were selected in accordance with the guidelines for conventional and

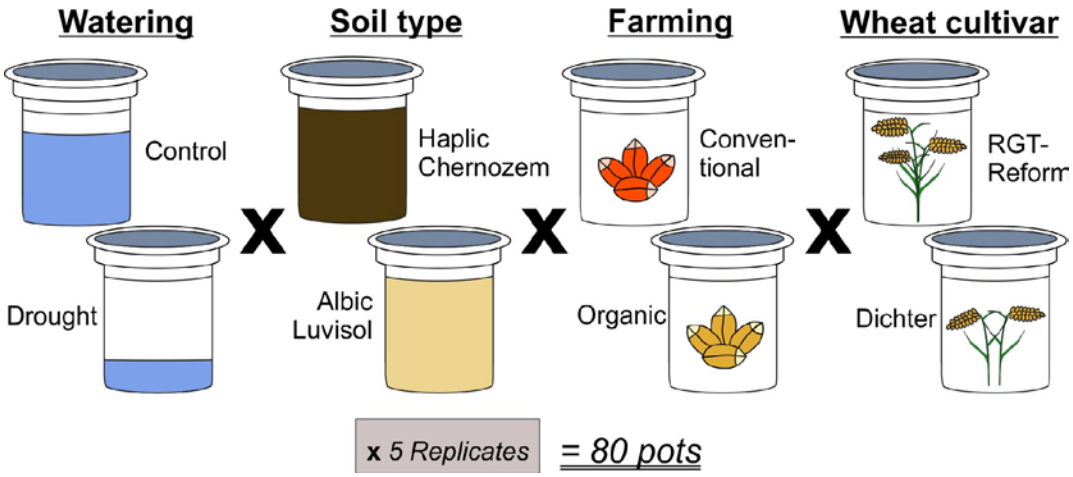


Fig 5. Experimental design including four factors: watering, soil type, farming system and wheat cultivar. Watering was identical for all pots from sowing (October 2016) until the tillering stage (March 2017). Drought was applied during the growing season (March 2017–July 2017).

organic farming (Table S4). Both conventional and organic farming pots were fertilized with 2 g N, 1 g P and 2 g K. The addition of 0.5 g Mg ($MgSO_4$), 0.15 g $FeCl_3$ and a mixture of micronutrients (A–Z solution by Hoagland and Snyder, 1933) was exclusive for conventional farming pots. For each treatment combination five replicates were prepared, which ended up in a total number of 80 pots (Fig. 5).

In October 2016, winter wheat was sown with either 16 fungicide-treated seeds per pot for the conventional farming treatment or 16 untreated seeds per pot for the organic farming treatment. The pots were randomly placed on trolleys in a cold greenhouse and kept at 60% of WHC_{max} over the winter 2016/17. In March 2017, plants were thinned and equally adjusted to a number of 12 per pot. After that, watering was stopped for half of the pots until they reached 25% of soil type-specific WHC_{max}. Subsequently, the watering regimes – 25% and 60% of WHC_{max}, reflecting limited and ample watering conditions – were maintained. The water contents of the pots were checked daily by weighing and adjusted to the targeted water content. Water was added to the cache pot in order to avoid leaching of nutrients by top irrigation and the amount of added water was recorded for each pot. During harvest in July 2017, we collected soil and wheat rhizosphere samples. For the measurement of soil abiotic parameters, five soil cores were taken from each pot, pooled, sieved to 2 mm and frozen at -20 °C. Subsequently, the roots of all plants were harvested and loose soil was removed by shaking. The remaining soil closely attached to the roots (defined as rhizosphere soil) was collected for functional and structural community analysis and stored at -80 °C.

Plant and abiotic soil parameters

Fresh and dry (60 °C, 24 h) weights of wheat were recorded for each pot at harvest. Water use by wheat plants (ml water/g biomass) was estimated for each pot, based on the total amount of added water and the final aboveground biomass. Dried straw and corn were grained to powder and total carbon and nitrogen contents were measured using an elemental analyser (Elementar Vario EL III, Elementar, Hanau, Germany).

Soil mineral nitrogen (nitrate and ammonium) was measured per flow injection analysis (FlAstar 5000, Foss GmbH, Rellingen, Germany). Briefly, 5 g of fresh soil was suspended in 20 ml of 1 M KCl solution, shaken for 1 h on a horizontal shaker and filtered through 0.45 µm cellulose nitrate filter (Sartorius Biolab Products, Göttingen, Germany). According to Schulz (2002), hot water extractable carbon and nitrogen from air-dried soil samples were measured using an elemental analyser for liquid samples (Multi N/C, Analytik Jena, Germany). Available phosphate in the soil was extracted with double lactate solution (1:50 wt./vol.) at pH 3.6 and phosphate concentrations were determined colorimetrically using the molybdenum blue method (Murphy and Riley, 1962). A halogen moisture analyser (Mettler Toledo, Gießen, Germany) was used to determine gravimetric soil water contents. For pH measurements, 12 g of air-dried soil was suspended in 30 ml of 0.01 M $CaCl_2$, shaken for 1 h and measured with pH electrode.

Soil enzyme activities

A modified fluorometric assay of Sinsabaugh *et al.* (2003) was used to determine potential bulk soil and rhizosphere

Environmental Microbiology, 23, 5866–5882

extracellular enzyme activities. The activity potentials of three hydrolytic soil enzymes involved in carbon acquisition (β-glucosidases, xylosidases and cellobiohydrolases) were measured as turnover rate of 4-methylumbelliferon (MUF)-coupled substrates (Table S5). The amount of released, fluorescent MUF can directly be related to enzymatic activity potentials. The substrate concentration (300 µM for all substrates) applied has been optimized for the Chernozem soil of Bad Lauchstädt and was also used for the Luvisol for proper comparison of the enzymatic activity between soil types. The activity potentials were measured for bulk soil samples at germination and harvest, as well as for rhizosphere soil at harvest.

For each sample, a separate black 96-well microplate was prepared. The plates contained all three substrates, MUF dilutions to calculate quench and extinction coefficients (1.25 and 2.5 µM), as well as substrate and soil suspension controls. Approximately 250 mg of fresh soil sample was suspended in 50 ml of 50 µM acetate buffer (pH 5) for analysis. To break up soil aggregates, the soil suspensions were sonicated for 5 min, then transferred to the prepared microplates and incubated at 25 °C for 60 min. The addition of 30 µl 1 M NaOH solution stopped the enzymatic reactions. Subsequently, fluorescence was measured for eight replicates after another 3 min using an Infinite 200 PRO instrument (Tecan Group, Männedorf, Switzerland) with 360 nm excitation and 465 nm emission filters. Enzyme activities are provided as turnover rate of substrate in nmol per gram dry soil and hour (nmol g $soil^{-1}$ h^{-1}) (German *et al*., 2011).

DNA extraction and next-generation sequencing (Illumina MiSeq)

DNA was extracted from original soil samples from September 2016 as well as from bulk and rhizosphere soil samples taken at the harvest of wheat in July 2017. The DNeasy PowerSoil kit (QIAGEN, Hilden, Germany) was used to extract DNA from 400 mg rhizosphere and bulk soil following the manufacturer's instructions. The approximate concentrations and quality of extracted DNA were analysed with a NanoDrop ND-8000 spectrophotometer (Thermo Fischer Scientific, Dreieich, Germany). Until amplification DNA was stored at -20 °C. Using the universal primer pair 515f and 806r (Caporaso *et al*., 2011), which was equipped with Illumina adapter sequences, the bacterial and archaeal 16S rRNA gene V4 region was amplified. Proofreading KAPA HiFi polymerase (KAPA Biosystems, Boston, MA, United States) ensured correct amplification of the sequences and running amplification in three repetitions to minimized amplification biases. The following program was used to run PCR: initial denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 98 °C for 20 s, annealing at 55 °C for 15 s and elongation at 72 °C for 15 s and final extension at 72 °C for 5 min.

PCR products were examined by gel electrophoresis, the three repetitions pooled and a first clean up performed using the Agencourt AMPure XP kit (Beckmann Coulter, Krefeld, Germany). In a second PCR, Illumina Nextera XT indices were attached to both ends of the bacterial fragments. The combination of indices is unique and assigns the sequences to the respective samples. The following program was used to run index PCR: initial denaturation at 95 °C for 3 min, followed by 8 cycles of denaturation at 98 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s and final extension at 72 °C for 5 min. In a second clean-up, PCR products were purified using AMPure beads. The final concentration of amplified DNA was quantified with the PicoGreen assay (Molecular Probes, Eugene, OR, United States). Bacterial amplicon libraries were set to defined volumes (corresponding to 60 ng DNA for each sample) to reach an equimolar representation of each sample in the sequencing approach. The libraries were pooled in one tube and again checked for their quality with an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, United States). Following the protocol of the MiSeq v3 reagent kit, sample libraries and PhiX control libraries were denatured and diluted to a final concentration of 10 pM, combined to a volume of 600 µl (30 µl of PhiX control library and 570 µl of bacterial amplicon library) and loaded onto MiSeq v3 reagent cartridge for sequencing. Paired-end sequencing of 2 × 300 bp was implemented on an Illumina MiSeq platform (Illumina, San Diego, CA, United States) at the Department of Soil Ecology of the Helmholtz-Centre for Environmental Research (UFZ, Halle/Saale, Germany). Demultiplexed sequences are accessible in the Sequence Read Archive under the Bioproject PRJNA680908.

Bioinformatics workflow

Demultiplexed sequences were processed using the house-intern pipeline dadasnake (Weißbecker *et al*., 2020), which uses the open-source program R's (v. 3.6.1; R Core Team 2017) DADA2 package (Callahan *et al*., 2016) in snakemake (Köster and Rahmann, 2012). 16S rDNA amplicon reads were cut and filtered using default settings of the pipeline with exception of truncation quality (set to 9) and maximum expected error (set to 0.5). Read pairs were merged with a minimum overlap of 12 bp and zero mismatches, and chimeric reads removed using the consensus algorithm. For taxonomical classification of the 16S rDNA gene amplicon sequences, the mothur implementation of the Bayesian Classifier (Schloss *et al*., 2009) and, as a follow up in the case of a

missing classification, BLASTn were applied, referring to the SILVA database (version 132, non-redundant at 99%; Quast *et al*., 2012). The final output was comprised of an OTU table with taxonomic classifications for all samples.

Functional gene abundances and normalization

The package Tax4Fun (R, Aßhauer *et al*., 2015) was used to predict functional capabilities of the bacterial communities. The approach is based on 16S rDNA datasets generated by next-generation sequencing and the cross-comparison with KEGG orthology (KO) database (Kanehisa and Goto, 2000). Running the Tax4Fun command line in R, the latest supported version of SILVA database was used (SILVA123, released July 2015) (Quast *et al*., 2012). The output table contained all functional gene abundances found in the community and provided KO numbers for gene annotations and Enzyme Commission (EC) number as object identifier for enzymes. The enzymes of interest involved in direct and indirect drought stress resistance were identified and selected by KO numbers and EC numbers (Table S6). Gene abundances of the selected enzymes were corrected with bacterial biomass, which was obtained for each sample using qRT-PCR. Briefly, a standard curve of pure bacterial genomic DNA was prepared from a *Phyllobacterium* isolate (ranging from 0.5 to 4 ng μl^{-1} genomic DNA). Environmental samples were diluted to 2 ng μl^{-1}. Measured C_t-values of environmental samples were related to the standard curve to estimate the amount of bacterial DNA in the environmental sample. Obtained bacterial DNA concentrations, including the dilution factor, were used for normalization of functional gene abundances.

Statistics

All statistical analyses and visualizations were performed in R (R Core Team, 2019). The output table of the dadasnake pipeline containing OTUs and taxonomy was loaded into R. In preparation, the dataset was cleaned from sequences assigned to mitochondria and chloroplasts, as well as from unassigned sequences. Furthermore, singletons were removed. Using the phyloseq package in R (McMurdie and Holmes, 2013), the OTU and taxonomy table, as well as the sample file with descriptions of the sample sites, were merged to a phyloseq object and used for further analysis. To correct different read numbers between the samples, the dataset was rarefied to the sample with minimum read number, sample B08 with 45 925 reads, and this resulted in a total number of 23 848 OTUs. PerMANOVA (vegan package, Oksanen *et al*., 2013) was used to indicate impacts of experimental factors watering regime, farming system, soil type, sampling time point, soil compartment and wheat cultivar on community composition. Diversity indices of observed species number, Shannon diversity and evenness of the community were calculated using phyloseq package. Influence of experimental factors on plant and soil parameters, diversity indices, enzyme activities and functional genes were examined using ANOVA followed by *post hoc* tests of Tukey's HSD. Single-sided *t*-test was applied to identify significant decreases in plant biomass production under drought compared to control conditions.

Acknowledgements

The work has been done in the frame of a scholarship funded by the 'Deutsche Bundesstiftung Umwelt' (DBU) with the topic 'Einfluss pflanzenwachstumsfördender Rhizobakterien auf die Stressresistenz der Kulturpflanze Weizen (*Triticum aestivum* L.)' (AZ:20015/391).

We thank the Helmholtz Association, the Federal Ministry of Education and Research, the State Ministry of Science and Economy of Saxony-Anhalt, and the State Ministry for Higher Education, Research and the Arts Saxony for funding the GCEF project. We thank the staff of the Bad Lauchstädt Experimental Research Station (especially Ines Merbach and Konrad Kirsch) and Martin Schädler for their work in maintaining the plots and infrastructures of the GCEF, and Stefan Klotz, Harald Auge and Martin Schädler for their roles in setting up the GCEF. A special thanks also to Michael Baumecker, leader of the experimental station in Thyrow, who provided us the Albic Luvisol, and Kerstin Hommel, who helped to set up and finally harvest the pot experiment. We would also like to thank the Helmholtz Association for its financial support for the sequencing costs. The sequencing data were analysed at the High-Performance Computing (HPC) Cluster EVE, a joint effort between the Helmholtz Centre for Environmental Research - UFZ and the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig. A special thanks thereby to Dr. Anna Heintz-Buschart, who set up, maintained and provided the pipeline for processing the sequencing data.

References

Acosta-Martinez, V., Cotton, J., Gardner, T., Moore- Kucera, J., Zak, J., Wester, D., and Cox, S. (2014) Predominant bacterial and fungal assemblages in agricultural soils during a record drought/heat wave and linkages to enzyme activities of biogeochemical cycling. *Appl Soil Ecol* 84: 69–82.

Altermann, M., Rinklebe, J., Merbach, I., Körschens, M., Langer, U., and Hofmann, B. (2005) Chernozem - soil of the year 2005. *J Plant Nutr Soil Sci* 168: 725–740.

Aßhauer, K.P., Wemheuer, B., Daniel, R., and Meinicke, P. (2015) Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. *Bioinformatics* 31: 2882–2884.

Azarbad, H., Tremblay, J., Giard-Laliberté, C., Bainard, L.D., and Yergeau, E. (2020) Four decades of soil water stress history together with host genotype constrain the response

of the wheat microbiome to soil moisture. *FEMS Microbiol Ecol* 96: fiaa098.

Bachar, A., Al-Ashhab, A., Soares, M.I.M., Sklarz, M.Y., Angel, R., Ungar, E.D., and Gillor, O. (2010) Soil microbial abundance and diversity along a low precipitation gradient. *Microb Ecol* 60: 453–461.

Bell, C., Carrillo, Y., Boot, C.M., Rocca, J.D., Pendall, E., and Wallenstein, M.D. (2014) Rhizosphere stoichiometry: are C: N: P ratios of plants, soils, and enzymes conserved at the plant species-level? *New Phytol* 201: 505–517.

Bodner, G., Nakhforoosh, A., and Kaul, H.-P. (2015) Management of crop water under drought: a review. *Agron Sustain Dev* 35: 401–442.

Bouskill, N.J., Wood, T.E., Baran, R., Ye, Z., Bowen, B.P., Lim, H., *et al*. (2016) Belowground response to drought in a tropical forest soil. I. Changes in microbial functional potential and metabolism. *Front Microbiol* 7: 525.

Breitkreuz, C., Buscot, F., Tarkka, M., and Reitz, T. (2020) Shifts between and among populations of wheat rhizosphere *Pseudomonas*, *Streptomyces* and *Phyllobacterium* suggest consistent phosphate mobilization at different wheat growth stages under abiotic stress. *Front Microbiol* 10: 3109.

Bu, X., Gu, X., Zhou, X., Zhang, M., Guo, Z., Zhang, J., *et al*. (2018) Extreme drought slightly decreased soil labile organic C and N contents and altered microbial community structure in a subtropical evergreen forest. *For Ecol Manage* 429: 18–27.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 13: 581–583.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., *et al*. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc Natl Acad Sci U S A* 108: 4516–4522.

Cho, S.M., Kang, B.R., Han, S.H., Anderson, A.J., Park, J.-Y., Lee, Y.-H., *et al*. (2008) 2R, 3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis O6*, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. *Mol Plant Microbe Interact* 21: 1067–1075.

Coleman-Derr, D., and Tringe, S.G. (2014) Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. *Front Microbiol* 5: 283.

De Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M., and Bardgett, R.D. (2012) Land use alters the resistance and resilience of soil food webs to drought. *Nat Climate Change* 2: 276–280.

De Vries, F.T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M.A., Bjørnlund, L., *et al*. (2013) Soil food web properties explain ecosystem services across European land use systems. *Proc Natl Acad Sci U S A* 110: 14296–14301.

Delgado-Baquerizo, M., Reich, P.B., Khachane, A.N., Campbell, C.D., Thomas, N., Freitag, T.E., *et al*. (2017) It is elemental: soil nutrient stoichiometry drives bacterial diversity. *Environ Microbiol* 19: 1176–1188.

DESTATIS. (2021) Statistisches Bundesamt. URL http://www.destatis.de (8th of April 2021).

Fan, K., Cardona, C., Li, Y., Shi, Y., Xiang, X., Shen, C., *et al*. (2017) Rhizosphere-associated bacterial network structure and spatial distribution differ significantly from bulk soil in wheat crop fields. *Soil Biol Biochem* 113: 275–284.

FAO. (2018) Food and Agriculture Organization. URL http://www.fao.org/ (3rd of November 2020).

Ferrario-Méry, S., Valadier, M.-H., and Foyer, C.H. (1998) Overexpression of nitrate reductase in tobacco delays drought-induced decreases in nitrate reductase activity and mRNA. *Plant Physiol* 117: 293–302.

Fierer, N., Bradford, M.A., and Jackson, R.B. (2007) Toward an ecological classification of soil bacteria. *Ecology* 88: 1354–1364.

Fierer, N., Schimel, J.P., and Holden, P.A. (2003) Variations in microbial community composition through two soil depth profiles. *Soil Biol Biochem* 35: 167–176.

Francioli, D., Schulz, E., Buscot, F., and Reitz, T. (2018) Dynamics of soil bacterial communities over a vegetation season relate to both soil nutrient status and plant growth phenology. *Microb Ecol* 75: 216–227.

German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., and Allison, S.D. (2011) Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biol Biochem* 43: 1387–1397.

Glick, B.R. (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiol Lett* 251: 1–7.

Goswami, D., Thakker, J.N., and Dhandhukia, P.C. (2016) Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. *Cogent Food Agric* 2: 1127500.

Hari, V., Rakovec, O., Markonis, Y., Hanel, M., and Kumar, R. (2020) Increased future occurrences of the exceptional 2018–2019 central European drought under global warming. *Sci Rep* 10: 1–10.

Hoagland, D.R., and Snyder, W.C. (1933) Nutrition of strawberry plant under controlled conditions. (a) Effects of deficiencies of boron and certain other elements, (b) susceptibility to injury from sodium salts. *Proc Am Soc Hortic Sci* 30: 288–294.

Hueso, S., García, C., and Hernández, T. (2012) Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils. *Soil Biol Biochem* 50: 167–173.

Hurkman, W.J., McCue, K.F., Altenbach, S.B., Korn, A., Tanaka, C.K., Kothari, K.M., *et al*. (2003) Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. *Plant Sci* 164: 873–881.

Iannucci, A., Fragasso, M., Beleggia, R., Nigro, F., and Papa, R. (2017) Evolution of the crop rhizosphere: impact of domestication on root exudates in tetraploid wheat (*Triticum turgidum* L.). *Frontiers in. Plant Sci* 8: 2124.

Kanehisa, M., and Goto, S. (2000) KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 28: 27–30.

Kasim, W.A., Osman, M.E., Omar, M.N., Abd El-Daim, I.A., Bejai, S., and Meijer, J. (2013) Control of drought stress in

wheat using plant-growth-promoting bacteria. *J Plant Growth Regul* 32: 122–130.

Kavamura, V.N., Mendes, R., Bargaz, A., and Mauchline, T. H. (2021) Defining the wheat microbiome: towards microbiome-facilitated crop production. *Comput Struct Biotechnol J* 19: 1200–1213.

Kim, Y.-C., Glick, B.R., Bashan, Y., and Ryu, C.-M. (2012) Enhancement of plant drought tolerance by microbes. In *Plant Responses to Drought Stress*, Aroca, R. (ed). Berlin, Heidelberg: Springer, pp. 383–413.

Köster, J., and Rahmann, S. (2012) Snakemake - a scalable bioinformatics workflow engine. *Bioinformatics* 28: 2520–2522.

Lauber, C.L., Strickland, M.S., Bradford, M.A., and Fierer, N. (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol Biochem* 40: 2407–2415.

Liu, J.-F., Arend, M., Yang, W.-J., Schaub, M., Ni, Y.-Y., Gessler, A., *et al*. (2017) Effects of drought on leaf carbon source and growth of European beech are modulated by soil type. *Sci Rep* 7: 42462.

Lüttger, A.B., and Feike, T. (2018) Development of heat and drought related extreme weather events and their effect on winter wheat yields in Germany. *Theor Appl Climatol* 132: 15–29.

Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., *et al*. (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. *PloS One* 7: e48479.

Martiny, J.B., Martiny, A.C., Weihe, C., Lu, Y., Berlemont, R., Brodie, E.L., *et al*. (2017) Microbial legacies alter decomposition in response to simulated global change. *ISME J* 11: 490–499.

McMurdie, P.J., and Holmes, S. (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One* 8: e61217.

Mendes, R., Garbeva, P., and Raaijmakers, J.M. (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol Rev* 37: 634–663.

Mganga, K.Z., Razavi, B.S., Sanaullah, M., and Kuzyakov, Y. (2019) Phenological stage, plant biomass, and drought stress affect microbial biomass and enzyme activities in the rhizosphere of *Enteropogon macro- stachyus*. *Pedosphere* 29: 259–265.

Murphy, J., and Riley, J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27: 31–36.

Nautiyal, C.S., and Dion, P. (2008) *Molecular Mechanisms of Plant and Microbe Coexistence*, Vol. 15. New York: *Springer*, pp. 3–15.

Naylor, D., and Coleman-Derr, D. (2018) Drought stress and root-associated bacterial communities. *Front Plant Sci* 8: 2223.

Nio, S., Cawthray, G., Wade, L., and Colmer, T. (2011) Pattern of solutes accumulated during leaf osmotic adjustment as related to duration of water deficit for wheat at the reproductive stage. *Plant Physiol Biochem* 49: 1126–1137.

Ochoa-Hueso, R., Collins, S.L., Delgado-Baquerizo, M., Hamonts, K., Pockman, W.T., Sinsabaugh, R.L., *et al*. (2018) Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. *Glob Chang Biol* 24: 2818–2827.

Oksanen, J., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'hara, R. *et al*. (2013) Package 'vegan'.

Community ecology package, version 2 (9). *The R founda- tion for statistical computing*. URL www r-project org.

Palta, J., and Gregory, P. (1997) Drought affects the fluxes of carbon to roots and soil in 13C pulse-labelled plants of wheat. *Soil Biol Biochem* 29: 1395–1403.

Philippot, L., Raaijmakers, J.M., Lemanceau, P., and Van Der Putten, W.H. (2013) Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 11: 789–799.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., *et al*. (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41: D590–D596.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

R Core Team. (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reguera, M., Peleg, Z., Abdel-Tawab, Y.M., Tumimbang, E. B., Delatorre, C.A., and Blumwald, E. (2013) Stress-induced cytokinin synthesis increases drought tolerance through the coordinated regulation of carbon and nitrogen assimilation in rice. *Plant Physiol* 163: 1609–1622.

Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C., and Kuzyakov, Y. (2011) Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. *Appl Soil Ecol* 48: 38–44.

Santos-Medellín, C., Edwards, J., Liechty, Z., Nguyen, B., and Sundaresan, V. (2017) Drought stress results in a compartment-specific restructuring of the rice root-associated microbiomes. *MBio* 8: e00764-17.

Schädler, M., Buscot, F., Klotz, S., Reitz, T., Durka, W., Bumberger, J., *et al*. (2019) Investigating the consequences of climate change under different land-use regimes: a novel experimental infrastructure. *Ecosphere* 10: e02635.

Schimel, J.P., and Bennett, J. (2004) Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 85: 591–602.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., *et al*. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75: 7537–7541.

Schulz, E. (2002) Influence of extreme management on decomposable soil organic matter pool. *Arch Agron Soil Sci* 48: 101–105.

Schweitzer, K. (2010) Naturnahe Böden im Berliner Stadtgebiet. Referenz für anthropogene Bodenentwicklung oder Beispiel für anthropogene Bodenveränderungen? Boden- und Standortverhältnisse auf den Versuchsfeldern der landwirtschaftlichen Lehr- und Forschungsstation der Humboldt-Universiät zu Berlin. *Berl Geogr Arb* 117: 13–22.

Shao, H.B., Liang, Z.S., Shao, M.A., and Sun, Q. (2005) Dynamic changes of anti-oxidative enzymes of 10 wheat genotypes at soil water deficits. *Colloids Surf B Biointerfaces* 42: 187–195.

Singh, D.P., Prabha, R., Gupta, V.K., and Verma, M.K. (2018) Metatranscriptome analysis deciphers multifunctional genes and enzymes linked with the degradation of aromatic compounds and pesticides in the wheat rhizosphere. *Front Microbiol* 9: 1331.

Sinsabaugh, R., Saiya-Cork, K., Long, T., Osgood, M., Neher, D., Zak, D., and Norby, R. (2003) Soil microbial activity in a liquidambar plantation unresponsive to CO_2-driven increases in primary production. *Appl Soil Ecol* 24: 263–271.

Song, F., Han, X., Zhu, X., and Herbert, S.J. (2012) Response to water stress of soil enzymes and root exudates from drought and non-drought tolerant corn hybrids at different growth stages. *Can J Soil Sci* 92: 501–507.

Thiele-Bruhn, S., Bloem, J., de Vries, F.T., Kalbitz, K., and Wagg, C. (2012) Linking soil biodiversity and agricultural soil management. *Curr Opin Environ Sustain* 4: 523–528.

Timmusk, S., and Nevo, E. (2011) Plant root associated biofilms: perspectives for natural product mining. In *Bacteria in Agrobiology: Plant Nutrient Management*, Maheshwari, D.
K. (ed). Berlin, Heidelberg: Springer, pp. 285–300.

Ullah, A., Akbar, A., Luo, Q., Khan, A.H., Manghwar, H., Shaban, M., and Yang, X. (2019) Microbiome diversity in cotton rhizosphere under normal and drought conditions. *Microb Ecol* 77: 429–439.

Van Bueren, E.L., Jones, S.S., Tamm, L., Murphy, K.M., Myers, J.R., Leifert, C., and Messmer, M. (2011) The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: a review. *NJAS-Wageningen J Life Sci* 58: 193–205.

van der Molen, M.K., Dolman, A.J., Ciais, P., Eglin, T., Gobron, N., Law, B.E., *et al*. (2011) Drought and ecosystem carbon cycling. *Agric For Meteorol* 151: 765–773.

Wagg, C., Hautier, Y., Pellkofer, S., Banerjee, S., Schmid, B., and van der Heijden, M.G. (2021) Diversity and asynchrony in soil microbial communities stabilizes ecosystem functioning. *Elife* 10: e62813.

Wang, J., Xue, C., Song, Y., Wang, L., Huang, Q., and Shen, Q. (2016) Wheat and rice growth stages and fertilization regimes alter soil bacterial community structure, but not diversity. *Front Microbiol* 7: 1207.

Weißbecker, C., Schnabel, B., and Heintz-Buschart, A. (2020) Dadasnake, a Snakemake implementation of DADA2 to process amplicon sequencing data for microbial ecology. *GigaScience* 9: giaa135.

Xu, L., Naylor, D., Dong, Z., Simmons, T., Pierroz, G., Hixson, K.K., *et al*. (2018) Drought delays development of the sorghum root microbiome and enriches for monoderm bacteria. *Proc Natl Acad Sci U S A* 115: E4284–E4293.

Xu, Z., and Zhou, G. (2006) Nitrogen metabolism and photosynthesis in Leymus chinensis in response to long-term soil drought. *J Plant Growth Regul* 25: 252–266.

Yadav, A.N., Verma, P., Kumar, S., Kumar, V., Kumar, M., Sugitha, T.C.K., *et al*. (2018) Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. In *New and Future Developments in Microbial Biotechnology and Bioengineering*, Singh, B., Gupta, V., and Passari, A. (eds). Amsterdam: Elsevier, pp. 13–41.

Yandigeri, M.S., Meena, K.K., Singh, D., Malviya, N., Singh, D.P., Solanki, M.K., *et al*. (2012) Drought-tolerant endophytic actinobacteria promote growth of wheat (*Triticum aestivum*) under water stress conditions. *Plant Growth Regul* 68: 411–420.

Yu, X., Li, B., Wang, L., Chen, X., Wang, W., Gu, Y., *et al*. (2016) Effect of drought stress on the development of endosperm starch granules and the composition and physicochemical properties of starches from soft and hard wheat. *J Sci Food Agric* 96: 2746–2754.

Zhang, W., Gu, J., Wang, Z., Wei, C., Yang, J., and Zhang, J. (2017) Comparison of structural and functional properties of wheat starch under different soil drought conditions. *Sci Rep* 7: 1–18.

Zhou, C., Ma, Z., Zhu, L., Xiao, X., Xie, Y., Zhu, J., and Wang, J. (2016) Rhizobacterial strain *Bacillus megaterium BOFC15* induces cellular polyamine changes that improve plant growth and drought resistance. *Int J Mol Sci* 17: 976.

Zia, R., Nawaz, M.S., Siddique, M.J., Hakim, S., and Imran, A. (2021) Plant survival under drought stress: impli- cations, adaptive responses, and integrated rhizosphere management strategy for stress mitigation. *Microbiol Res* 242: 1–16.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supplementary Information

Supplemental Material:

Interactions between soil properties, agricultural management and cultivar type drive structural and functional adaptations of the wheat rhizosphere microbiome under drought

[1]Claudia Breitkreuz, [1]Laura Herzig, [1,2]François Buscot, [1,2]Thomas Reitz, [1,2]Mika Tarkka

[1]UFZ - Helmholtz-Centre for Environmental Research, Soil Ecology Department, Theodor-Lieser-Straße 4, 06120 Halle, Germany

[2]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

Supplemental Tables

Table S 1 Gravimetric soil moistures (%) at wheat harvest. Standard deviations are provided in brackets. Significant differences (ANOVA and Tukey's HSD test; $p<0.05$) were calculated for each soil type separately and indicated by different letters.

System	Cultivar	Watering	Haplic Chernozem	Albic Luvisol
Conventional farming	„Dichter“	control	16.27 (1.00) a	11.28 (0.41) bc
		drought	9.26 (1.12) bc	5.85 (0.68) de
	„RGT“	control	16.13 (0.68) a	11.69 (0.96) b
		drought	9.79 (1.15) b	6.54 (0.56) d
Organic farming	„Dichter“	control	16.49 (0.42) a	10.94 (0.19) bc
		drought	8.07 (0.80) c	5.38 (0.23) e
	„RGT“	control	15.80 (0.44) a	10.49 (0.45) c
		drought	9.47 (1.18) bc	5.24 (0.57) e

Table S 2 Relative abundances of phyla in Haplic Chernozem. Abundances have been calculated for each sample and are given as average for each treatment combination. Empty spots mark relative abundances of phyla <1% in the respective treatments.

Haplic Chernozem			Harvest															
			Conventional farming (CF)								Organic farming (OF)							
			Bulk soil				Rhizosphere				Bulk soil				Rhizosphere			
	Start		Dichter		RGT		Dichter		RGT		Dichter		RGT		Dichter		RGT	
	CF	OF	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*
Acidobacteria	0.14	0.16	0.11	0.12	0.10	0.11	0.06	0.08	0.06	0.09	0.13	0.13	0.11	0.13	0.08	0.10	0.08	0.11
Actinobacteria	0.20	0.17	0.21	0.26	0.20	0.24	0.33	0.36	0.30	0.33	0.19	0.26	0.23	0.25	0.29	0.32	0.29	0.30
Bacteroidetes	0.06	0.07	0.06	0.04	0.07	0.04	0.07	0.04	0.09	0.05	0.06	0.04	0.05	0.04	0.06	0.04	0.08	0.05
Chloroflexi	0.08	0.07	0.09	0.09	0.08	0.09	0.09	0.10	0.08	0.11	0.07	0.08	0.08	0.09	0.07	0.09	0.07	0.09
Firmicutes	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.02		0.02
Gemmatimonadetes	0.05	0.05	0.05	0.05	0.06	0.05	0.04	0.03	0.04	0.03	0.06	0.04	0.05	0.04	0.04	0.03	0.04	0.03
Latescibacteria	0.01	0.02	0.01	0.01	0.01	0.01		0.01			0.02	0.01	0.01	0.01		0.01	0.01	0.01
Patescibacteria			0.01		0.01		0.01		0.01		0.01				0.01		0.01	
Planctomycetes	0.04	0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Proteobacteria	0.30	0.30	0.31	0.29	0.34	0.30	0.28	0.25	0.29	0.25	0.32	0.29	0.32	0.29	0.31	0.27	0.31	0.27
Rokubacteria	0.02	0.02	0.01	0.01	0.01	0.01		0.01		0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01
Thaumarchaeota	0.04	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.02	0.03
Verrucomicrobia	0.04	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03
Others	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

Table S 3 Relative abundances of phyla in Albic Luvisol. Abundances have been calculated for each sample and are given as average for each treatment combination. Empty spots mark relative abundances of phyla <1% in the respective treatments.

Albic Luvisol			Harvest															
			Conventional farming (CF)								Organic farming (OF)							
			Bulk soil				Rhizosphere				Bulk soil				Rhizosphere			
	Start		Dichter		RGT		Dichter		RGT		Dichter		RGT		Dichter		RGT	
	CF	OF	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*	*control*	*drought*
Acidobacteria	0.14	0.15	0.09	0.11	0.09	0.10	0.03	0.02	0.03	0.02	0.09	0.08	0.08	0.07	0.03	0.02	0.03	0.02
Actinobacteria	0.21	0.22	0.24	0.20	0.20	0.24	0.25	0.48	0.21	0.46	0.14	0.21	0.14	0.20	0.25	0.60	0.19	0.61
Armatimonadetes																		
Bacteroidetes	0.05	0.04	0.03	0.04	0.04	0.03	0.08	0.05	0.12	0.07	0.06	0.04	0.06	0.04	0.10	0.01	0.10	0.01
Chloroflexi	0.08	0.08	0.08	0.06	0.08	0.07	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.03	0.03	0.02	0.02	0.02
Firmicutes	0.01	0.01	0.04	0.01	0.03	0.03	0.04	0.03	0.01	0.02	0.02	0.05	0.04	0.04	0.03	0.09	0.02	0.06
Gemmatimonadetes	0.05	0.05	0.05	0.06	0.06	0.06	0.03	0.02	0.03	0.02	0.05	0.05	0.06	0.05	0.02		0.02	0.01
Patescibacteria	0.01		0.02	0.01	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.02		0.01	
Planctomycetes	0.03	0.04	0.05	0.05	0.06	0.05	0.05	0.02	0.04	0.02	0.04	0.03	0.04	0.03	0.03		0.03	
Proteobacteria	0.35	0.31	0.34	0.40	0.35	0.35	0.42	0.28	0.46	0.30	0.50	0.44	0.48	0.50	0.47	0.23	0.55	0.25
Thaumarchaeota	0.02	0.02	0.01		0.01	0.01	0.01	0.01										
Verrucomicrobia	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.02		0.02	
Others	0.02	0.04	0.03	0.03	0.03	0.02	0.02	0.01	0.03	0.01	0.02	0.02	0.02	0.01	0.02	0.03	0.02	0.02

Table S 4 Applied fertilizers.

	Conventional Farming	Organic Farming
Phosphorus	Triple Super Phosphate	Granulated raw phosphate (Physalg 25)
Potassium	60 % K_2O (60er Kali)	Muriate of potash (Patentkali)
Nitrogen	Ammonium nitrate	Urea

Table S 5 Measured enzyme activities involved in carbon acquisition.

Enzyme	Substrate
β-glucosidase (EC 3.2.1.21)	4-MUF-β-D-glucopyranoside
cellobiohydrolase (EC 3.2.1.91)	4-MUF-β-D-cellobioside
xylosidase (EC 3.2.1.37)	4-MUF-β-D-xylopyranoside

Table S 6 Bacterial enzyme categories related to direct and direct drought stress mitigation for plants. For each enzyme, the respective KEGG orthology (KO) identifiers and Enzyme Commission (EC) numbers were extracted from the output of the Tax4Fun prediction tool.

KEGG/EC-numbers	Description	Activity
Direct response to drought:		
K00689 [EC:2.4.1.5] **K03332 [EC:3.2.1.80]**	dextransucrase fructan beta-fructosidase	degradation of complex plant polysaccharides under drought (Martiny et al. 2016)
K11931 K11935 K11937	biofilm PGA synthesis lipoprotein	biofilm production (Timmusk & Nevo, 2011; Kim et al., 2013)
Drought-induced, indirect stress response:		
K00383 [EC:1.8.1.7]	glutathione reductase (NADPH)	involved in the plant's ascorbate–glutathione cycle, higher resilience against drought for wheat (Kasim et al. 2013)
K01505 [EC:3.5.99.7]	1-aminocyclopropane-1-carboxylate deaminase	control of stress hormone ethylene (Glick, 2005)
K03366 [EC:1.1.1.4 1.1.1.303]	(R,R)-butanediol dehydrogenase / diacetyl reductase	Butanediol is involved in stomata closure, SA signaling pathway (Cho et al., 2008)
K00316 [EC:1.5.99.6]	spermidine dehydrogenase	spermidine scavenges ROS, upregulates ABA biosynthesis and response genes, and by extension augments photosynthesis and root system architecture (Zhou et al., 2016)

Supplemental Figures

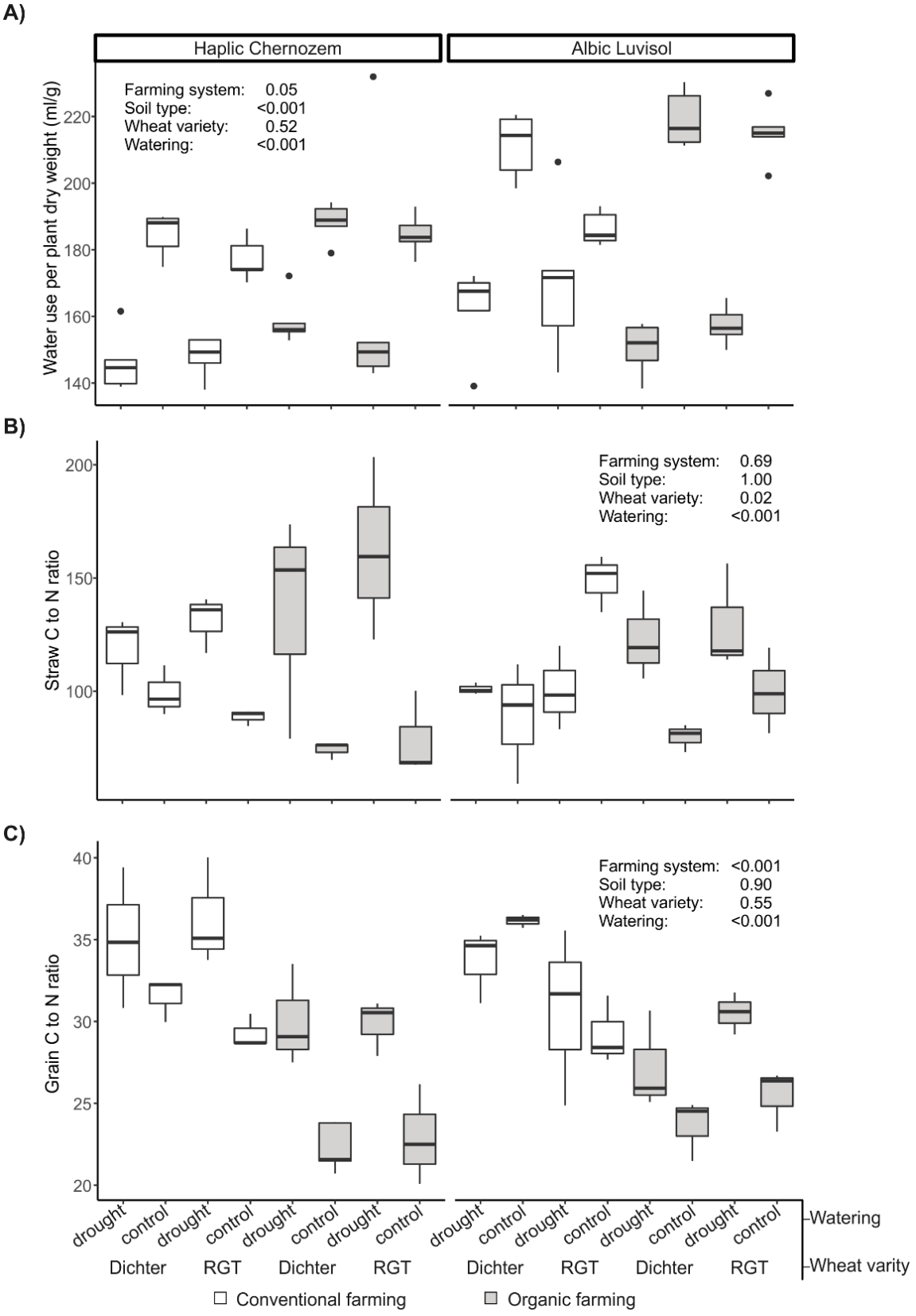


Figure S 1 Influence of experimental factors watering regime, farming system, soil type and wheat variety on C to N ratios of straw (A) and corn (B). ANOVA was applied to test for significant differences between the treatments and calculated p-values are given in each subfigure.

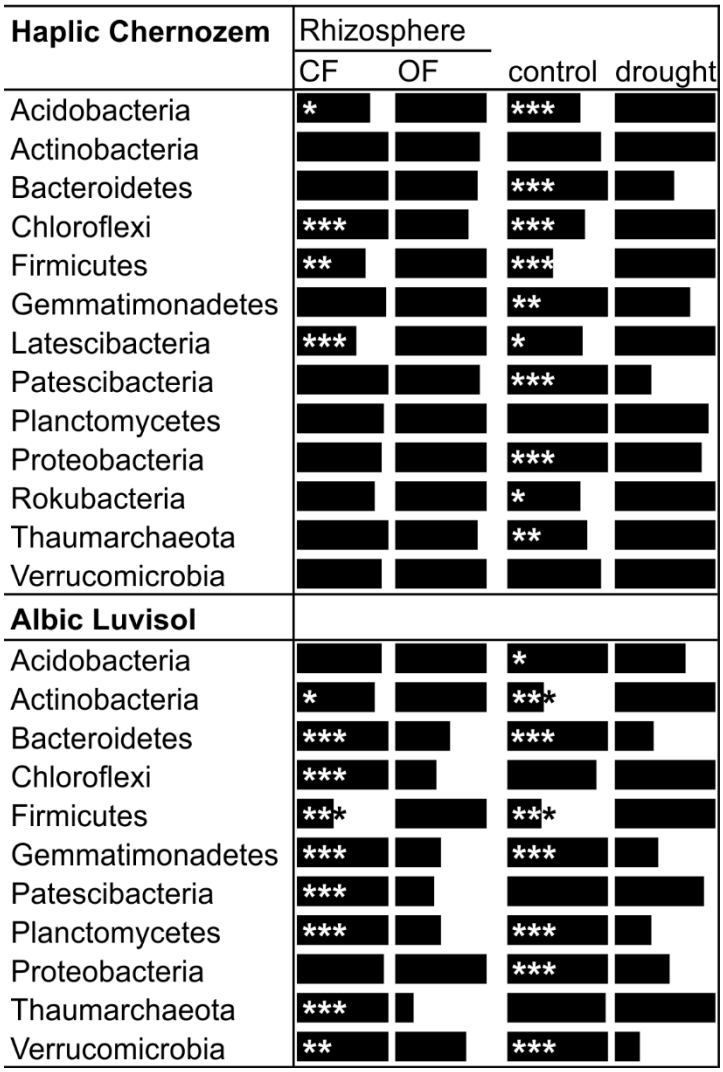


Figure S 2 Bars showing the relative abundances of phyla in the rhizosphere of “RGT-Reform” at the harvest (separated by the two soil types). ANOVA was applied to test for differences between farming systems - conventional farming (CF) vs. organic farming (OF) - and watering regimes - control vs. drought - in the rhizosphere: $p<0.001$***, $p<0.01$** and $p<0.05$*.

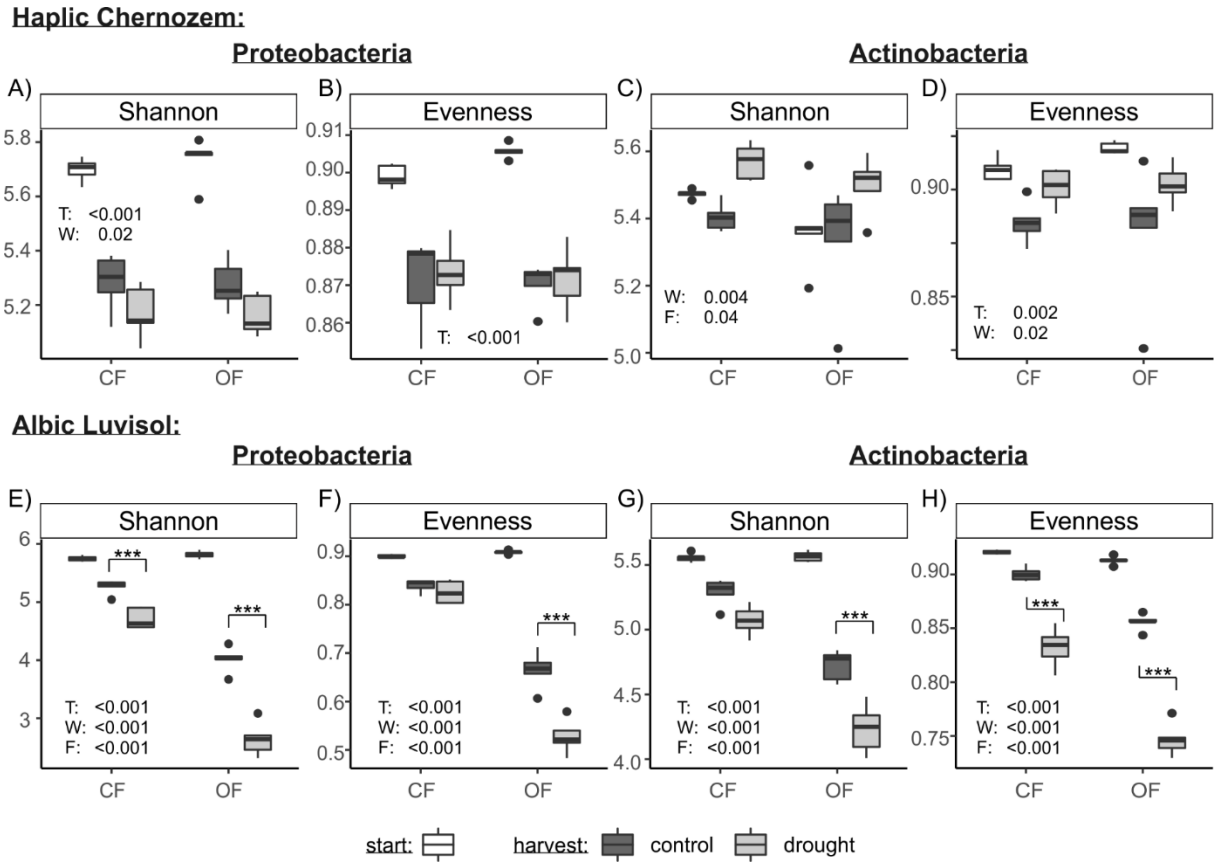


Figure S 3 Alpha-diversity indices for Proteobacteria (subfigures A,B,E,F) and Actinobacteria (subfigures C,D,G,H) in the rhizosphere of “RGT-Reform” in dependency of sampling time (T), watering regime (W) and farming system (F) (each treatment, n=5). Panels A-D and E-H illustrate differences in Chernozem and Luvisol, respectively. Significant differences induced by the treatments are given in each panel as p-values (ANOVA). Significant differences within treatments are marked according to TukeyHSD as following: p<0.001***, p<0.01** and p<0.05*.

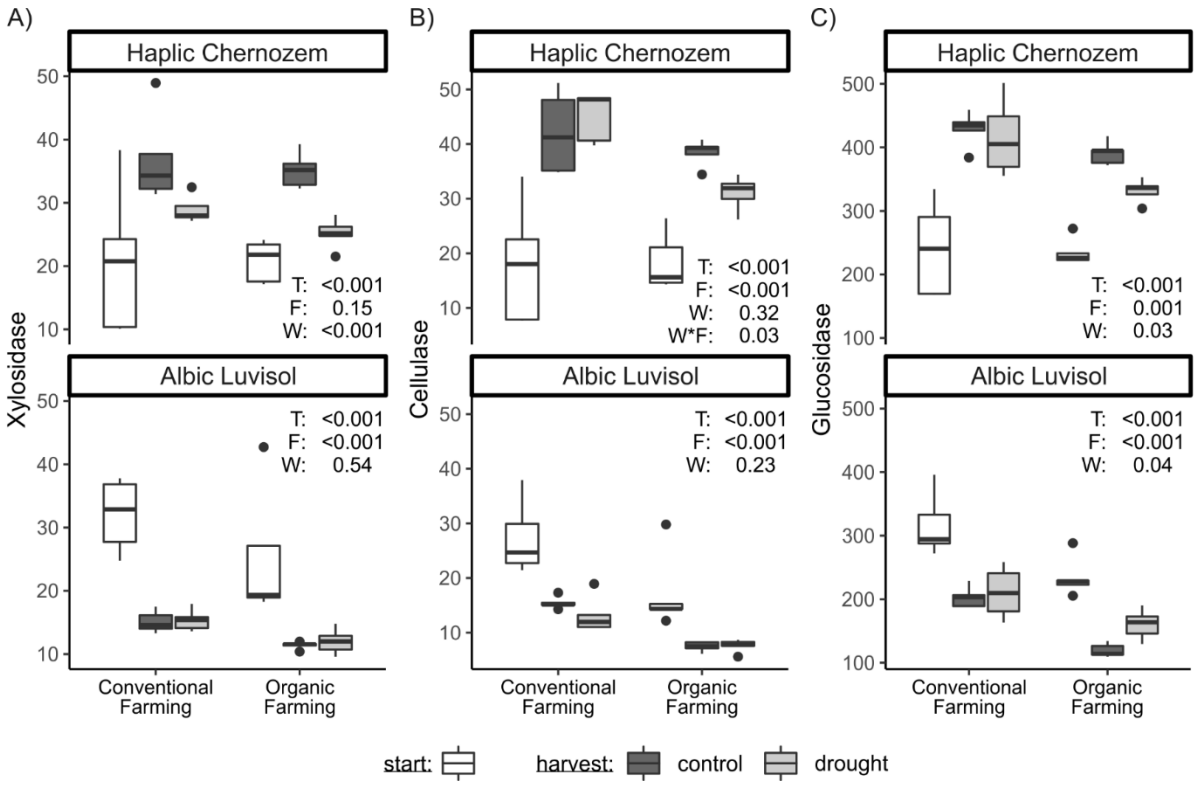


Figure S 4 Activity potentials of C-cycling enzymes in the rhizosphere of wheat. A) xylosidase, B) cellulase and C) glucosidase activities are presented for the wheat cultivar "RGT-Reform". Significant impacts of experimental factors were calculated using ANOVA and are given as p-values for each enzyme and soil type. Thereby, sampling time effect (T) was tested between start and harvest, while watering (W) and farming (F) effects as well as interaction of the two factors were evaluated at harvest.

CHAPTER 2

Can We Estimate Functionality of Soil Microbial Communities from Structure-Derived Predictions? A Reality Test in Agricultural Soils

Claudia Breitkreuz, Anna Heintz-Buschart, François Buscot, Sara Fareed Mohamed Wahdan, Mika Tarkka, Thomas Reitz

Microbiology Spectrum (2021), published

Accepted: July 12th 2021

RESEARCH ARTICLE

Can We Estimate Functionality of Soil Microbial Communities from Structure-Derived Predictions? A Reality Test in Agricultural Soils

Claudia Breitkreuz,[a] Anna Heintz-Buschart,[a,b] François Buscot,[a,b] Sara Fareed Mohamed Wahdan,[a] Mika Tarkka,[a,b] Thomas Reitz[a,b]

[a]Department of Soil Ecology, UFZ—Helmholtz Centre for Environmental Research, Halle, Germany
[b]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

ABSTRACT Computational approaches that link bacterial 16S rRNA gene amplicon data to functional genes based on prokaryotic reference genomes have emerged. This study aims to validate or refute the applicability of the functional gene predic- tion tools for assessment and comparison of community functionality among experi- mental treatments, inducing either fast or slow responses in rhizosphere microbial community composition and function. Rhizosphere samples of wheat and barley were collected in two consecutive years at active and mature growth phases from organic and conventional farming plots with ambient or future-climate treatments of the Global Change Experimental Facility. Bacterial community composition was determined by 16S rRNA gene amplicon sequencing, and the activities of five extrac- ellular enzymes involved in carbon (b-glucosidases, cellobiohydrolase, and xylosi- dase), nitrogen (N-acetylglucosaminidase), and phosphorus (acid phosphatase) cycles were determined. Structural community data were used to predict functional pat- terns of the rhizosphere communities using Tax4Fun and PanFP. Subsequently, the predictions were compared with the measured activities. Despite the fact that differ- ent treatments mainly drove either community composition (plant growth phase) or measured enzyme activities (farming system), the predictions mirrored patterns in the treatments in a qualitative but not quantitative way. Most of the discrepancies between measured and predicted values resulted from plant growth stages (fast community response), followed by farming management and climate (slower com- munity response). Thus, our results suggest the applicability of the prediction tools for comparative investigations of soil community functionality in less-dynamic envi- ronmental systems.

IMPORTANCE Linking soil microbial community structure to its functionality, which is important for maintaining health and services of an ecosystem, is still challenging. Besides great advances in structural community analysis, functional equivalents, such as metagenomics and metatranscriptomics, are still time and cost intensive. Recent computational approaches (Tax4Fun and PanFP) aim to predict functions from struc- tural community data based on reference genomes. Although the usability of these tools has been confirmed with metagenomic data, a comparison between predicted and measured functions is so far missing. Thus, this study comprises an expansive reality test on the performance of these tools under different environmental condi- tions, including relevant global change factors (land use and climate). The work pro- vides a valuable validation of the applicability of the prediction tools for comparison of soil community functions across different sufficiently established soil ecosystems and suggest their usability to unravel the broad spectrum of functions provided by a given community structure.

Citation Breitkreuz C, Heintz-Buschart A, Buscot F, Wahdan SFM, Tarkka M, Reitz T. 2021. Can we estimate functionality of soil microbial communities from structure-derived predictions? A reality test in agricultural soils. Microbiol Spectr 9:e00278-21. https://doi.org/10.1128/Spectrum.00278-21.
Editor Jeffrey A. Gralnick, University of Minnesota

Address correspondence to Claudia Breitkreuz, claudia.breitkreuz@ufz.de, or Anna Heintz-Buschart, anna.heintz-buschart@idiv.de.
Received 29 June 2021
Accepted 12 July 2021
Published 4 August 2021

KEYWORDS GCEF, PanFP, Tax4Fun, agriculture, bacterial communities, barley, climate change, enzymes, wheat

Over the last decades, we experienced a rapid advancement of molecular approaches to explore structural diversity of soil microbial communities. The use of next-generation amplicon sequencing allows for high-resolution analyses of microbial community structure, e.g., on its temporal dynamics and adaptation to different environmental conditions (1, 2). Corresponding studies revealed that soil microbial communities change over the growing season (3, 4) and are dependent on the plant species (5–8) as well as on the plant development stage (8–11). Moreover, soil type as well as land-use- and management-related variations in pH and available nutrient concentrations shape soil microbial communities (12–15). However, it remains challenging to determine the functional traits of a given microbial community in order to estimate resultant soil processes and ecosystem services (16). This is because soil processes and functions can be maintained in spite of community shifts by functional redundancy (reviewed in reference 17), while others may be lost by losing individual, possibly even low-abundant, key species (18). Thus, it is crucial to have information on the traits of all present taxa to derive the functionality of the whole community (19–21).

Cultivation has been traditionally used to cross-examine the taxonomic and functional properties of bacteria. Even though the longstanding "1% cultivability paradigm" has been questioned in recent discussions (22, 23), cultivation-based approaches are hardly meaningful for functional trait assessment in environmental samples, since trait variation is strongly reduced by studying only few isolates (24). When measuring aggregated functional properties of the microbial community, e.g., by analyzing community enzyme activities (25–27) or gene expression profiles (28, 29), it is often difficult to assign activities to certain taxa. Available methods that link structural and functional information of bacteria include stable isotope labeling of substrates and subsequent amplicon sequencing of isotope-enriched DNA or RNA (28, 30) or using genome-resolved metagenomics or metatranscriptomics (31, 32). Nevertheless, capturing the functional diversity of whole microbial communities in depth and breadth with these methods remains cost and time intensive (32).

Computational prediction tools in microbial ecology, such as Tax4Fun and PanFP, offer the possibility to translate structural community data into ecosystem functions in a cost-effective way (33–35). These approaches use the link between bacterial 16S rRNA gene amplicon sequencing and functional gene annotations of prokaryotic reference genomes. As output, the programs provide abundance estimates of functional genes. The applicability of both tools has been validated by comparison of the predicted functional gene abundance with the number of detected genes in the respective metagenome (36–38). Median Spearman rank correlation coefficients range up to

0.87 for Tax4Fun (36) and 0.80 for PanFP (37), suggesting good approximations of func- tional profiles. At the same time, assessment of whether and how well such predicted functional profiles mirror microbial community trait expression and thus allow estima- tion of ecosystem processes is still missing.

In this study, the activity potentials of five extracellular microbial enzymes (*b*-glucosidase, cellobiohydrolase, xylosidase, *N*-acetylglucosaminidase, and acid phosphatase) were measured and compared to the abundances of the respective genes predicted with PanFP and Tax4Fun based on Illumina MiSeq amplicon sequencing data. These enzymes were chosen because (i) they play a crucial role in soil C, N, and P cycling, (ii) their activities are commonly measured in environmental studies as representative of soil function, and (iii) the protocols and assays for activity determination are standardized and well established.

We expected that a linear link might not be conceivable, since a direct correlation of gene abundance and its related function would require that (i) the genes of interest are constitutively transcribed to mRNA, (ii) the mRNAs are translated into proteins, (iii) all proteins responsible for the same reaction have the same kinetics and optimal conditions for

activity, and (iv) all enzymes have the same life span (28). In reality, gene expression and enzyme secretion are not consistent but are regulated in response to soil conditions. Moreover, the life span of extracellular enzymes in soil can range from hours to months, depending on local biotic and abiotic soil parameters (28, 39). Thus, enzyme activity measurements depict the situation in soil at a certain point in time but do not necessarily reflect short-term changes in microbial community composition (39, 40). Nevertheless, we assumed that patterns of measured enzyme activities follow those of the corresponding functional gene abundances in the microbial communities along treatments or environmental gradients that exert a continuous and steady impact.

This study aimed to evaluate this assumption and to validate or refute the applicability of the functional gene prediction tools for assessment and comparison of soil processes. Since the functional predictions by Tax4Fun and PanFP refer exclusively to bacterial genomes, we selected croplands as study systems. Agricultural soils are usually dominated by bacteria, whereby the fungal contribution to enzyme profiles is minimized (41, 42). We collected rhizosphere soils, i.e., the hot spot for abundance, activity, and turnover of soil bacteria (43), of wheat (and barley in the subsequent year) from agricultural plots of the Global Change Experimental Facility (GCEF) (44). This experimental field platform cross-manipulates climatic conditions (ambient versus future) and farming management (conventional versus organic farming). Both experimental treatments are known to steer structure and function of bacterial communities (12–14). The small but continuous impact of changed climatic conditions induces a slow response of the soil community, whereas the adaptation to different management measures induces quicker community responses. To account for very rapid responses, we collected rhizosphere samples at two different plant growth phases: active biomass production and mature phase. The dynamics of roots from active to mature growth stages (45, 46) are known to cause rapid temporal changes in rhizobac- terial community structures (reviewed in reference 47). From all collected samples, we determined the rhizobacterial community composition using 16S rRNA gene amplicon sequencing, estimated the functional gene abundances by the prediction tools Tax4Fun and PanFP, and measured the enzyme activity potentials.

We hypothesized that (i) deviations between the predicted traits and the measured enzyme activities show a positive correlation with the speed of the community's response to the treatments. Thus, the strongest deviations should be related to plant growth phases (strong dynamics, rapid adaptation) followed by the impact of the farming management, while the most concurrent patterns should be observed along the climate treatments (slow, consistent community adaptation). We further hypothesized that (ii) across growth phases, the deviations are more pronounced during the mature growth phase. Plants stimulate rhizobacterial growth and activity by a gradually increasing release of rhizodeposits during active growth but strongly reduce rhizodeposition when reaching maturity, inducing a reduction of bacterial biomass (9). Accordingly, functional gene abundance drops quickly, while there is a delay for enzyme activity. We also hypothesized that (iii) deviations in measured and predicted values are more pronounced in conventional farming soil, as these systems experience more disturbances by, e.g., pesticide application, compared to that of organic soil. Finally, we hypothesized that (iv) under future climatic conditions, with larger variability of annual precipitation, the deviations between measured and predicted functions are more pronounced than under ambient climatic conditions.

RESULTS

Experimental treatments drive rhizosphere community composition and enzyme activities. (i) Impact on the bacterial community composition. The effect of the experimental treatments and the related differences in abiotic soil parameters (for more information on edaphic parameters and impact on community composition refer to Material S1 and S2, respectively, in the supplemental material) on the rhizobacterial community composition was studied for both crops in the two consecutive years of cultivation (Fig. 1). In the first year, when wheat was cultivated, growth phase was the main driver for bacterial community composition (permutational multivariate analysis

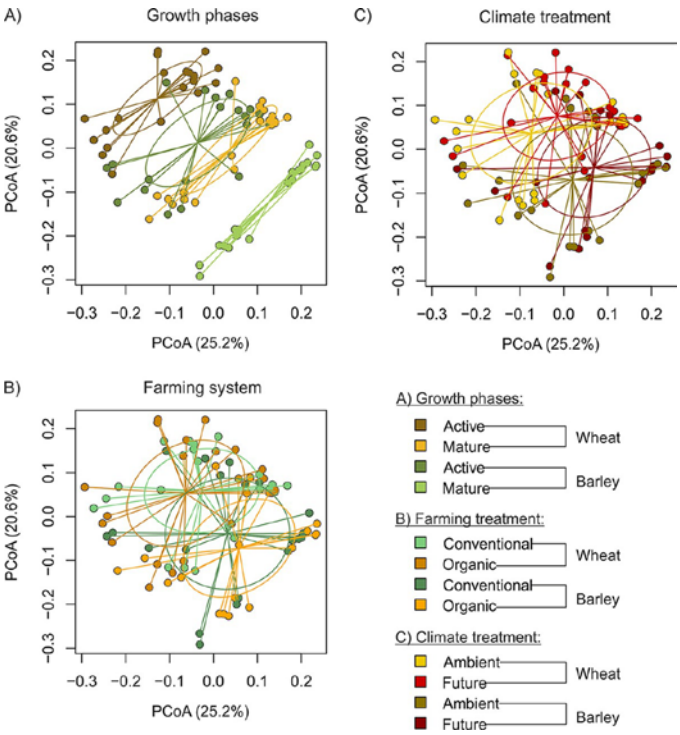


FIG 1 Principal coordinates analysis for beta-diversity of bacterial rhizosphere communities. The points are colored and circled according to growth phases (A), farming system (B), and climate treatment (C).

of variance [PERMANOVA], R2 = 0.26, P , 0.001) (Fig. 1A), followed by farming system (PERMANOVA, R2 = 0.08, P = 0.003) (Fig. 1B) and climate treatment (PERMANOVA, R2 = 0.03, P = 0.18) (Fig. 1C). In a comparable way, rhizobacterial community composition of barley in the subsequent year was affected in decreasing order by growth phase (PERMANOVA, R2 = 0.22, P , 0.001) (Fig. 1A), farming system (PERMANOVA, R2 = 0.04, P = 0.08) (Fig. 1B), and climate treatment (PERMANOVA, R2 = 0.03, P = 0.33) (Fig. 1C). In line with these results, analysis and visualization of indicator species in a bipartite net- work indicated a strong grouping of species according to the growth phase and farm- ing system in the wheat rhizosphere as well as according to the growth phase in the barley rhizosphere (Material S3). Besides, wheat and barley strongly differed in their rhizobacterial community composition (PERMANOVA, R2 = 0.13, P , 0.001).

(ii) Impact on rhizosphere enzyme activities. Farming system and the related differences in edaphic parameters (Material S1) were the main drivers of enzyme activities (Table 1; Material S2). Thereby, higher enzyme activities were found in rhizosphere soil from conventional farming than in the ones from organic farming, which was evident for wheat at both growth phases, while for barley, it was mainly observed in the active growth phase (see the blue boxes in Fig. 2). The effects of the growth phase and of the climate treatment on rhizosphere enzyme activities were comparably weak, with signif- icant impacts of individual extracellular enzymes and in a crop-specific manner (Table 1). The growth phase affected chitinase activity in wheat (active , mature) (Fig. 2C) and acid phosphatase activity in barley rhizosphere (active . mature) (Fig. 2D) (Table 1). Climate treatment effects were found for the activities of xylosidases and acid phospha- tases in the rhizosphere of wheat and for the activity of cellulases in the rhizosphere of barley (Table 1). Besides, all enzyme activities strongly differed (P , 0.001) between wheat and barley rhizospheres, with higher enzyme activities in the wheat rhizosphere (Fig. 2).

TABLE 1 Drivers of rhizosphere enzyme activities[a]

	P value[b]			
Enzyme	Farming	Growth phase	Climate	Growth phase × farming
Wheat				
Glucosidases	*<0.001*	0.76	0.11	0.57
Xylosidases	*0.007*	*0.09*	*0.04*	0.38
Chitinases	*<0.001*	*0.002*	0.41	0.19
Phosphatases	*<0.001*	0.19	*0.04*	0.21
Cellulases	*<0.001*	0.84	0.77	0.91
Barley				
Glucosidases	*<0.001*	0.71	0.32	*0.007*
Xylosidases	*<0.001*	0.15	0.67	*0.03*
Chitinases	*0.02*	0.24	0.19	0.14
Phosphatases	*<0.001*	*,0.001*	0.79	*<0.001*
Cellulases	*<0.001*	0.85	*0.04*	*0.05*

[a]Activities of *b*-glucosidases, xylosidases, *N*-acetylglucosaminidases (chitinases), acid phosphatases, and cellobiohydrolases (cellulases) were tested against the factors farming system, growth phase, climate, and interaction of farming system and growth phase.

[b]Significant impacts according to ANOVA are indicated by italic font.

Patterns of measured enzyme activities compared to predicted enzyme gene abundances in the rhizosphere. (i) Correlations along and relative changes between factors of growth phase, farming system, and climate. Spearman rank correlations were tested to identify common and specific patterns of predicted functional gene abundances and measured enzyme activities over both growth phases ($n = 40$) (Table S1). In the wheat rhizosphere, a positive correlation between measured and Tax4Fun- as well as PanFP-predicted values was found for xylosidases and with a trend observed for acid phosphatases. Contrary to that, in the rhizosphere of barley, functional gene abundances predicted by Tax4Fun were found to be positively correlated with the measured enzyme activities of glucosidases, xylosidases, chitinases, and cellulases. Regarding PanFP predictions, significant correlations with the measured activities were indicated for xylosidases and chitinases and with a trend observed also for glucosidases and cellulases.

Assessing the two growth phases separately, the significance level of correlations was commonly reduced, mainly due to the lower number of samples ($n = 20$). Nevertheless, we

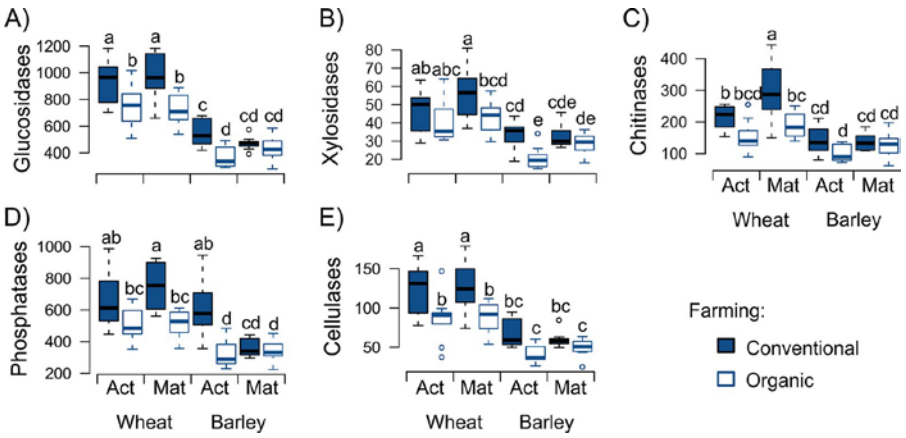


FIG 2 Impacts of farming system, crop species, and crop growth phase on measured enzyme activities (nmol g soil^{21} h^{21}). *b*-glucosidases (A), xylosidases (B), *N*-acetylglucosaminidases (chitinases) (C), acid phosphatases (D), and cellobiohydrolases (cellulases) (E). Measured enzyme activities at the active (Act) and mature (Mat) growth phases in conventional and organic farming soils are given. Different lowercase letters within each panel indicate significant differences between the treatments (P , 0.05) according to Tukey's HSD.

TABLE 2 Relative changes between factors of growth phase (active versus mature), farming system (conventional versus organic), and climate (ambient versus future) treatment[a]

	Relative change								
	Active vs mature			Conventional vs organic			Ambient vs future		
Enzyme	Tax	Pan	Enzymes	Tax	Pan	Enzymes	Tax	Pan	Enzymes
Wheat									
Glucosidase	0.27	0.53	0.02	-0.23	-0.23	-0.23	-0.20	-0.18	-0.08
Xylosidase	0.52	0.55	0.14	-0.25	-0.24	-0.19	-0.24	-0.18	-0.14
Chitinase	0.15	0.36	0.31	-0.27	-0.23	-0.32	-0.17	-0.21	-0.07
Phosphatase	0.89	0.59	0.09	-0.26	-0.25	-0.28	-0.27	-0.22	-0.13
Cellulase	0.84	0.40	0.02	-0.22	0.02	-0.31	-0.30	0.01	-0.02
Barley									
Glucosidase	0.60	1.15	-0.02	-0.28	-0.22	-0.22	0.00	0.02	0.06
Xylosidase	0.73	1.30	0.12	-0.26	-0.19	-0.25	-0.03	0.01	0.03
Chitinase	0.16	1.12	0.11	-0.33	-0.20	-0.19	-0.06	-0.02	0.12
Phosphatase	1.27	1.19	-0.26	-0.25	-0.22	-0.31	0.00	0.02	0.02
Cellulase	1.03	0.77	0.01	-0.26	-0.23	-0.28	0.00	-0.02	0.16

[a]Relative changes are given for predicted gene abundances of Tax4Fun (Tax) and PanFP (Pan), as well as for measured enzyme activities in the rhizosphere of wheat and barley.

observed stronger positive correlations between the predictions and the measured activity for xylosidases and acid phosphatases in the wheat rhizosphere at maturity than at the active growth phase. In contrast, for barley, these correlations were stronger at the active growth phase than at crop maturity. Furthermore, the predictions by Tax4Fun at the active growth phase of barley, as well as the predictions by PanFP at both growth phases of barley were highly positively correlated with the measured phosphatase activities (Table S1). Relative differences between active and mature growth phases, conventional and organic farming, and ambient and future climate for predicted and measured values are presented in Table 2. Thereby, relative differences of predicted functional gene abundances mostly mirrored the measured enzyme activities in a qualitative way, i.e., in terms of the direction (positive, negative, or no difference). Exceptions were found for acid phosphatases and, to a lower extent, also for glucosidases in the rhizosphere of barley. While predictions indicated higher gene abundances of the respective functional genes at the active phase than at the mature growth phase, measured enzyme activities showed an opposing pattern (Table 2).

(ii) Concordance and discordance between the measured and predicted values. To be able to quantitatively compare the patterns of predicted gene abundances and measured enzyme activities, z-transformed data were used.

The degrees of over- or underestimations of functions varied between the tested experimental conditions (Fig. 3 and Fig. S1). Regarding the wheat rhizosphere, measured enzyme activities were mostly underestimated by the predictions at the active growth phase (Fig. S1), whereby the strongest deviations were found for conventional farming (CF) under ambient-climate conditions (Fig. 3) (Tax4Fun, standard deviation [SD] = 0.51; PanFP, SD = 0.63) and organic farming (OF) under future climatic conditions (Fig. 3) (Tax4Fun, SD = 0.65; PanFP, SD = 0.54). At crop maturity, we found strong concordances between activities and predictions (Fig. S1). They were particularly strong in OF under ambient-climate conditions (Fig. 3) (SD ≤ 0.20 for both prediction tools) as well as in CF in an ambient climate (Fig. 3) (Tax4Fun, SD = 0.23; PanFP, SD = 0.36). Under future-climate conditions, the activities were underestimated in CF and overestimated in OF (Fig. 3). Overall, we found a better fit between measured activities and predictions in OF than in CF (Fig. S1) and an overall good fit for future- and ambient-climate treatments (Fig. S1).

For the barley rhizosphere, a clear pattern emerged with almost perfect fits of measured activities and predicted gene abundances at the active growth phase (Fig. 3) (SD ≤ 0.2 for both prediction tools) (Fig. S1, red lines indicate zero deviations). The strongest deviations occurred in CF under future-climate conditions, when mainly phosphatase

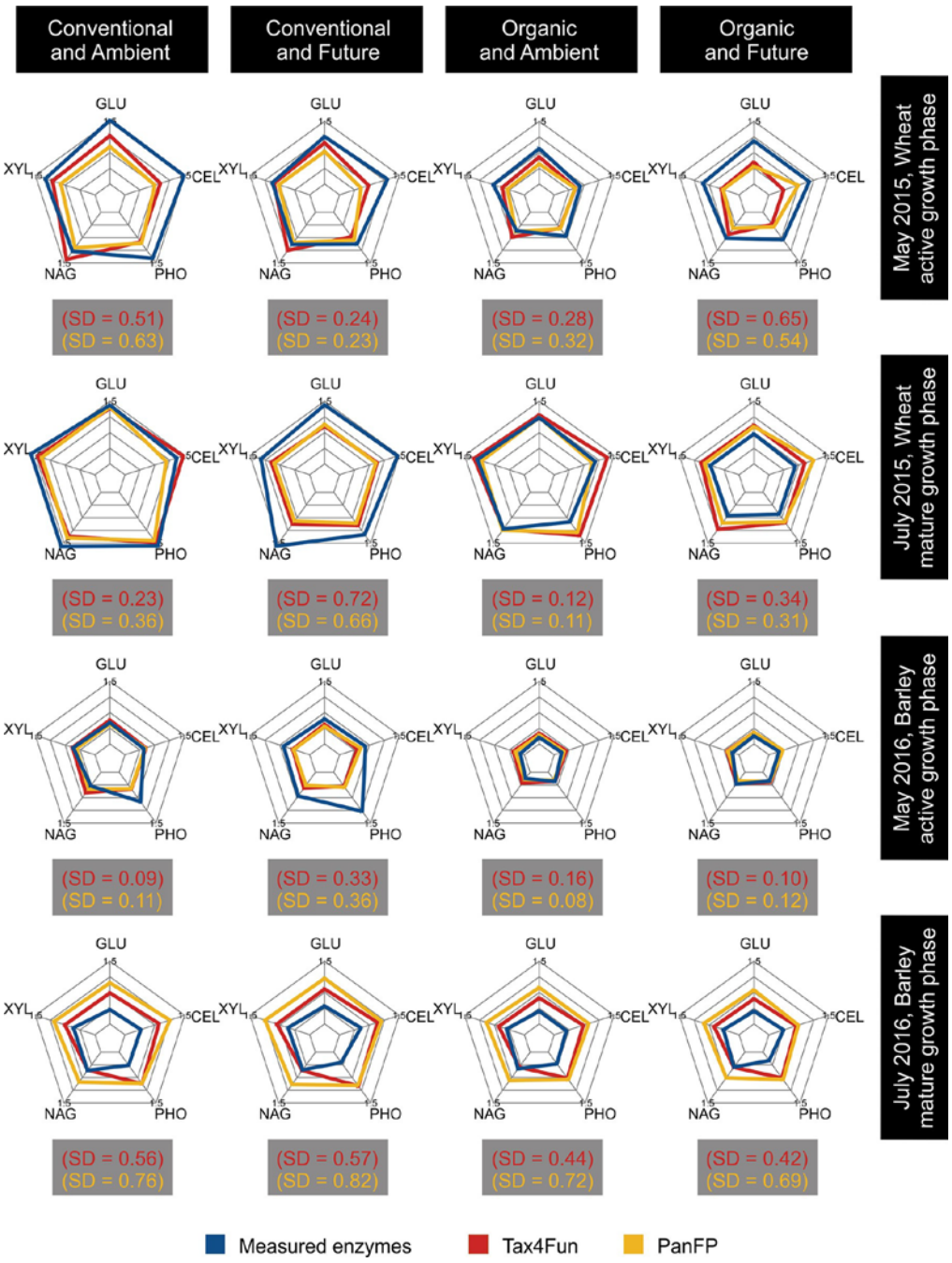


FIG 3 Measured enzyme activities and predicted functional gene abundances of Tax4Fun and PanFP, arranged by growth phases of the two crops (horizontal) and by the experimental treatment (vertical). Data were normalized by z-transformation. The spider charts represent the measured enzyme activity levels (blue), and gene abundance levels estimated by Tax4Fun (red) and PanFP (yellow). Higher values are more distant from the center of the web. The median of the standard deviations between measured and predicted values for all enzymes is given for both prediction tools separately in brackets (SD). GLU, *b*-glucosidases; XYL, xylosidases; NAG, *N*- acetylglucosaminidases (chitinases); PHO, acid phosphatases; CEL, cellobiohydrolases (cellulases).

TABLE 3 Significance of deviations in measured to predicted values with respect to the experimental factors[a]

	P value[b]								
	Growth phase			Farming system			Climate		
Enzyme	Both	Wheat	Barley	Both	Wheat	Barley	Both	Wheat	Barley
Tax4Fun vs enzymes									
Glucose	*0.01*	0.25	*<0.001*	0.44	0.41	0.87	0.52	0.63	0.52
Xylosidase	0.06	0.31	*0.02*	0.39	0.71	0.20	0.79	0.96	0.58
Chitinase	0.21	0.24	0.67	0.22	0.25	0.72	0.40	0.73	0.13
Phosphatase	*<0.001*	*0.04*	*<0.001*	0.05	0.21	0.05	0.71	0.73	0.84
Cellulase	*<0.001*	*0.02*	*<0.001*	0.15	0.16	0.54	0.10	0.18	0.20
PanFP vs enzymes									
Glucose	*<0.001*	*0.03*	*<0.001*	0.40	0.34	0.76	0.71	0.77	0.70
Xylosidase	*0.003*	0.26	*<0.001*	0.39	0.64	0.28	0.87	0.72	0.81
Chitinase	0.18	0.72	*<0.001*	0.24	0.20	0.88	0.32	0.52	0.27
Phosphatase	*<0.001*	0.15	*<0.001*	0.06	0.24	0.05	0.90	0.92	0.92
Cellulase	*0.001*	0.05	*<0.001*	0.05	*0.00*	0.90	0.74	0.80	0.37

[a]The deviation between z-transformed values of measured and predicted indices for the activity of five enzymes was calculated and tested for significance across growth phases, farming systems, and climate treatments.
[b]The *P* values are given according to ANOVA for the total data set (both wheat and barley, $n = 80$) as well as separately for wheat ($n = 40$) and barley ($n = 40$). Significant impacts according to ANOVA are indicated by italic font.

activities were heavily underestimated by the predictions (Tax4Fun, SD = 0.33; PanFP, SD = 0.36). At the mature growth phase of barley, enzyme activities were overestimated by the predictions (Fig. 3 and Fig. S1), except for chitinases, where Tax4Fun predictions perfectly matched measured activities (Fig. 3). Overall, we observed less deviations of the predicted from the measured values in OF than in CF (Fig. S1), while the deviations were similar across the two climate treatments (Fig. S1).

Testing the effect of different treatments on deviations, growth phase definitely exerted the strongest impact on the concordance between predicted and measured enzyme indices for individual enzymes in the wheat rhizosphere and for all of them in the barley rhizo- sphere (Table 3). Thereby, variance partitioning revealed that 22% of the total variations in the deviations can be explained by this treatment. In contrast, the experimental factors farming system and climate did not significantly affect the accuracy of the predictions (Table 3) (analysis of variance [ANOVA], P . 0.05; variance partitioning, 0.9% and 0.1%), and *P values* were smaller for farming system than for climate (Table 3). The decreasing differences in deviations of predictions from measured values, growth phase . farming system . climate treatment, was more obvious in the barley than in the wheat rhizosphere (Table 3).

DISCUSSION

Structure and function of microbial communities in agroecosystems are affected by different drivers. Plant growth phase was the most prominent driver of community composition in the rhizospheres of wheat and barley. This result agrees with findings of Houlden et al. (48) and Francioli et al. (3), who demonstrated a strong shift in rhizobacterial community composition according to plant growth stages in crop plants such as wheat, pea, and sugar beet (48) but also in grassland species (3). Such differences can be explained by quantitative and qualitative changes in rhizodeposition that are related to the different plant development phases (46, 49).

In contrast, measured enzyme activities were mainly driven by farming practice and associated differences in mineral nitrogen and total C and N contents (see results in Material S1 in the supplemental material). Farming practice is an important driver of soil enzyme activity, which drastically changes soil structure (50, 51) and soil chemical parameters (25). We found higher enzyme activities in CF than in OF. In line with that, Arcand and colleagues (52) observed increased activity and production of enzymes in conventional farming soil compared to that in organic farming soil. The pattern may be caused by a higher availability of nitrogen in CF, which is known to foster the production and activity of polysaccharide-degrading enzymes (53–55).

Our results revealed that shifts in composition and functions of the rhizobacterial com- munity are caused by different drivers, indicating a decoupling of community composition and function. In concordance, Francioli et al. (56) found that mineral and organic fertilizers mainly affect either activity or composition of the microbiome in an agricultural soil. Additionally, Bowles et al. (57) indicated that structurally highly similar bacterial communities can show very contrasting enzyme activities in differently managed organic fields.

Measured enzyme activities partly confirmed by predictions. The predicted gene abundances in our study responded to the drivers of both community composition and activity and were thus affected by the growth stage and by the farming system (Table S2, Fig. S2). In line with the first hypothesis, the performance of the prediction tools was driver dependent, whereby the strongest deviations could be related to the crop growth phase, followed by farming system and climate treatment. Furthermore, a remarkable impact of the crop species on the level of concordance between the predicted and measured activities was indicated.

For barley, in accordance with hypothesis two, strong correlations and concordances between measured activities and predicted gene abundances of both tools were observed at the active growth phase. In contrast, the enzyme activities were overestimated by both prediction tools at the mature growth phase. This finding is likely based on a faster response of the community composition than of enzyme activity. To promote plant growth, plants exudate carbon compounds into their rhizosphere that stimulate growth and activity of soil microorganisms (58 and reviewed in reference 59). Depending on plant development stage, root exudation patterns differ and thus strongly influence the rhizobacterial community (9, 46). Root exudation of carbon-rich compounds (sugar) is at its strongest in the juvenile growth phase, represented by the active growth phase of this experiment, and decreases thereafter (46). The overestimation of activity by the prediction tools at the mature growth phase of barley may therefore be a result of accumulated, mostly inactive rhizobacterial genes.

Deviation patterns of measured and predicted activities were more heterogeneous in the wheat rhizosphere. Three possible reasons may explain the discordance between predictions and measured activities:

(i) A major impact on enzyme activities was attributed to soil mineral N concentrations, which were positively correlated (Material S2) and significantly higher in the rhi- zosphere of wheat than in that of barley (Material S1). This relation has already been demonstrated for activities of cellulase and *b*-glucosidase (54, 60) as well as of acid phosphatase (61) and chitinase (62). The high mineral N concentration in the wheat rhizosphere likely fostered enzyme production without microbial growth, especially at the early active growth phase, and may explain deviations between measured activ- ities and predictions.

(ii) For wheat, we observed an interaction effect of the experimental climate and farming system treatment on enzyme activities, which was not reflected in the predictions. Supporting our fourth hypothesis, we observed strong deviations under a future-climate condition at the mature growth phase and under organic farming conditions also at the active growth phase of wheat. With conventional farming at the active growth phase of wheat, the pattern of deviations was inverse. As such a pattern was not observed in the barley rhizosphere, this may indicate a plant-specific drought effect on the enzyme activity that could not be mirrored by the prediction tools. When Kosová et al. (63) summarized the knowledge about wheat and barley responses to drought, they stated no strong advantages or disadvantages for either of the two but variations along different genotypes. It is therefore likely that the cultivated wheat ge- notype expressed a different adaption capacity to drought than the barley genotype, which may feedback to structure and functionality of the rhizosphere community and thus functional predictions.

(iii) Indicator species analysis and a bipartite network indicated a clear effect of the growth phase for barley, which was also the main driver for community composition (Material S3). In contrast, the impact of wheat growth phase on indicator species was

surpassed by farming system, as we found high numbers of shared indicator species between active and mature growth phases under conventional farming conditions. Thus, the contradicting drivers for overall community and indicator species in the wheat rhizosphere may contribute to deviations between predictions and measured activities.

Interestingly, and against our assumptions of hypothesis three, farming system-related deviations between measured and predicted values were not obvious either in the wheat or in the barley rhizosphere. An explanation could be the normalization of the predicted functional gene abundances with 16S rRNA gene abundances, which were strongly biased by farming system. Another possibility would be that the effect of farming system was outcompeted by the overall stronger effect of growth phases. Besides the deviations between measured and predicted values, the correlations along the treatments and relative changes between factors of treatments were not affected, suggesting that the prediction tools mirrored the impacts of the experimental factors in a qualitative way.

Limitations. (i) Prediction tools. Although the applied tools were created for universal use, their predictive power depends on the quality of the databases. Another commonly used prediction tool, PiCrust (phylogenetic investigation of communities by reconstruction of unobserved states [64]), is tailored to functional predictions in the human microbiome by using the Integrated Microbial Genomes database (65) containing genomes from the Human Microbiome Project (The Human Microbiome Jumpstart Reference Strains Consortium [66]) and Greengenes database (67). For soil microbiomes, Tax4Fun (36) and PanFP (37) outperform predictions of PiCrust (36, 37). These tools rely on the bacterial sequences of the SILVA database (68) which comprises quality-controlled aligned rRNA gene sequences. Nevertheless, all tools are subject to some restrictions which have to be considered for analysis.

First, the contribution of other organisms, including plants and fungi, to the extracellular enzyme production cannot be estimated by the tools. While extracellular enzymes in the soil are mainly attributed to origination from edaphic microorganisms and the contribution of plants may be thus negligible (39, 69), the contribution of fungal communities is of considerable importance, especially in extensively managed grassland and forest ecosystems (70). For comparison of predicted and measured activities, we therefore performed our study in agricultural systems which are known to be dominated by bacterial communities (41, 42).

Second, the predictive power of the tools relies on the integrity of the databases. SILVA (68) and KEGG Orthology (KO) (71) databases facilitate annotation of bacteria preferably to the genus level (36), thereby loosing information about functional differences on species level. Aßhauer et al. (36) further noticed that the members of the highly diverse soil community are not sufficiently represented in the KEGG database. Since publication of the prediction tools in 2015, the SILVA and KO databases have been updated frequently. Notwithstanding, the tools do not implement the latest versions of KO and SILVA databases. While PanFP is based on SILVA v128 (released in 2017), Tax4Fun supports only SILVA v123 (released in 2015). The KO database has been licensed and, thus, allows only free access to version 64.0 released in October 2012. In our analysis, we used the latest applicable versions for Tax4Fun and PanFP analysis. For Tax4Fun predictions, we were able to trace back the percentage of bacterial sequences used for predictions and the distribution of KO identifiers representing the five enzymatic classes among samples (Fig. S3). Only 37% and 32% of bacterial sequences could be used for the functional predictions in the rhizospheres of wheat and barley, respectively. Regarding indicator species, the numbers improved to at least 43% for wheat and 42% for barley. It would, therefore, be desirable to integrate updated databases and more recent metagenomic data to improve predictions.

Third, the expected discrepancy between gene presence and expression is a major constraint. Next-generation sequencing does not discriminate between the fractions of living and dead cells. Furthermore, in a given soil, only a certain proportion of

microorganisms are active at a certain time point (reviewed in references 72). The iden- tity and number of active or inactive taxa depend on external conditions and stimuli (73). In our study, we specifically investigated the impact of land use/management, cli- mate, and plant growth. Our results imply that the various proportions of active taxa and the contributions of dead organisms may partly explain discrepancy between measured and predicted activities and may be the main reason for the missing quanti- tative concordance.

(ii) Evaluation of enzyme activities as functional indicators. Besides database-related deficiencies, discrepancies in the comparisons may also emerge due to the approach used to determine enzyme activities. The measurement of soil enzyme activ- ities cannot distinguish between recently secreted enzymes and enzymes that were produced earlier by taxa whose relative abundance may have declined. These so-called abiontic enzymes are protected against their degradation in clay complexes (39). The persistence and accumulation of enzymes in the soil matrix may strongly influence overall enzyme activity measurement. The amount of immobilized enzymes strongly depends on the soil type and respective clay and organic matter content (74). We found a strong relationship between 16S rRNA gene abundances and enzyme activities at the active growth phase of both crops but a decrease of enzyme activity accompa- nied by an increase of gene abundances in the mature growth phase. This indicates a minor role of abiontic enzymes for the measured activities and, rather, suggests differ- ent root exudation rates at active and mature growth phases as the main driver of enzyme secretion.

A second methodological aspect that should be considered is that we determined enzyme activities under standardized conditions which are close to the optima of the different enzymes (pH 5, 25°C). Moreover, the high substrate concentration in the assay (300 *m*M) ensured no limitation due to substrate availability (75). While the used temperature (25°C) represents reasonable daytime temperatures at the sampling dates in late May and July, the used pH was much lower than the average from all samples (pH 6.3). Since we did not compare absolute values but only differential expression of enzymes, and the pH was comparable between all treatments, differences along the treatments should be maintained. In contrast, farming system-specific substrate availability may result in different enzyme patterns than those obtained under saturating substrate concentrations. These methodological issues plead for further studies measuring enzyme activities at realistic temperature and pH as well as particularly under substrate concentrations reflecting the availability in the respective system.

Conclusions and perspectives. Our results demonstrate that Tax4Fun and PanFP provide cost-effective tools to estimate functional patterns of rhizobacterial communities in a qualitative (i.e., direction of response) but not in a quantitative (i.e., extent of response) way. The response of the studied activities to experimental drivers was predominantly predicted correctly by both tools in terms of direction, i.e., increase or decrease. This is particularly noteworthy, since drivers of community structure and measured activities differed from each other. Moreover, we observed a gradual decrease in predictability the faster the treatments acted on community structure. The tools do not provide a one-size-fits-all solution, and interpretation of predicted functions has to be performed thoroughly. To trace mechanisms behind concordances and discordances, a deeper understanding of the underlying drivers of functions and structure is necessary. We emphasize the importance of more studies on predicted and measured functional traits to explore relevant drivers for functions and structure in different environments. These studies should be combined with transcriptomics data to explore whether the link between predicted and real soil processes will be strengthened, as assumed for this study, or, rather, diminished.

Nevertheless, our finding provides a valuable validation of the applicability and robustness of these prediction tools for comparison of soil community functions across stable soil ecosystems. While the enzymes used in this approach address solely activities related to nutrient cycling, the positive validation would plead for further research

on the possibility to predict other, more intractable functions of microbial communities in a simple and quick way.

MATERIALS AND METHODS

Soil sampling and sample preparation. Samples were obtained from the Global Change Experimental Facility (GCEF) situated at the research station in Bad Lauchstädt, Central Germany (51°23′35″N 11°52′55″E, 118 m above sea level [a.s.l.]). The site is characterized by a temperate climate with an average temperature of 9.7°C (1993 to 2013) and a mean annual precipitation of 525 mm (1993 to 2013). The soil type is a fertile loamy soil (haplic chernozem) (76). The experimental platform of the GCEF was established in 2013 and combines land use and climate treatments as described by Schädler et al. (44). In our study, we focused on the cultivated cropland systems, organic farming (OF; 10 plots) and conventional farming (CF; 10 plots). The grown crop was identical for CF and OF in 2015 (winter wheat) and 2016 (winter barley) but differed in 2014 (CF, winter rape seed; OF, field bean). In CF, synthetic fertilizers (N, P, and K), growth regulators, and pesticides are applied. The use of pesticides in OF is restricted, and fertilization is realized by including legumes in the crop rotation as well as by the application of rock phosphate (P-Ca-Mg) and patent kali (K-Mg-S) every 3 years. Half of the plots experience ambient climate (A), while the other half is exerted to simulated future-climate conditions (F) comprising a warming (+0.55°C on average) and a changed precipitation pattern (-20% in summer, +10% in spring and fall) (for details refer to Schädler et al. [44]). Cereal roots with closely adherent soil were sampled in the active cereal growth phase in May and at the mature state in July in 2015 (3 wheat plants per plot, 20 plots) and 2016 (3 barley plants, 20 plots). Samples were transported in cooling boxes to the field station and immediately frozen. Simultaneous to root sampling, surrounding bulk soil was sampled for the analysis of soil parameters. For this, six soil cores (diameter [Ø] 15 mm, 0- to 15-cm depth) were taken from each plot, pooled, sieved to 2 mm, manually cleaned from organic material, and frozen at -20°C.

To separate rhizosphere soil from roots, the roots with adherent soil were crushed and put in 50-ml Falcon tubes with 40 ml of 0.5% NaCl solution. Tubes were vortexed for 1 min to loosen adherent soil from the roots. Subsequently, roots were transferred to a second set of 50-ml Falcon tubes. Soil suspensions without roots were centrifuged at 12,851 × *g* for 10 min. Then, the supernatants were filled into the tubes with the roots and used for a second washing step. After vortexing, soil suspensions were transferred into the Falcon tubes with the pellets from the first washing step. The procedure of washing and centrifuging was repeated three times. Rhizosphere soil pellets were frozen at -20°C.

Soil parameters. Since the amount of rhizosphere soil was limited to 2 to 3 g per sample, basic soil parameters were determined using respective bulk soil. For pH analysis, 12 g of air-dried soil was suspended in 30 ml of 0.01 M $CaCl_2$ solution (1:2.5 [wt/vol]). The soil suspension was equilibrated at room temperature and thoroughly mixed every 20 min. After 1 h, the pH was measured with a pH electrode. Total carbon and nitrogen contents were determined from air-dried soil using an elemental analyzer (Elementar Vario EL III; Elementar, Hanau, Germany). For analysis of mineral nitrogen, 5 g of fresh soil was suspended in 20 ml of 1 M KCl solution and measured via flow injection analysis (FIAstar 5000; Foss GmbH, Rellingen, Germany). Available phosphorus was extracted from fresh soil with double lactate (1:50 [wt/vol], pH 3.6) and quantified using the colorimetrical molybdenum blue method (77).

Soil enzymes. The activity potentials of hydrolytic soil enzymes were measured using a modified fluorometric assay introduced by Sinsabaugh et al. (78). The analyzed enzymes are involved in phosphorus acquisition (phosphatases), nitrogen acquisition (*N*-acetylglucosaminidases), and carbon acquisition (*b*-glucosidases, xylosidases, and cellobiohydrolases). Enzymatic activities were determined as turnover rate of 4-methylumbelliferon (MUF)-coupled substrates (Table S3 in the supplemental material), where the amount of released fluorescent MUF was directly related to enzymatic activity potentials. To avoid underestimation of enzyme activities (79), the substrate concentration was optimized for the haplic chernozem soil and set to 300 *m*M for all substrates.

For each sample, a black 96-well microplate was prepared containing all five substrates, MUF dilutions to calculate quench and extinction coefficients (1.25 *m*M and 2.5 *m*M), and the substrate and soil suspension controls. For analysis, approximately 250 mg of fresh rhizosphere soil was suspended in 50 ml of 50 *m*M acetate buffer (pH 5) and sonicated for 5 min to break up soil aggregates. Subsequently, the soil suspension was added to the substrates and incubated at 25°C for 60 min. The enzyme reaction was stopped by the addition of 1 M NaOH solution. After 3 min, fluorescence was measured for eight replicates using an Infinite 200 PRO instrument (Tecan Group Ltd., Männedorf, Switzerland) with 360-nm excitation and 465-nm emission filters. Enzyme activity was calculated as turnover rate of substrate in nanomoles per gram dry soil per hour (nmol g $soil^{-1}$ h^{-1}) (80).

DNA extraction and next-generation sequencing (Illumina MiSeq). The extraction of soil bacterial genomic DNA was performed using the PowerSoil DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). The protocol was slightly modified by increasing the soil amount from 250 to 400 mg. The concentration of extracted DNA was examined with a NanoDrop ND-8000 spectrophotometer (Thermo Fischer Scientific, Dreieich, Germany), and the DNA was then stored at 220°C. Before running the PCR, the concentrations of DNA extracts were adjusted to 10 to 15 ng/*m*l. The amplification of the bacterial 16S rRNA gene V4 region was performed with the universal primers 515f and 806r (81), which were equipped with Illumina adapter sequences. To ensure correct amplification of the sequences, all PCRs were performed using proofreading KAPA HiFi polymerase (KAPA Biosystems, Boston, MA, USA). The conditions of the PCR are summarized in Table S4 (PCR 1).

PCR products were tested by gel electrophoresis and purified using the Agencourt AMPure XP kit (Beckmann Coulter, Krefeld, Germany). To assign the sequences to the respective samples, Illumina Nextera XT indices were attached to both ends of the bacterial fragments in a second PCR. The

conditions of the index PCR are presented in Table S4 (PCR 2). PCR products were purified using AMPure beads, and DNA was quantified with the PicoGreen assay (Molecular Probes, Eugene, OR, USA). For an equimolar representation of each sample, defined volumes of prepared bacterial amplicon libraries (corresponding to 80 ng DNA for each sample) were pooled in one tube. The fragment sizes and the quality of DNA sequencing libraries were again checked with the Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Sample libraries and PhiX control libraries were denatured according to the protocol of the MiSeq v3 reagent kit and diluted to a final concentration of 10 pM. Denatured and diluted libraries were combined to a volume of 600 *m*l (30 *m*l of PhiX control library, and 570 *m*l of bacte- rial amplicon library) and loaded onto MiSeq v3 reagent cartridge for sequencing. Finally, paired-end sequencing of 2 by 300 bp was implemented on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the Department of Soil Ecology of the Helmholtz Centre for Environmental Research (UFZ, Halle, Germany).

Bioinformatics workflow, functional predictions, and normalization. In total, 13,912,979 demultiplexed sequencing reads were processed using an in-house pipeline (described in reference 82, with modifications) based on mothur (83) and OBITools (84). In brief, reads without the 515f and 806r primers were discarded, and the primers were clipped from the remaining sequences using cutadapt (85). Read pairs were assembled using PANDAseq (86) and quality trimmed to an average Phred score of 26, retaining 59% of the reads. After preclustering at 99% identity using CD-HIT-454 (87), chimeric reads were removed using the UCHIME algorithm (88), and the remaining reads were clustered into operational taxonomic units (OTUs) at 97% identity using vsearch (89). To safeguard against artifacts, singletons were removed corresponding to 2.5% of the reads. The representative reads of all OTUs were examined for chimeric reads, which were removed using the UCHIME algorithm. Subsequently, the representative reads were taxonomically assigned based on the reference sequences from the SILVA database (version 128, nonredundant at 99% [90]) using the mothur implementation of the naive Bayesian classifier (91). OTUs of chloroplasts and mitochondria and those not assigned to the kingdoms *Bacteria* or *Archaea* were removed. The final sequencing depth per sample (± standard deviation) was 96,000 ± 14,000 reads.

Functional predictions of the bacterial communities were performed with two programs, PanFP (37) and Tax4Fun (36), working on the basis of the OTU abundance and taxonomy data. Tax4Fun (36) and PanFP (37) create functional profiles of bacterial communities using two related approaches for the analyses. Tax4Fun assigns operational taxonomic units (OTUs) to reference sequences in the SILVA database (SILVA database [68]) and converts the counts of SILVA-labeled OTUs to a taxonomic profile of organisms in the KEGG database (71). PanFP creates pangenomes of taxonomically related genomes with their identity also obtained via the SILVA database and subsequently weighs the pangenome's functional profile with OTU abundances. The authors' instructions were followed to run Tax4Fun program line in R (version 3.4.0 [92]) using the latest supported version of SILVA database (SILVA123, released July 2015). Taxonomic assignment was adapted for Tax4Fun analysis to SILVA123. PanFP was executed on a suitably formatted OTU table. The output tables provided KEGG orthology (KO) numbers for gene annotations and Enzyme Commission (EC) number as object identifier for enzymes. Gene abundances of the enzymes of interest were extracted from the output tables of Tax4Fun and PanFP predictions (Table S5). Gene abundances of the three acid phosphatases and the two *b*-glucosidases, which belong to the same enzymatic class according to EC numbers, were summed up from the Tax4Fun and PanFP output tables, respectively, for further analysis.

Both programs provide abundance estimates of functional genes which are compromised by methodical restraints of 16S rRNA gene sequencing. To allow a balanced reading, the input samples had to be adjusted to a certain DNA concentration, vanishing actual differences between samples. To correct predictions of gene abundances for biomass differences in the samples (36, 37), we estimated bacterial DNA concentrations of rhizosphere samples by quantitative real-time PCR (qPCR) analysis. In conformity with the Illumina sequencing, the reactions were performed with the primer pair 515f and 806r (81) tar- geting the 16S rRNA gene V4 region of the bacterial genomes. All samples were diluted to 2-ng/*m*l DNA input concentrations, as measured by the PicoGreen assay (Molecular Probes, Eugene, OR, USA), and the dilution factor was recorded. As reference, a dilution series of 0.05, 0.125, 0.5, 1, and 4 ng/*m*l genomic DNA of a *Phyllobacterium* isolate from the same soil was prepared to generate a standard curve. Quantitative PCR was run with the Bio-Rad iCycler (Bio-Rad Laboratories GmbH, Munich, Germany) under the conditions listed in Table S4 (qPCR). Measured threshold cycle (*CT*) values of the rhizosphere samples were related to the standard curve to calculate the mass of bacterial DNA. Subsequently, the obtained DNA concentrations were multiplied with the dilution factors to yield the relative bacterial DNA concen- trations for each sample. The obtained values were used as factors to normalize gene abundances in each sample and are further given as DNA concentrations in micrograms per gram dry soil in Table S6.

Statistics. All analyses were performed with the open-source software R (version 3.4.0, R Core Team). The impacts of farming system, climate, and growth phases were tested separately for OTU data to identify the main drivers of the bacterial community. The factors were then ordered by decreasing impact. PERMANOVA ("adonis" R package vegan) was run, separately for crop species, using the following model: Bray-Curtis dissimilarity (OTU table) ::: growth phase × farming system × climate. PERMANOVA ("adonis," R package vegan) was also performed to analyze the influence of soil abiotic parameters on community structure. The stratification by crop species ensured permutations only within groups of samples belonging to wheat or barley rhizosphere. To visualize significant grouping factors of bacterial community composition, principal-coordinate analysis (PCoA) was performed. For this, absolute abundances of each OTU were normalized to the total read counts in the samples, a Bray-Curtis dissimi- larity matrix was calculated, and the first two axes of the PCoA were plotted.

Indicator species analysis was performed to identify OTUs that were either specific or shared between wheat and barley among the two growth stages and the two farming systems. According to Hartman et al. (93), we applied two different approaches to test for indicator species using R (version 3.4.0 [92]). The correlation-based approach calculates point-biserial correlation coefficients (R package indicspecies [94]) indicating positive associations of OTUs to one or various conditions of farming system and plant growth phases. Associations were considered significant at a *P* value of ,0.05. A likelihood ra- tio test evaluated differences in abundances of OTUs between plant growth phases in the two different farming systems (R package edgeR [95]). Differences in abundances were considered significant by a false-discovery rate (FDR)-corrected *P* value of ,0.05. OTUs, when confirmed by both tests to be signifi- cant, were regarded as indicator species and further implemented in bipartite network analysis. The net- work was constructed with the R package igraph (93, 96).

Subsequently, Fisher tests were performed identifying enrichments of phyla within the indicator species compared to the overall community composition in wheat and barley rhizospheres to examine if indicator species were a random subset of the overall community (R package rcompanion [97]).

The measured enzyme activities of glucosidases, xylosidases, chitinases, phosphatases, and cellulases were evaluated using the following linear model: test variable ::: (crop species) × farming system × growth phase × climate.

The crop species was included when analyzing effects across both years/crop species. To test for significant impacts of single factors and for interaction effects, but also for the influence of abiotic soil parameters, an ANOVA was run with the respective models followed by Tukey's honestly significant differ- ence (HSD) *post hoc* tests. Significance levels were classified as highly significant (P , 0.001), strongly significant (P , 0.01), significant (P , 0.05), and tendency (P , 0.1.).

Spearman rank correlation tests were applied to assess correlations of measured enzyme activities with the respective predicted gene abundances within climate and farming system treatments and also within and among the different growth phases. To compare expression levels of measured and predicted enzyme activities, values were z-transformed. The differences between measured and predicted Tax4Fun and PanFP, as well as relative changes between factors of the treatment, growth phase, farming system, and climate, were calculated for each enzyme. Significant deviations within and among experimental factors tested with ANOVA and variance partitioning are given (R package *vegan*).

Data availability Demultiplexed sequences are accessible in the Sequence Read Archive under BioProject accession PRJNA605022. The pipeline used for analysis of metabarcoding raw read libraries is available at https://github.com/lentendu/DeltaMP. Tax4Fun and PanFP are open access, author's descriptions can be found for Tax4Fun under http://tax4fun.gobics.de/ and for PanFP under https://github.com/srjun/PanFP.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 2.1 MB.

ACKNOWLEDGMENTS

We thank the Helmholtz Association, the Federal Ministry of Education and Research, the State Ministry of Science and Economy of Saxony-Anhalt, and the State Ministry for Higher Education, Research and the Arts Saxony to fund the Global Change Experimental Facility (GCEF) project. We also thank the staff of the Bad Lauchstädt Experimental Research Station (especially Ines Merbach and Konrad Kirsch) and Martin Schädler for their work in maintaining the plots and infrastructures of the GCEF as well as Harald Auge, Stefan Klotz, and Martin Schädler for their role in setting up the GCEF. We thank Beatrix Schnabel for performing the sequencing. We thank Guillaume Lentendu for establishing the sequence processing workflow. The community composition data were computed at the High-Performance Computing (HPC) Cluster EVE, a joint effort of both the Helmholtz Centre for Environmental Research—UFZ and the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig.

Claudia Breitkreuz was supported by funding from the Deutsche Bundesstiftung Umwelt (DBU; AZ 20015/391) and sequencing costs by the Helmholtz Centre for Environmental Research—UFZ. Anna Heintz-Buschart was funded by the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig of the German Research Foundation (FZT 118, 202548816). Sara Fareed Mohamed Wahdan is financially supported by the Egyptian Scholarship (Ministry of Higher Education, external missions 2016/2017 call).

F.B., M.T., T.R., A.H.-B., and C.B. conceived and designed the experiment. T.R. and C.B. performed the field experiments. C.B., T.R., and S.F.M.W. performed the laboratory work. A.H.-B. ran bioinformatics. C.B. and A.H.-B. analyzed data. Results were interpreted by

M.T., T.R., F.B., A.H.-B., and C.B. M.T., T.R., A.H.-B., and C.B. wrote the manuscript with input from F.B. and S.F.M.W.

REFERENCES

1. Liu W, Li L, Khan MA, Zhu F. 2012. Popular molecular markers in bacteria. Mol Genet Microbiol Virol 27:103–107. https://doi.org/10.3103/S0891416812030056.
2. Raja HA, Miller AN, Pearce CJ, Oberlies NH. 2017. Fungal identification using molecular tools: a primer for the natural products research community. J Nat Prod 80:756–770. https://doi.org/10.1021/acs.jnatprod.6b01085.
3. Francioli D, Schulz E, Buscot F, Reitz T. 2018. Dynamics of soil bacterial communities over a vegetation season relate to both soil nutrient status and plant growth phenology. Microb Ecol 75:216–227. https://doi.org/10.1007/s00248-017-1012-0.
4. Wolsing M, Priemé A. 2004. Observation of high seasonal variation in community structure of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle manure by determining T-RFLP of *nir* gene fragments. FEMS Microbiol Ecol 48:261–271. https://doi.org/10.1016/j.femsec.2004.02.002.
5. Berg G, Smalla K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x.
6. Burns JH, Anacker BL, Strauss SY, Burke DJ. 2015. Soil microbial community variation correlates most strongly with plant species identity, followed by soil chemistry, spatial location and plant genus. AoB Plants 7:plv030. https://doi.org/10.1093/aobpla/plv030.
7. Ehrenfeld JG, Ravit B, Elgersma K. 2005. Feedback in the plant-soil system. Annu Rev Environ Resour 30:75–115. https://doi.org/10.1146/annurev.energy.30.050504.144212.
8. Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G. 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl Environ Microbiol 67:4742–4751. https://doi.org/10.1128/AEM.67.10.4742-4751.2001.
9. Micallef SA, Channer S, Shiaris MP, Colón-Carmona A. 2009. Plant age and genotype impact the progression of bacterial community succession in the *Arabidopsis* rhizosphere. Plant Signal Behav 4:777–780. https://doi.org/10.4161/psb.4.8.9229.
10. Grayston SJ, Wang S, Campbell CD, Edwards AC. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30:369–378. https://doi.org/10.1016/S0038-0717(97)00124-7.
11. Haichar FZ, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent J, Heulin T, Achouak W. 2008. Plant host habitat and root exudates shape soil bacterial community structure. ISME J 2:1221–1230. https://doi.org/10.1038/ismej.2008.80.
12. Marschner P, Solaiman Z, Rengel Z. 2005. Growth, phosphorus uptake, and rhizosphere microbial-community composition of a phosphorus-efficient wheat cultivar in soils differing in pH. J Plant Nutr Soil Sci 168:343–351. https://doi.org/10.1002/jpln.200424101.
13. Rousk J, Brookes PC, Bååth E. 2010. The microbial PLFA composition as affected by pH in an arable soil. Soil Biol Biochem 42:516–520. https://doi.org/10.1016/j.soilbio.2009.11.026.
14. Li R, Khafipour E, Krause DO, Entz MH, de Kievit TR, Fernando WD. 2012. Pyrosequencing reveals the influence of organic and conventional farming systems on bacterial communities. PLoS One 7:e51897. https://doi.org/10.1371/journal.pone.0051897.
15. Zhao Z, Ge T, Gunina A, Li Y, Zhu Z, Peng P, Wu J, Kuzyakov Y. 2019. Carbon and nitrogen availability in paddy soil affects rice photosynthate allocation, microbial community composition, and priming: combining continuous ^{13}C labeling with PLFA analysis. Plant Soil 445:137–152. https://doi.org/10.1007/s11104-018-3873-5.
16. Fleishman E, Noss RF, Noon BR. 2006. Utility and limitations of species richness metrics for conservation planning. Ecol Indic 6:543–553. https://doi.org/10.1016/j.ecolind.2005.07.005.
17. Grządziel J. 2017. Functional redundancy of soil microbiota – does more always mean better? Pol J Soil Sci 50:75. https://doi.org/10.17951/pjss.2017.50.1.75.
18. van der Voort M, Kempenaar M, van Driel M, Raaijmakers JM, Mendes R. 2016. Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. Ecol Lett 19:375–382. https://doi.org/10.1111/ele.12567.
19. Cardenas E, Tiedje JM. 2008. New tools for discovering and characterizing microbial diversity. Curr Opin Biotechnol 19:544–549. https://doi.org/10.1016/j.copbio.2008.10.010.
20. Krause S, Le Roux X, Niklaus PA, Van Bodegom PM, Lennon JT, Bertilsson S, Grossart H, Philippot L, Bodelier PLE. 2014. Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Front Microbiol 5:251. https://doi.org/10.3389/fmicb.2014.00251.
21. Torsvik V, Øvreås L. 2002. Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5:240–245. https://doi.org/10.1016/s1369-5274(02)00324-7.
22. Martiny AC. 2019. High proportions of bacteria are culturable across major biomes. ISME J 13:2125–2128. https://doi.org/10.1038/s41396-019-0410-3.
23. Steen AD, Crits-Christoph A, Carini P, DeAngelis KM, Fierer N, Lloyd KG, Thrash C. 2019. High proportions of bacteria and archaea across most biomes remain uncultured. ISME J 13:3126–3130. https://doi.org/10.1038/s41396-019-0484-y.
24. Green JL, Bohannan BJ, Whitaker RJ. 2008. Microbial biogeography: from taxonomy to traits. Science 320:1039–1043. https://doi.org/10.1126/science.1153475.
25. Bandick AK, Dick RP. 1999. Field management effects on soil enzyme activities. Soil Biol Biochem 31:1471–1479. https://doi.org/10.1016/S0038-0717(99)00051-6.
26. Caldwell BA. 2005. Enzyme activities as a component of soil biodiversity: a review. Pedobiologia 49:637–644. https://doi.org/10.1016/j.pedobi.2005.06.003.
27. García-Ruiz R, Ochoa V, Hinojosa MB, Carreira JA. 2008. Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biol Biochem 40:2137–2145. https://doi.org/10.1016/j.soilbio.2008.03.023.
28. Saleh-Lakha S, Miller M, Campbell RG, Schneider K, Elahimanesh P, Hart MM, Trevors JT. 2005. Microbial gene expression in soil: methods, applications and challenges. J Microbiol Methods 63:1–19. https://doi.org/10.1016/j.mimet.2005.03.007.
29. Yergeau E, Sanschagrin S, Maynard C, St-Arnaud M, Greer CW. 2014. Microbial expression profiles in the rhizosphere of willows depend on soil contamination. ISME J 8:344–358. https://doi.org/10.1038/ismej.2013.163.
30. Aoyagi T, Hanada S, Itoh H, Sato Y, Ogata A, Friedrich M, Kikuchi Y, Hori T. 2015. Ultra-high-sensitivity stable-isotope probing of rRNA by high-throughput sequencing of isopycnic centrifugation gradients. Environ Microbiol Rep 7:282–287. https://doi.org/10.1111/1758-2229.12243.
31. Aguiar-Pulido V, Huang W, Suarez-Ulloa V, Cickovski T, Mathee K, Narasimhan G. 2016. Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis: supplementary issue: bioinformatics methods and applications for big metagenomics data. Evol Bioinform Online 12 Suppl 1:5–16. https://doi.org/10.4137/EBO.S36436.
32. Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J, Gonzalez A, Kosciolek T, McCall L, McDonald D, Melnik AV, Morton JT, Navas J, Quinn RA, Sanders JG, Swafford AD, Thompson LR, Tripathi A, Xu ZZ, Zaneveld JR, Zhu Q, Caporaso JG, Dorrestein PC. 2018. Best practices for analysing microbiomes. Nat Rev Microbiol 16:410–422. https://doi.org/10.1038/s41579-018-0029-9.
33. Dukunde A, Schneider D, Schmidt M, Veldkamp E, Daniel R. 2019. Tree species shape soil bacterial community structure and function in temperate deciduous forests. Front Microbiol 10:1519. https://doi.org/10.3389/fmicb.2019.01519.
34. Kaiser K, Wemheuer B, Korolkow V, Wemheuer F, Nacke H, Schöning I, Schrumpf M, Daniel R. 2016. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. Sci Rep 6:33696. https://doi.org/10.1038/srep33696.
35. Stefanini I, Cavalieri D. 2018. Metagenomic approaches to investigate the contribution of the vineyard environment to the quality of wine fermentation: potentials and difficulties. Front Microbiol 9:991. https://doi.org/10.3389/fmicb.2018.00991.

36. Aßhauer KP, Wemheuer B, Daniel R, Meinicke P. 2015. Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics 31:2882–2884. https://doi.org/10.1093/bioinformatics/btv287.
37. Jun SR, Robeson MS, Hauser LJ, Schadt CW, Gorin AA. 2015. PanFP: pangenome-based functional profiles for microbial communities. BMC Res Notes 8:479. https://doi.org/10.1186/s13104-015-1462-8.
38. Jenkins DA, Sperrin M, Martin GP, Peek N. 2018. Dynamic models to predict health outcomes: current status and methodological challenges. Diagn Progn Res 2:23. https://doi.org/10.1186/s41512-018-0045-2.
39. Dick RP, Kandeler E. 2005. Enzymes in soils, p 448–455. *In* Hillel D (ed), Encyclopedia of soils in the environment. Elsevier, Amsterdam, Netherlands.
40. Baldrian P. 2019. The known and the unknown in soil microbial ecology. FEMS Microbiol Ecol 95:fiz005. https://doi.org/10.1093/femsec/fiz005.
41. de Vries FT, Thébault E, Liiri M, Birkhofer K, Tsiafouli MA, Bjørnlund L, Jørgensen HB, Brady MV, Christensen S, de Ruiter PC, d'Hertefeldt T, Frouz J, Hedlund K, Hemerik L, Hol WHG, Hotes S, Mortimer SR, Setälä H, Sgardelis SP, Uteseny K, van der Putten WH, Wolters V, Bardgett RD. 2013. Soil food web properties explain ecosystem services across European land use systems. Proc Natl Acad Sci U S A 110:14296–14301. https://doi.org/10.1073/pnas.1305198110.
42. Hendrix PF, Parmelee RW, Crossley D, Jr, Coleman DC, Odum EP, Groffman PM. 1986. Detritus food webs in conventional and no-tillage agroecosystems. Bioscience 36:374–380. https://doi.org/10.2307/1310259.
43. Kuzyakov Y, Blagodatskaya E. 2015. Microbial hotspots and hot moments in soil: concept & review. Soil Biol Biochem 83:184–199. https://doi.org/10.1016/j.soilbio.2015.01.025.
44. Schädler M, Buscot F, Klotz S, Reitz T, Durka W, Bumberger J, Merbach I, Michalski SG, Kirsch K, Remmler P, Schulz E, Auge H. 2019. Investigating the consequences of climate change under different land-use regimes: a novel experimental infrastructure. Ecosphere 10:e02635. https://doi.org/10.1002/ecs2.2635.
45. Malhi S, Johnston A, Schoenau J, Wang Z, Vera C. 2006. Seasonal biomass accumulation and nutrient uptake of wheat, barley and oat on a black chernozem soil in Saskatchewan. Can J Plant Sci 86:1005–1014. https://doi.org/10.4141/P05-116.
46. Chaparro JM, Badri DV, Vivanco JM. 2014. Rhizosphere microbiome assemblage is affected by plant development. ISME J 8:790–803. https://doi.org/10.1038/ismej.2013.196.
47. Nannipieri P, Ascher J, Ceccherini M, Landi L, Pietramellara G, Renella G. 2003. Microbial diversity and soil functions. European J Soil Science 54:655–670. https://doi.org/10.1046/j.1351-0754.2003.0556.x.
48. Houlden A, Timms-Wilson TM, Day MJ, Bailey MJ. 2008. Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. FEMS Microbiol Ecol 65:193–201. https://doi.org/10.1111/j.1574-6941.2008.00535.x.
49. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006. The role of root exudates in the rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159.
50. Kandeler E, Tscherko D, Spiegel H. 1999. Long-term monitoring of microbial biomass, N mineralisation and enzyme activities of a chernozem under different tillage management. Biol Fertil Soils 28:343–351. https://doi.org/10.1007/s003740050502.
51. Mijangos I, Pérez R, Albizu I, Garbisu C. 2006. Effects of fertilization and tillage on soil biological parameters. Enzyme Microb Technol 40:100–106. https://doi.org/10.1016/j.enzmictec.2005.10.043.
52. Arcand MM, Helgason BL, Lemke RL. 2016. Microbial crop residue decomposition dynamics in organic and conventionally managed soils. Appl Soil Ecol 107:347–359. https://doi.org/10.1016/j.apsoil.2016.07.001.
53. Henriksen T, Breland T. 1999. Nitrogen availability effects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. Soil Biol Biochem 31:1121–1134. https://doi.org/10.1016/S0038-0717(99)00030-9.
54. Shi B, Zhang J, Wang C, Ma J, Sun W. 2018. Responses of hydrolytic enzyme activities in saline-alkaline soil to mixed inorganic and organic nitrogen addition. Sci Rep 8:4543. https://doi.org/10.1038/s41598-018-22813-9.
55. Schleuss PM, Widdig M, Heintz-Buschart A, Guhr A, Martin S, Kirkman K, Spohn M. 2019. Stoichiometric controls of soil carbon and nitrogen cycling after long-term nitrogen and phosphorus addition in a mesic grassland in South Africa. Soil Biol Biochem 135:294–303. https://doi.org/10.1016/j.soilbio.2019.05.018.
56. Francioli D, Schulz E, Lentendu G, Wubet T, Buscot F, Reitz T. 2016. Mineral vs. organic amendments: microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. Front Microbiol 7:1446. https://doi.org/10.3389/fmicb.2016.01446.
57. Bowles TM, Acosta-Martínez V, Calderón F, Jackson LE. 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biol Biochem 68:252–262. https://doi.org/10.1016/j.soilbio.2013.10.004.
58. Zhang X, Dippold MA, Kuzyakov Y, Razavi BS. 2019. Spatial pattern of enzyme activities depends on root exudate composition. Soil Biol Biochem 133:83–93. https://doi.org/10.1016/j.soilbio.2019.02.010.
59. Jones DL, Hodge A, Kuzyakov Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480. https://doi.org/10.1111/j.1469-8137.2004.01130.x.
60. Geisseler D, Horwath WR. 2009. Relationship between carbon and nitrogen availability and extracellular enzyme activities in soil. Pedobiologia 53:87–98. https://doi.org/10.1016/j.pedobi.2009.06.002.
61. Piotrowska A, Wilczewski E. 2012. Effects of catch crops cultivated for green manure and mineral nitrogen fertilization on soil enzyme activities and chemical properties. Geoderma 189–190:72–80. https://doi.org/10.1016/j.geoderma.2012.04.018.
62. Banerjee S, Bora S, Thrall PH, Richardson AE. 2016. Soil C and N as causal factors of spatial variation in extracellular enzyme activity across grassland-woodland ecotones. Appl Soil Ecol 105:1–8. https://doi.org/10.1016/j.apsoil.2016.04.002.
63. Kosová K, Urban MO, Vítámvás P, Prášil IT. 2016. Drought stress response in common wheat, durum wheat, and barley: transcriptomics, proteomics, metabolomics, physiology, and breeding for an enhanced drought tolerance, p 277–314. Drought stress tolerance in plants, vol 2. Springer, Cham, Switzerland.
64. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RLV, Knight R, Beiko RG, Huttenhower C. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31:814–821. https://doi.org/10.1038/nbt.2676.
65. Markowitz VM, Chen I-MA, Chu K, Szeto E, Palaniappan K, Grechkin Y, Ratner A, Jacob B, Pati A, Huntemann M, Liolios K, Pagani I, Anderson I, Mavromatis K, Ivanova NN, Kyrpides NC. 2012. IMG/M: the integrated metagenome data management and comparative analysis system. Nucleic Acids Res 40:D123–D129. https://doi.org/10.1093/nar/gkr975.
66. Human Microbiome Jumpstart Reference Strains Consortium, Nelson KE, Weinstock GM, Highlander SK, Worley KC, Creasy HH, Wortman JR, Rusch DB, Mitreva M, Sodergren E, Chinwalla AT, Feldgarden M, Gevers D, Haas BJ, Madupu R, Ward DV, Birren BW, Gibbs RA, Methe B, Petrosino JF, Strausberg RL, Sutton GG, White OR, Wilson RK, Durkin S, Giglio MG, Gujja S, Howarth C, Kodira CD, Kyrpides N, Mehta T, Muzny DM, Pearson M, Pepin K, Pati A, Qin X, Yandava C, Zeng Q, Zhang L, Berlin AM, Chen L, Hepburn TA, Johnson J, McCorrison J, Miller J, Minx P, Nusbaum C, Russ C, Sykes SM, Tomlinson CM, Young S, et al. 2010. A catalog of reference genomes from the human microbiome. Science 328:994–999. https://doi.org/10.1126/science.1183605.
67. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072. https://doi.org/10.1128/AEM.03006-05.
68. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:7188–7196. https://doi.org/10.1093/nar/gkm864.
69. Dunn C, Jones TG, Girard A, Freeman C. 2014. Methodologies for extracellular enzyme assays from wetland soils. Wetlands 34:9–17. https://doi.org/10.1007/s13157-013-0475-0.
70. Bardgett RD, Lovell RD, Hobbs PJ, Jarvis SC. 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biol Biochem 31:1021–1030. https://doi.org/10.1016/S0038-0717(99)00016-4.
71. Kanehisa M, Goto S. 2000. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 28:27–30. https://doi.org/10.1093/nar/28.1.27.
72. Blagodatskaya E, Kuzyakov Y. 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol Biochem 67:192–211. https://doi.org/10.1016/j.soilbio.2013.08.024.
73. Wahdan SFM, Heintz-Buschart A, Sansupa C, Tanunchai B, Wu YT, Schädler M, Noll M, Purahong W, Buscot F. 2021. Targeting the active rhizosphere microbiome of *Trifolium pratense* in grassland evidences a stronger-than-

expected belowground biodiversity-ecosystem functioning link. Front Microbiol 12:73. https://doi.org/10.3389/fmicb.2021.629169.
74. Burns RG, Dick RP. 2002. Enzymes in the environment: activity, ecology, and applications. CRC Press, Boca Raton, FL.
75. Bell CW, Fricks BE, Rocca JD, Steinweg JM, McMahon SK, Wallenstein MD. 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. J Vis Exp 81:e50961. https://doi.org/10.3791/50961.
76. Altermann M, Rinklebe J, Merbach I, Körschens M, Langer U, Hofmann B. 2005. Chernozem—soil of the year 2005. J Plant Nutr Soil Sci 168:725–740. https://doi.org/10.1002/jpln.200521814.
77. Murphy J, Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.
78. Sinsabaugh R, Saiya-Cork K, Long T, Osgood M, Neher D, Zak D, Norby RJ. 2003. Soil microbial activity in a Liquidambar plantation unresponsive to CO2-driven increases in primary production. Appl Soil Ecol 24:263–271. https://doi.org/10.1016/S0929-1393(03)00002-7.
79. Nannipieri P, Gianfreda L. 1998. Kinetics of enzyme reactions in soil environment. John Wiley, New York, NY.
80. German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biol Biochem 43:1387–1397. https://doi.org/10.1016/j.soilbio.2011.03.017.
81. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci U S A 108:4516–4522. https://doi.org/10.1073/pnas.1000080107.
82. Schöps R, Goldmann K, Herz K, Lentendu G, Schoning I, Bruelheide H, Wubet T, Buscot F. 2018. Land use intensity rather than plant functional identity shapes bacterial and fungal rhizosphere communities. Front Microbiol 9:2711. https://doi.org/10.3389/fmicb.2018.02711.
83. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541. https://doi.org/10.1128/AEM.01541-09.
84. Boyer F, Mercier C, Bonin A, Le Bras Y, Taberlet P, Coissac E. 2016. obitools: a unix-inspired software package for DNA metabarcoding. Mol Ecol Resour 16:176–182. https://doi.org/10.1111/1755-0998.12428.
85. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10–12. https://doi.org/10.14806/ej.17.1.200.
86. Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. 2012. PANDAseq: paired-end assembler for Illumina sequences. BMC Bioinformatics 13:31. https://doi.org/10.1186/1471-2105-13-31.
87. Fu L, Niu B, Zhu Z, Wu S, Li W. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics 28:3150–3152. https://doi.org/10.1093/bioinformatics/bts565.
88. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200. https://doi.org/10.1093/bioinformatics/btr381.
89. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584. https://doi.org/10.7717/peerj.2584.
90. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219.
91. Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. https://doi.org/10.1128/AEM.00062-07.
92. R Core Development Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
93. Hartman K, van der Heijden MG, Wittwer RA, Banerjee S, Walser JC, Schlaeppi K. 2018. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. Microbiome 6:74. https://doi.org/10.1186/s40168-018-0456-x.
94. De Cáceres M, Legendre P, Moretti M. 2010. Improving indicator species analysis by combining groups of sites. Oikos 119:1674–1684. https://doi.org/10.1111/j.1600-0706.2010.18334.x.
95. Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. https://doi.org/10.1093/bioinformatics/btp616.
96. Csardi G, Nepusz T. 2006. The igraph software package for complex network research. InterJournal 1695:1–9.
97. Mangiafico S. 2017. Package 'rcompanion'. https://cran.r-project.org/web/packages/rcompanion/index.html.

Supplemental Material:

Can we estimate functionality of soil microbial communities from structure-derived predictions? A reality test in agricultural soils

Claudia Breitkreuz[a]#, Anna Heintz-Buschart[b,a]#, François Buscot[a,b], Sara Fareed Mohamed Wahdan[a], Mika Tarkka[a,b], Thomas Reitz[a,b]

[a]Department of Soil Ecology, UFZ – Helmholtz Centre for Environmental Research, Halle/Saale, Germany

[b]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Supplementary Tables

Table S 1 Correlations between functional gene abundances predicted by Tax4Fun and PanFP and measured enzyme activities of β-glucosidases, xylosidases, N-acetylglucosaminidases (chitinases), acid phosphatases and cellobiohydrolases (cellulases). The correlation coefficients (Spearman's rho) and respective significance levels were calculated for wheat and barley rhizospheres for combined and separated growth phases. Significant impacts according to ANOVA are indicated by italic p values and significance levels according to Spearman rank correlation test are given as follows: <0.001(***), <0.01(**), <0.05(*) and p<0.1(.).

Spearman rank correlation		Wheat						Barley					
		Total (n=40)		active (n=20)		mature (n=20)		Total (n=40)		active (n=20)		mature (n=20)	
		ρ	p value	ρ	p value	ρ	p value	ρ	p value	ρ	p value	ρ	p value
Tax4Fun vs. Enzymes	Glucosidases	0.22	0.15	0.35	0.12	0.18	0.44	*0.44*	*0.003***	*0.55*	*0.01**	*0.41*	*0.06.*
	Xylosidases	*0.45*	*0.003***	*0.41*	*0.06.*	*0.44*	*0.04**	*0.41*	*0.008***	*0.57*	*0.008***	0.16	0.49
	Chitinases	0.19	0.23	0.32	0.16	0.009	0.97	*0.41*	*0.007***	*0.40*	*0.08.*	*0.39*	*0.08.*
	Phosphatases	*0.30*	*0.05.*	0.17	0.46	*0.50*	*0.02**	0.08	0.58	*0.59*	*0.006***	0.36	0.11
	Cellulases	0.17	0.28	0.15	0.51	0.28	0.22	*0.33*	*0.03**	*0.38*	*0.09.*	*0.46*	*0.04**
PanFP vs. Enzymes	Glucosidases	0.23	0.13	0.34	0.14	0.29	0.20	*0.29*	*0.06.*	*0.51*	*0.02**	*0.46*	*0.04**
	Xylosidases	*0.45*	*0.003***	0.34	0.13	*0.53*	*0.01**	*0.33*	*0.03**	*0.42*	*0.06.*	0.24	0.29
	Chitinases	0.20	0.21	0.18	0.43	0.09	0.67	*0.41*	*0.007***	*0.38*	*0.09.*	*0.38*	*0.09.*
	Phosphatases	*0.27*	*0.08.*	0.17	0.44	*0.41*	*0.07.*	0.14	0.36	*0.60*	*0.005***	*0.45*	*0.04**
	Cellulases	0.05	0.71	0.33	0.15	-0.08	0.70	*0.27*	*0.08.*	0.33	0.15	0.27	0.24

Table S 2 Drivers of Tax4Fun- and PanFP-predicted functional gene abundances. Abundances of genes encoding for β-glucosidases, xylosidases, N-acetylglucosaminidases (chitinases), acid phosphatases and cellobiohydrolases (cellulases) were tested against the factors farming system, growth phase, climate and interaction of farming system and growth phase. Significant impacts according to ANOVA are indicated by italic p values and significance levels are given as follows: p <0.001(***), p <0.01(**), p< 0.05(*) and p<0.1(.).

Predicted: Tax4Fun		**Farming**	**Growth Phase**	**Climate**	**Growth Phase: Farming**	**Crop Species**
Wheat	Glucosidases	0.12	0.15	0.18	0.25	
	Xylosidases	0.10	*0.02**	0.13	0.27	
	Chitinases	*0.09.*	0.45	0.31	0.12	
	Phosphatases	0.10	*0.001***	*0.08.*	0.47	
	Cellulases	0.20	*0.003***	*0.06.*	0.42	
Barley	Glucosidases	*0.01**	*<0.001****	0.98	0.64	
	Xylosidases	*0.02**	*<0.001****	0.81	0.74	
	Chitinases	*0.006***	0.29	0.62	0.25	
	Phosphatases	*0.02**	*<0.001****	0.97	0.84	
	Cellulases	*0.02**	*<0.001****	0.99	0.88	
Wheat & Barley	Glucosidases					*<0.001****
	Xylosidases					*<0.001****
	Chitinases					*<0.001****
	Phosphatases					*0.004***
	Cellulases					*0.002***

Predicted: PanFP		**Farming**	**Growth Phase**	**Climate**	**Growth Phase:Farming**	**Crop Species**
Wheat	Glucosidases	0.10	*0.01**	0.21	0.46	
	Xylosidases	*0.09.*	*0.01**	0.22	0.41	
	Chitinases	0.13	*0.07.*	0.16	0.30	
	Phosphatases	0.10	*0.01**	0.16	0.42	
	Cellulases	0.87	*0.01**	0.94	0.80	
Barley	Glucosidases	*0.05.*	*<0.001****	0.89	0.97	
	Xylosidases	0.10	*<0.001****	0.96	0.91	
	Chitinases	*0.08.*	*<0.001****	0.86	0.66	
	Phosphatases	*0.06.*	*<0.001****	0.88	0.70	
	Cellulases	*0.08.*	*<0.001****	0.88	0.81	
Wheat & Barley	Glucosidases					*0.02**
	Xylosidases					*0.09.*
	Chitinases					*0.003***
	Phosphatases					*0.008***
	Cellulases					0.17

Table S3 Measured enzyme categories and respective 4-methylumbelliferon (MUF)-coupled substrates

Enzyme	Substrate
β-glucosidase (EC 3.2.1.21)	4-MUF-β-D-glucopyranoside
cellobiohydrolase (EC 3.2.1.91)	4-MUF-β-D-cellobioside
xylosidase (EC 3.2.1.37)	4-MUF-β-D-xylopyranoside
N-acetylglucosaminidase (EC 3.2.1.14)	4-MUF-N-acetyl-β-D-glucosaminide
acid phosphatase (EC 3.1.3)	4-MUF-phosphate

Table S4 Settings of PCRs used for next generation sequencing with Illumina and qPCR. Conditions for amplification of 16S rRNA gene region (PCR 1), index PCR (PCR 2) and qPCR are listed.

	Step	Temperature (°C)	Time (min:sec)
PCR 1			
	Initial denaturation	95	**3:00**
25 cycles	Denaturation	98	**0:20**
	Annealing	55	**0:15**
	Elongation	72	**0:15**
	Final extension	72	**5:00**
PCR 2			
	Initial denaturation	95	**3:00**
8 cycles	Denaturation	98	**0:30**
	Annealing	55	**0:30**
	Elongation	72	**0:30**
	Final extension	72	**5:00**
qPCR			
	Initial denaturation	95	**5:00**
40 cycles	Denaturation	95	**0:10**
	Annealing	**55**	**0.30**

Table S5 Enzyme categories extracted from output of Tax4Fun and PanFP prediction tools. The respective KEGG orthology (KO) identifiers and Enzyme Commission (EC) identifiers for each enzyme are given.

KO	Description (EC number)
K05349	beta-glucosidase [EC:3.2.1.21]
K05350	beta-glucosidase [EC:3.2.1.21]
K01198	1,4-beta-xylosidase [EC:3.2.1.37]
K01205	alpha-N-acetylglucosaminidase [EC:3.2.1.50]
K01078	acid phosphatase [EC:3.1.3.2]
K03788	acid phosphatase (class B) [EC:3.1.3.2]
K09474	acid phosphatase (class A) [EC:3.1.3.2]
K01225	1,4-beta-cellobiosidase [EC:3.2.1.91]

Table S6 Treatment-specific 16S rDNA gene abundances (given as µg DNA per g dry soil) in the rhizosphere. Treatment-specific means (± standard deviation in brackets) of DNA concentrations were calculated for both crops separately. Different small letters indicate significant differences between the treatments (ANOVA and Tukey HSD).

Crop/ Year	Growth Phase	Farming system†	Concentrations (µg DNA per g dry soil)
Wheat 2015	Active	CF	3.13 ab (1.22)
		OF	1.94 bc (1.20)
	Mature	CF	3.63 a (1.33)
		OF	3.26 ab (1.86)
Barley 2016	Active	CF	2.01 bc (0.90)
		OF	1.25 c (0.49)
	Mature	CF	3.28 ab (1.29)
		OF	2.69 abc (0.60)

† CF = conventional farming, OF = organic farming

Supplemental Figures

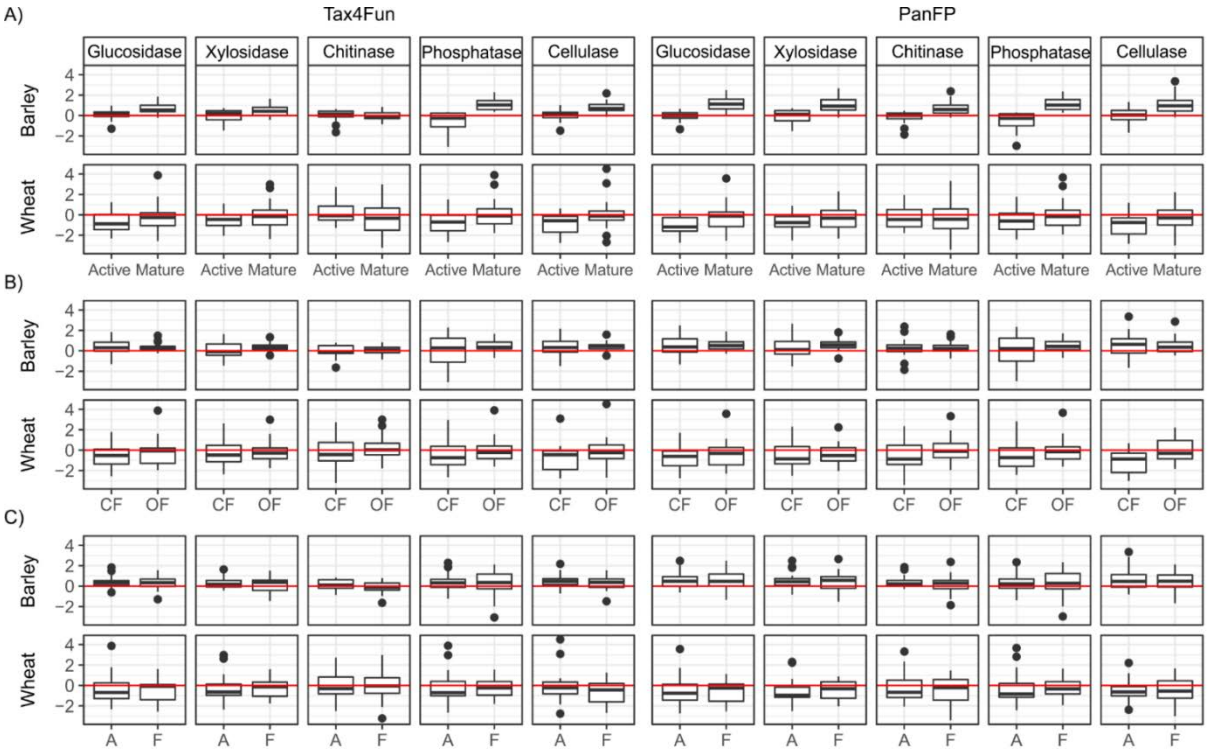


Figure S 1 Difference between measured and Tax4Fun and PanFP predicted z-transformed values. Differences are given for barley and wheat relative to A) active and mature growth phases, B) conventional (CF) and organic (OF) farming systems and C) ambient (A) and future (F) climate treatments. The red lines indicate zero deviations in levels of measured and predicted values, while positive values indicate overestimation and negative values underestimation of predictions compared to measured enzyme activities.

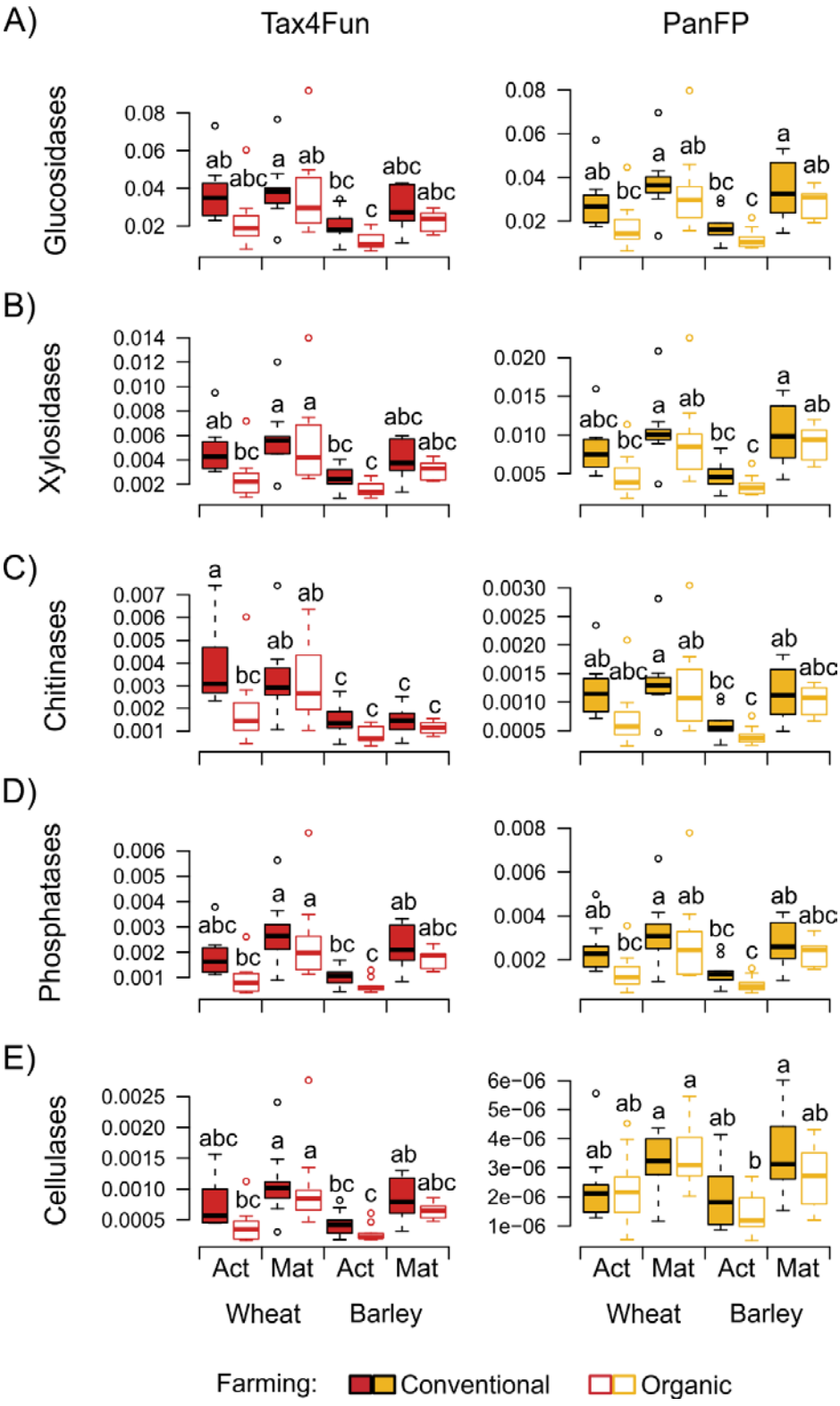


Figure S 5 Impacts of crop growth phase, farming system and crop species on predicted functional gene abundances. A) *β*-glucosidases, B) xylosidases, C) N-acetylglucosaminidases (chitinases), D) acid phosphatases, and E) cellobiohydrolases (cellulases). Predicted gene abundances by Tax4Fun (red) and PanFP (yellow) at the active (Act) and Mature (Mat) growth phases in conventional and organic farming soils are given. Different small letters within each subfigure indicate significant differences between the treatments (p<0.05) according to Tukey HSD.

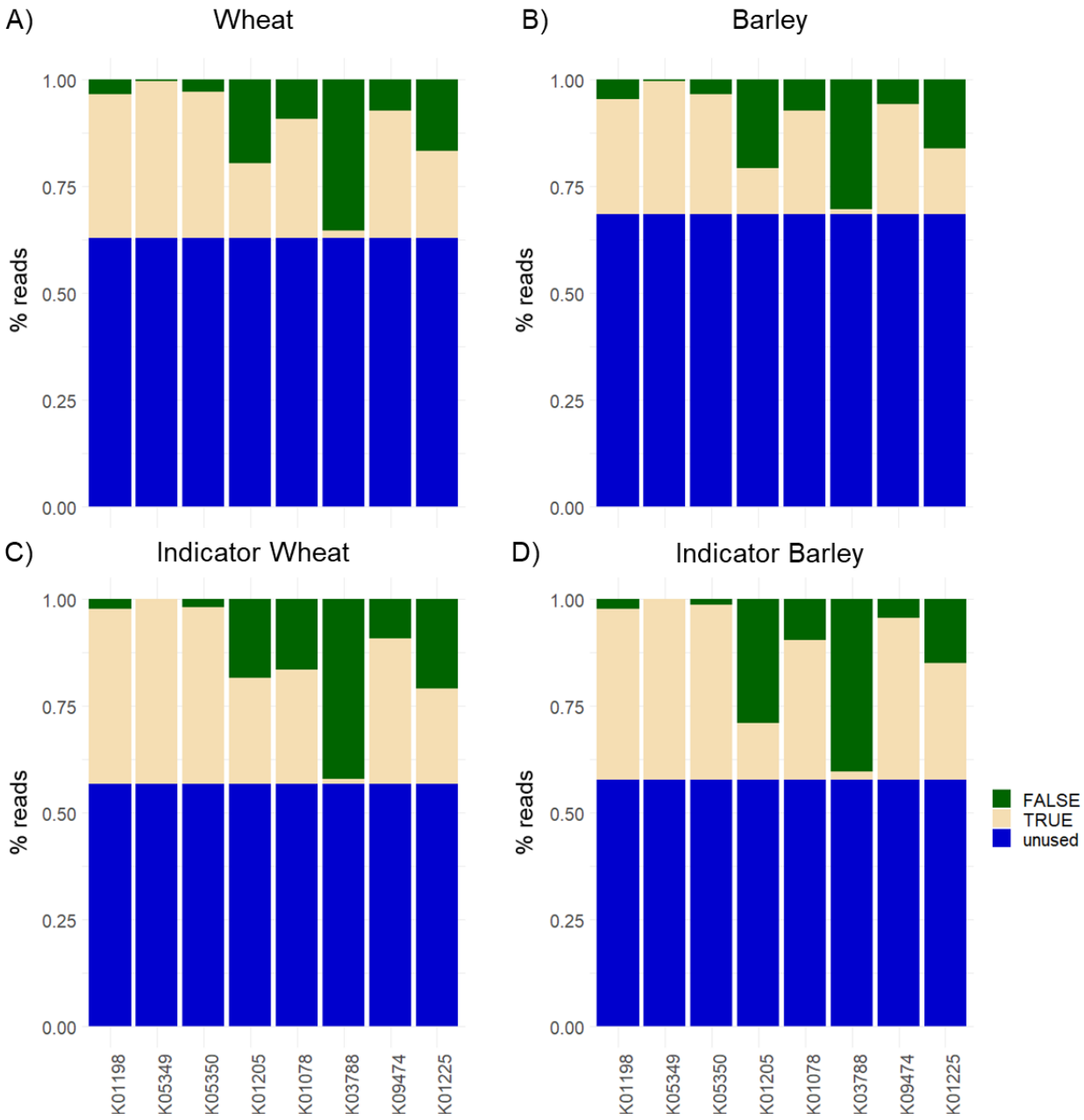


Figure S 6 Proportion of bacterial sequences used for functional predictions with Tax4Fun and containing the enzyme gens. Kegg orthology identifiers represent the five enzymatic classes referred to within this study (see Table S3 for more information on enzymes). Analysis was performed at OTU level, which were weighted by average number of reads in rhizospheres of A) wheat and B) barley, as well as by the respective indicator species abundance in C) wheat and D) barley. FALSE: gene not represented, TRUE: gene represented, unused: no information about presence or absence of gene.

Supplemental Material 1:

Variation in edaphic parameters according to experimental factors

Among the tested abiotic soil parameters, mineral nitrogen concentration (ANOVA, $F = 62.74$, $p < 0.001$) as well as total carbon (ANOVA, $F = 11.52$, $p = 0.001$) and total nitrogen content (ANOVA, $F = 7.47$, $p = 0.008$) in the soil were influenced by farming practice. Thereby, the concentrations were higher in conventional than in organic farming systems (Table SM 1). Moreover, mineral nitrogen concentration (ANOVA, $F = 51.96$, $p < 0.001$) and soil moisture (ANOVA, $F = 1894.94$, $p < 0.001$) differed significantly between the two growth phases. Mineral nitrogen concentration and soil moisture were higher at the mature than the active growth phase (Table SM 1). Measured soil moisture rather displays short-term changes due to rainfall events at sampling dates than overall climate treatment. An effect of the crop species in each year was found for total nitrogen content (ANOVA, $F = 8.20$, $p = 0.006$) and mineral nitrogen concentrations (ANOVA, $F = 43.33$, $p < 0.001$). For pH and P neither an effect of the farming system nor of crop species or growth phase could be detected.

Table SM 1 Abiotic soil parameters. Treatment-specific means (± standard deviation in brackets) are given for mineral nitrogen (N_{min}), total carbon (TC), total nitrogen (TN), pH, soil moisture, available phosphor (P_{av}). Different small letters within each column indicate significant differences between the treatments.

Crop/ Year	Growth Phase	Farming system[†]	N_{min} (mg/kg)	TC (%)	TN (%)	pH	Moisture (%)	P_{av} (mg/kg)
Wheat 2015	Active	CF	10.79 ab (5.15)	2.05 a (0.07)	0.17 a (0.01)	6.79 (0.50)	10.45 a (0.91)	82.4 (40.6)
		OF	4.35 def (0.89)	1.94 ab (0.07)	0.16 ab (0.01)	6.64 (0.56)	10.57 a (0.33)	73.4 (39.7)
	Mature	CF	13.45 a (2.31)	1.95 ab (0.20)	0.16 ab (0.02)	6.82 (0.52)	15.94 b (0.39)	83.1 (40.6)
		OF	7.49 bcd (1.27)	1.85 b (0.28)	0.14 b (0.03)	6.71 (0.59)	15.74 b (0.56)	72.4 (36.4)
Barley 2016	Active	CF	3.96 ef (1.02)	2.01 ab (0.09)	0.17 a (0.01)	6.79 (0.48)	9.85 a (0.59)	75.0 (37.3)
		OF	2.71 f (0.41)	1.92 ab (0.09)	0.16 ab (0.01)	6.66 (0.55)	10.58 a (0.75)	71.2 (40.9)
	Mature	CF	9.26 bc (2.78)	2.00 ab (0.07)	0.18 a (0.01)	6.81 (0.52)	16.08 b (0.73)	73.6 (36.9)
		OF	6.53 cde (2.26)	1.86 ab (0.09)	0.16 ab (0.01)	6.64 (0.58)	15.79 b (0.68)	70.0 (37.7)

[†] CF = conventional farming, OF = organic farming

Supplemental Material 2

Effect of abiotic soil parameters on bacterial community structure and function

Plot-specific soil pH was found to be the most prominent edaphic factor shaping rhizosphere bacterial community composition (Figure SM 1, PERMANOVA, R^2=0.20, p<0.001), followed by soil moisture (PERMANOVA, R^2=0.16, p<0.001), available phosphate concentration (PERMANOVA, R^2=0.14, p<0.001), mineral nitrogen concentration (PERMANOVA, R^2=0.05, p=0.003) and total carbon content (PERMANOVA, R^2=0.05, p=0.003). No effect of total nitrogen content on community composition was found (PERMANOVA, R^2=0.02, p=0.33).

Contrary to the community composition, enzyme activities in the rhizosphere of wheat and barley were mainly driven by mineral nitrogen and TC (Table SM 1). While the effect of mineral nitrogen was mainly pronounced in the rhizosphere of wheat, the effect of TC was mainly driving enzyme activities in the barley rhizosphere (Table SM 1). TN influenced enzyme activities of glucosidases and cellulases in the rhizospheres of wheat and barley. Soil moisture had only effects on phosphatases in the barley rhizosphere and on chitinases in the wheat rhizosphere. Available phosphate concentration and pH did not affect enzyme activities (Table SM 1).

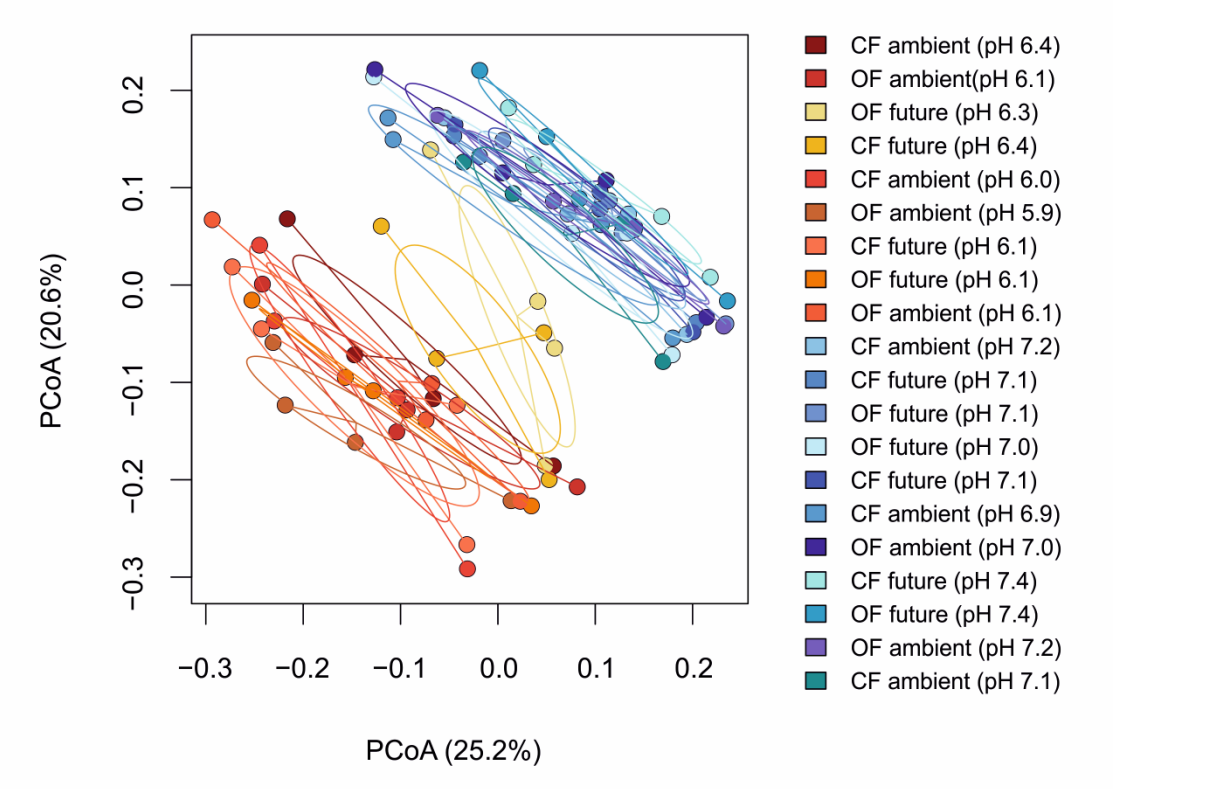


Figure SM 1 Comparison of the beta diversity of the rhizosphere bacterial communities between all plots (n = 80). Principal component analysis based on OTU abundances. Points are colored and connected according to origin of samples from the plots of the GCEF and respective treatments of farming - conventional farming (CF) and organic farming (OF) - and climate - ambient and future climate. Means of the respective pH values at the four time points sampled are given in brackets.

Table SM 1 Impact of abiotic soil parameters on rhizosphere enzyme activities. Activities of beta-glucosidases, xylosidases, N-acetylglucosaminidases (chitinases), acid phosphatases and cellobiohydrolases (cellulases) were tested against soil pH, soil moisture, mineral nitrogen (N_{min}), available phosphor (P), total carbon (TC) and total nitrogen (TN). Significant impacts according to ANOVA are indicated by italic p values and significance levels according to ANOVA are given as follows: p < 0.001(***), p < 0.01(**), p < 0.05(*) and p < 0.1(.).

Measured		pH	Moisture	N_{min}	P	TC	TN
Wheat	Glucosidases	0.50	0.60	*0.01**	0.20	*0.02**	*0.005***
	Xylosidases	0.35	*0.09.*	0.11	0.67	0.17	*0.05.*
	Chitinases	0.60	*0.01**	*0.002***	0.95	0.17	0.17
	Phosphatases	0.31	0.23	*0.005***	*0.09.*	0.11	*0.06.*
	Cellulases	0.53	0.80	*0.01**	0.28	*0.01**	*0.01**
Barley	Glucosidases	0.36	0.44	0.44	0.40	*0.002***	*0.03**
	Xylosidases	0.46	0.40	0.18	0.43	*0.01**	0.16
	Chitinases	0.58	0.51	0.21	0.92	*0.05.*	0.58
	Phosphatases	0.41	*0.001***	0.28	0.86	*0.08.*	0.92
	Cellulases	0.39	0.72	0.32	0.36	*<0.001****	*0.03**

Supplemental Material 3

Indicator species analysis

To identify OTUs that drove the observed separation according to experimental factors, indicator species analysis was performed (Figure SM 1). Over the four sampling times and in relation to the farming system treatment 950 and 564 indicator species were identified for wheat and barley, respectively. The phylum-level composition of the set of indicator species was comparable to the total community (Fisher test; Table SM 1 and SM 2). For barley, we found a clear separation between growth phases and across the two farming systems with 122 (21.6%) at active and 143 (25.4%) common indicator species at mature growth phases (Venn diagram, Figure SM 1). Only 24 (4.3%) and 14 (2.5%) indicator species were shared within CF and OF at different growth phases. In contrast, indicator species distribution for wheat showed a partially different pattern (Figure SM 1). While we observed 75 (7.9%) and 84 (8.8%) growth phase-specific shared indicator species, we identified 225 (23.7%) and 72 (7.8%) shared indicator species at different growth phases in CF and OF, respectively (Venn diagram, Figure SM 1).

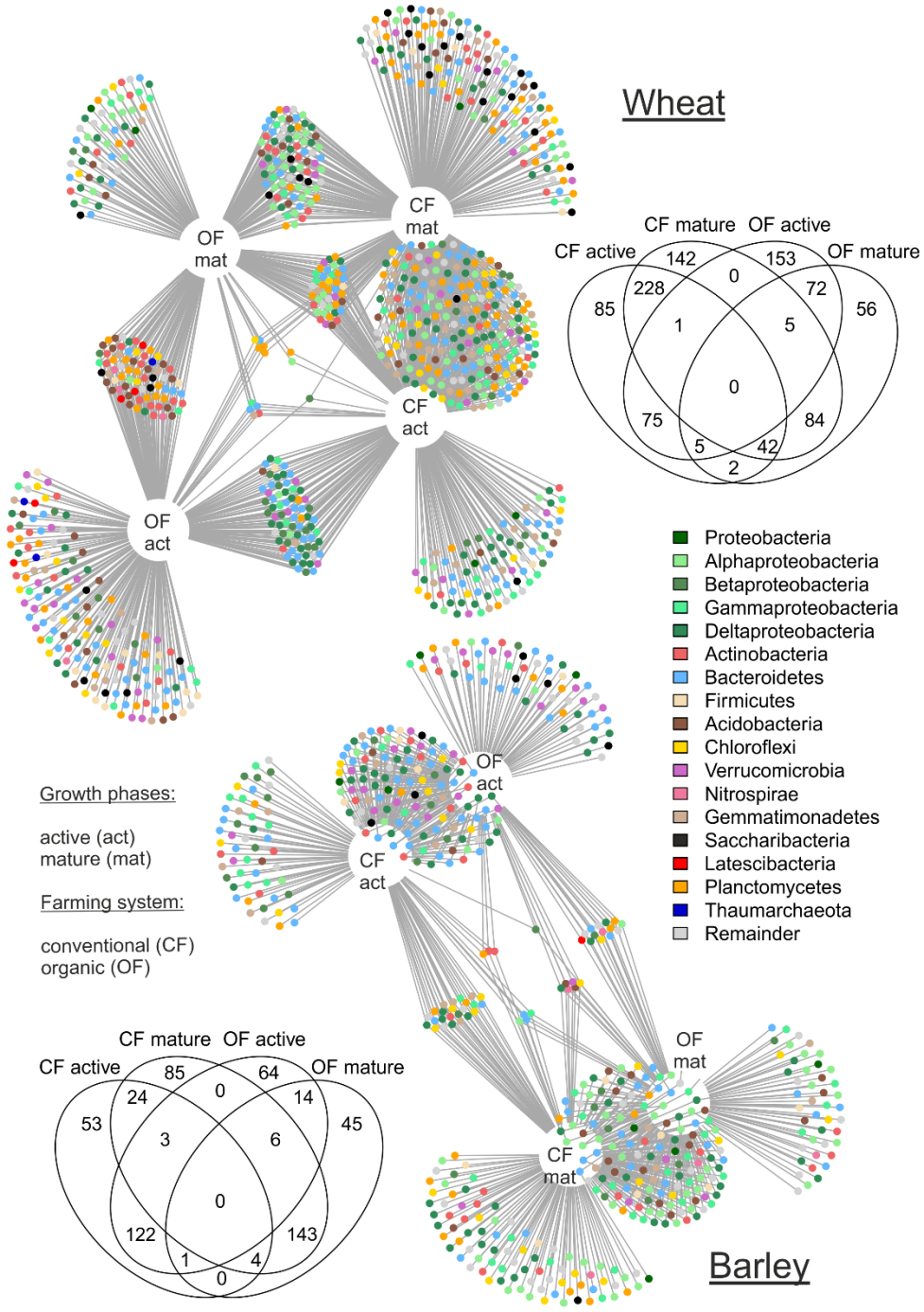


Figure SM 1 Indicator species analysis visualized as bipartite networks and Venn diagrams summarizing indicator species number and their phylogenetic assignment at different growth phases of wheat and barley in conventional and organic farming soils. Circles represent bacterial OTUs significantly associated with single or combined treatments according to consistent results of correlation and likelihood ratio tests ($p<0.05$). Circles are colored according to the phylum. Remainder comprises low abundant species (cumulative cut-off was set to 98 %).

Table SM 2 Distribution of indicator species among treatments, given at the phylum level for wheat. Enrichments in the occurrence of indicator species within treatments compared to overall community in the rhizosphere of wheat (Total OTU) were estimated by Fisher test. Significant differences are given as different letters and marked by grey background.

Wheat		Indicator species (OTU)						
			Conventional Farming		Organic Farming		Shared	Shared
	Total OTU	Total	active	mature	active	mature	two	three
Acidobacteria	**7917**	55 ab	2 a	10 ab	7 a	3 a	31	2
Actinobacteria	**9965**	64 a	2 a	11 a	13 a	5 a	29	4
Alphaproteobacteria	**2916**	85 cdefg	4 abc	1 a	14 b-f	11 a	49	6
ARKDMS-49	**2**	1 a-m	1 abc	0 abcd	0 a-f	0 a	0	0
Armatimonadetes	**311**	14 cdehikl	1 abc	2 abcd	2 a-f	0 a	8	1
Bacteria_unclassified	**1167**	12 abj	1 abc	1 abc	0 abc	5 a	5	0
Bacteroidetes	**3155**	144 hikl	16 b	25 cd	21 bdef	7 a	66	9
Bathyarchaeota	**2**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Betaproteobacteria	**5458**	39 ab	5 ac	8 ab	3 a	2 a	20	1
BJ-169	**11**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
BRC1	**40**	5 chkl	1 abc	1 abcd	3 d	0 a	0	0
Candidatus_Berkelbacteria	**81**	0 a-jm	0 abc	0 abcd	0 a-f	0 a	0	0
Chlamydiae	**105**	0 abd-gijm	0 abc	0 abcd	0 a-f	0 a	0	0
Chlorobi	**44**	7 k	0 abc	0 abcd	1 a-f	1 a	4	1
Chloroflexi	**2539**	40 jm	4 abc	9 abcd	4 abc	2 a	19	2
Cyanobacteria	**109**	11 kl	1 abc	0 abcd	4 de	1 a	5	0
Deferribacteres	**1**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Deinococcus-Thermus	**17**	1 a-m	0 abc	0 abcd	0 a-f	0 a	1	0
Deltaproteobacteria	**2275**	118 hikl	17 b	12 bcd	12 b-f	9 a	65	3
Elusimicrobia	**130**	5 c-m	0 abc	3 bcd	0 a-f	0 a	2	0
Euryarchaeota	**32**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
FBP	**58**	2 a-m	0 abc	0 abcd	1 a-f	0 a	0	1
FCPU426	**4**	1 a-m	0 abc	1 abcd	0 a-f	0 a	0	0
Fibrobacteres	**91**	4 a-m	1 abc	1 abcd	1 a-f	0 a	1	0
Firmicutes	**705**	20 cdefghijm	1 abc	11 d	4 a-f	0 a	3	1
Fusobacteria	**2**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
GAL15	**4**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Gammaproteobacteria	**2886**	53 dfjm	9 bc	6 abc	2 ac	3 a	29	4
Gemmatimonadetes	**1486**	54 ceghil	9 bc	8 abcd	4 abcf	1 a	30	2
Gracilibacteria	**13**	1 a-m	0 abc	0 abcd	0 a-f	0 a	1	0
Hydrogenedentes	**15**	2 a-m	0 abc	0 abcd	1 a-f	0 a	1	0
Ignavibacteriae	**1**	1 a-m	0 abc	1 abcd	0 a-f	0 a	0	0
JTB23	**1**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Latescibacteria	**115**	5 c-m	0 abc	2 abcd	0 a-f	0 a	3	0
Lentisphaerae	**1**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Microgenomates	**30**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Nitrospirae	**545**	4 abjm	0 abc	1 abcd	0 abcf	0 a	3	0
Omnitrophica	**48**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Parcubacteria	**173**	0 abfgjm	0 abc	0 abcd	0 a-f	0 a	0	0
Peregrinibacteria	**40**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Planctomycetes	**2056**	112 hikl	3 abc	16 cd	22 def	3 a	55	13
Proteobacteria_unclassified	**193**	5 a-m	1 abc	0 abcd	2 a-f	2 a	0	0
RBG-1_(Zixibacteria)	**1**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Saccharibacteria	**1307**	30 cdefgjm	1 abc	5 abcd	15 def	1 a	8	0
Spirochaetae	**3**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
SR1_(Absconditabacteria)	**11**	1 a-m	0 abc	0 abcd	0 a-f	0 a	1	0
Synergistetes	**1**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Tectomicrobia	**53**	2 a-m	0 abc	0 abcd	0 a-f	0 a	2	0
Tenericutes	**15**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Thaumarchaeota	**361**	3 abdefgjm	0 abc	2 abcd	0 abcef	0 a	1	0
TM6_(Dependentiae)	**121**	2 a-m	0 abc	1 abcd	0 a-f	0 a	1	0
Verrucomicrobia	**1811**	47 cdefgm	5 abc	15 cd	6 abcef	0 a	18	3
Woesearchaeota_(DHVEG-6)	**14**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
WS2	**20**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
WWE3	**8**	0 a-m	0 abc	0 abcd	0 a-f	0 a	0	0
Total	**48462**	**950**	**85**	**153**	**142**	**56**	**461**	**53**

Table SM 3 Distribution of indicator species among treatments, given at the phylum level for barley. Enrichments in occurrence of indicator species within treatments compared to overall community in the rhizosphere of barley (Total OTU) were estimated by Fisher test. Significant differences are given as letters and additionally marked by grey background.

Barley		Indicator species (OTU)						
			Conventional Farming		Organic Farming		Shared	Shared
	Total OTU	Total	active	mature	active	mature	two	three
Acidobacteria	**8463**	23 a	1 a	1 a	0 a	3 a	16	2
Actinobacteria	**9218**	35 a	3 ab	6 abc	4 ab	3 a	17	2
Alphaproteobacteria	**3085**	63 b-g	1 abc	14 de	2 abcd	10 a	35	1
ARKDMS-49	**2**	1 a-j	0 abc	0 a-f	1 bcd	0 a	0	0
Armatimonadetes	**346**	8 b-j	1 abc	0 a-f	3 bcd	2 a	2	0
Bacteria_unclassified	**1281**	20 bcefhi	1 abc	4 abcde	6 cd	2 a	7	0
Bacteroidetes	**2881**	93 dgj	11 c	5 abcd	14 c	5 a	56	2
Bathyarchaeota	**1**	0 a-j	0 abc	0 a-f	0 abcd	0	0	0
Betaproteobacteria	**5369**	26 a	9 abc	1 ab	1 ab	1 a	13	1
BJ-169	**12**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
BRC1	**43**	1 a-j	0 abc	1 a-f	0 abcd	0 a	0	0
Candidatus_Berkelbacteria	**111**	1 a-j	0 abc	0 a-f	0 abcd	1 a	0	0
Chlamydiae	**122**	0 abcdhi	0 abc	0 a-f	0 abcd	0 a	0	0
Chlorobi	**48**	3 b-hj	0 abc	3 ef	0 abcd	0 a	0	0
Chloroflexi	**2683**	30 bhi	3 abc	8 cd	0 abd	2 a	16	1
Cyanobacteria	**125**	11 j	1 abc	5 f	0 abcd	1 a	4	0
Deferribacteres	1	0 a-j	0 abc	0 a-f	0 abcd	0	0	0
Deinococcus-Thermus	**18**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Deltaproteobacteria	**2723**	79 cdeg	2 abc	14 def	8 bcd	5 a	49	1
Diapherotrites	**2**	0 a-j	0 abc	0 a-f	0 abcd	0	0	0
Elusimicrobia	**137**	2 a-j	0 abc	0 a-f	0 abcd	0 a	2	0
Epsilonproteobacteria	**1**	0 a-j	0 abc	0 a-f	0 abcd	0	0	0
Euryarchaeota	**36**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
FBP	**59**	2 a-j	2 abc	0 a-f	0 abcd	0 a	0	0
FCPU426	**4**	1 a-j	0 abc	0 a-f	1 abcd	0 a	0	0
Fibrobacteres	**118**	3 a-j	0 abc	0 a-f	0 abcd	1 a	2	0
Firmicutes	**698**	12 b-i	0 abc	2 a-f	1 abcd	1 a	8	0
Fusobacteria	**2**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
GAL15	**6**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Gammaproteobacteria	**3009**	46 bfhi	9 bc	7 bcd	3 abcd	4 a	22	1
Gemmatimonadetes	**1600**	20 bhi	4 abc	0 abcd	0 abcd	2 a	14	0
Gracilibacteria	**26**	1 a-j	0 abc	0 a-f	0 abcd	0 a	1	0
Hydrogenedentes	**16**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Ignavibacteriae	**1**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
JTB23	**1**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Latescibacteria	**126**	1 a-j	0 abc	0 a-f	0 abcd	0 a	1	0
Lentisphaerae	**1**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Microgenomates	**36**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Nitrospirae	**620**	5 abhi	0 abc	1 abcde	0 abcd	1 a	2	1
Omnitrophica	**65**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Parcubacteria	**329**	2 abcdhi	0 abc	0 a-f	0 abcd	0 a	2	0
Peregrinibacteria	**72**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Planctomycetes	**2165**	28 bhi	3 abc	9 de	6 bcd	0 a	9	1
Proteobacteria_unclassified	**242**	7 b-hj	0 abc	1 a-f	1 abcd	1 a	4	0
RBG-1_(Zixibacteria)	**1**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Saccharibacteria	**1256**	8 ahi	0 abc	1 abcd	4 bcd	0 a	3	0
Spirochaetae	**5**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
SR1_(Absconditabacteria)	**16**	3 efgj	0 abc	1 a-f	1 abcd	0 a	1	0
Synergistetes	**1**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Tectomicrobia	**59**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Tenericutes	**10**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Thaumarchaeota	**376**	0 ai	0 abc	0 abcde	0 abcd	0 a	0	0
TM6_(Dependentiae)	**138**	1 a-i	0 abc	0 a-f	0 abcd	0 a	1	0
Verrucomicrobia	**1935**	28 bfhi	2 abc	1 abcd	8 cd	0 a	16	1
Woesearchaeota_(DHVEG-6)	**22**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
WS2	**24**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
WWE3	**14**	0 a-j	0 abc	0 a-f	0 abcd	0 a	0	0
Total	**49747**	**564**	**53**	**85**	**64**	**45**	**303**	**14**

CHAPTER 3

Shifts Between and Among Populations of Wheat Rhizosphere Pseudomonas, Streptomyces and Phyllobacterium Suggest Consistent Phosphate Mobilization at Different Wheat Growth Stages Under Abiotic Stress

Claudia Breitkreuz, François Buscot, Mika Tarkka, Thomas Reitz

Frontiers in Microbiology (2020), published

Accepted: December 23rd 2019

frontiers in Microbiology

ORIGINAL RESEARCH
published: 22 January 2020
doi: 10.3389/fmicb.2019.03109

Shifts Between and Among Populations of Wheat Rhizosphere *Pseudomonas*, *Streptomyces* and *Phyllobacterium* Suggest Consistent Phosphate Mobilization at Different Wheat Growth Stages Under Abiotic Stress

Claudia Breitkreuz[1]*, *François Buscot*[1,2], *Mika Tarkka*[1,2] and *Thomas Reitz*[1,2]

[1] Department of Soil Ecology, UFZ – Helmholtz Centre for Environmental Research, Halle/Saale, Germany, [2] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

OPEN ACCESS

Edited by:
Yusuke Saijo,
Nara Institute of Science and Technology (NAIST), Japan

Reviewed by:
Usman Irshad,
COMSATS University Islamabad, Abbottabad Campus, Pakistan
Kei Hiruma,
Nara Institute of Science and Technology (NAIST), Japan

***Correspondence:**
Claudia Breitkreuz
claudia.breitkreuz@ufz.de

Specialty section:
This article was submitted to Plant Microbe Interactions, a section of the journal Frontiers in Microbiology

Received: 18 March 2019
Accepted: 23 December 2019
Published: 22 January 2020

Citation:
Breitkreuz C, Buscot F, Tarkka M and Reitz T (2020) Shifts Between and Among Populations of Wheat Rhizosphere Pseudomonas, Streptomyces and Phyllobacterium Suggest Consistent Phosphate Mobilization at Different Wheat Growth Stages Under Abiotic Stress. Front. Microbiol. 10:3109. doi: 10.3389/fmicb.2019.03109

Climate change models predict more frequent and prolonged drought events in Central Europe, which will exert extraordinary pressure on agroecosystems. One of the consequences is drought-related nutrient limitations for crops negatively affecting agricultural productivity. These effects can be mitigated by beneficial plant growth promoting rhizobacteria. In this study, we investigated the potential of cultivable bacterial species for phosphate solubilization in the rhizosphere of winter wheat at two relevant growth stages - stem elongation and grain filling stages. Rhizosphere samples were collected in the Global Change Experimental Facility in Central Germany, which comprises plots with conventional and organic farming systems under ambient and future climate. Phosphate-solubilizing bacteria were selectively isolated on Pikovskaya medium, phylogenetically classified by *16S rRNA* sequencing, and tested for *in vitro* mineral phosphate solubilization and drought tolerance using plate assays. The culture isolates were dominated by members of the genera *Phyllobacterium*, *Pseudomonas* and *Streptomyces*. Cultivation-derived species richness and abundance of dominant taxa, especially within the genera *Phyllobacterium* and *Pseudomonas*, as well as composition of *Pseudomonas* species were affected by wheat growth stage. *Pseudomonas* was found to be more abundant at stem elongation than at grain filling, while for *Phyllobacterium* the opposite pattern was observed. The abundance of *Streptomyces* isolates remained stable throughout the studied growth stages. The temporal shifts in the cultivable fraction of the community along with considerable P solubilization potentials of *Phyllobacterium* and *Pseudomonas* species suggest functional redundancy between and among genera at different wheat growth stages. Phosphate-solubilizing *Phyllobacterium* species were assigned to *Phyllobacterium*

ifriqiyense and *Phyllobacterium sophorae.* It is the first time that phosphate solubilization potential is described for these species. Since *Phyllobacterium* species showed the highest drought tolerance along all isolates, they may play an increasingly important role in phosphate solubilization in a future dryer climate.

Keywords: climate change, agriculture, wheat, PGPR, phosphate solubilization, drought tolerance

INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is the most commonly grown crop in Germany accounting for 54% of the total agricultural area in 2017 (Schmeling, 2017). At the same time wheat is the second most produced crop worldwide after maize (Statista, 2018). The production of winter wheat depends on frequent precipitation and sufficient nutrient supply (Fischer, 2003). Its cultivation is hence restricted to appropriate regions and is commonly supported by sufficient fertilization. Besides nitrogen and potassium, phosphorus (P) has to be applied, since the plant-available P in the rooted topsoil often represents a growth limiting factor. However, P fertilization does not necessarily increase P availability. Up to 90% of fertilized mineral P is quickly immobilized by reactive cations, such as Al^{3+} or Fe^{3+} in acidic or Ca^{2+} in calcareous or neutral soils (Oehl et al., 2002). At a global scale, models suggest an increase in P input into agricultural systems of 51–86% by the year 2050 (Mogollón et al., 2018) to maintain productivity. In parallel, the source for P fertilizer – natural rock phosphate – is expected to deplete in 50–100 years (Cordell et al., 2009). Regarding the application of organic fertilizers phosphorus is bound into complex compounds and first has to be released before plant uptake.

Processes that release P from mineral and organic compounds in the soil are mainly driven by microorganisms. Therefore, to counteract P limitation, plants enrich a variety of beneficial microbes in their rhizospheres by secreting nourishing, carbon-containing rhizodeposits. Besides mycorrhizal fungi, plant growth promoting rhizobacteria (PGPR) can enhance plant P uptake. PGPR thereby stimulate root growth, release P from immobilized inorganic and organic P pools in the soil, and transport solved P compounds to the plant's roots (Artursson et al., 2006). However, these mutual interactions between the plant and its rhizosphere community are highly dynamic and related to the plant growth stage as well as to environmental conditions.

Seasonal shifts in rhizosphere microbiomes related to plant growth stages are well documented for canola and grasses (Dunfield and Germida, 2003; Wolsing and Priemé, 2004; Francioli et al., 2018). The changes are mainly caused by variations in the quantity and quality of carbon-derived rhizodeposits serving as energy sources for soil microbes (reviewed in Bais et al., 2006). These rhizodeposits are predominantly released into the rhizosphere when plants are actively growing (Badri and Vivanco, 2009). Thus, flowering plants (Aulakh et al., 2001; Lucas García et al., 2001) mainly secrete root exudates during stem elongation, booting and flowering stages, and much less during fruit development and ripening stages. Malhi et al. (2006) also found stem elongation and tillering to be the stages with maximum nutrient uptake of cereals. Investigations of the wheat rhizobiome often focus on the stages of tillering and flowering, but also on grain filling (predominantly in field experiments, e.g., Juhnke et al., 1987; Creus et al., 2004; Naiman et al., 2009; Ilyas and Bano, 2010) or are independent of certain growth stages throughout the first weeks of wheat development (predominantly in pot experiments, e.g., Kasim et al., 2013; Timmusk et al., 2014). Clearly, more information on the interrelationships between plant growth stages and the PGPR are needed.

Besides the seasonal dynamics, microbial communities in agricultural soils have to cope with frequent disturbances. Agricultural management practices such as fertilization, tillage, pesticide application and crop rotation including brown fallow periods cause dramatic changes of C supply patterns within the soil rooting zone. It was shown that tillage (Frey et al., 1999; Kladivko, 2001) and crop rotation (Tiemann et al., 2015) lead to a decrease in fungal biomass (Smith and Read, 2010) and diversity (Verbruggen et al., 2010). A recent study of Banerjee et al. (2019) also demonstrated that land use intensification reduces fungal network complexity in conventional and even in no tillage land use systems. In contrary, bacterial biomass was nearly unaffected (Elliott et al., 1988), leading to establishment of a primarily bacteria-based food web in managed agroecosystems (Hendrix et al., 1986; de Vries and Shade, 2013). Therefore, we focused on the bacterial rather than the fungal community in this study.

The high adaptation potential of soil bacteria to changing conditions is related to their high growth and mutation rates, as well as rapid recombination by lateral gene transfer (Badri and Vivanco, 2009). Moreover, to maintain crucial soil processes after disturbances and under changing conditions, the huge bacterial diversity allows quick and diverse reordering of the active community (Kennedy, 1999). According to Sheik et al. (2011), stress conditions such as the combination of warming and drought effects lead to less diverse microbial populations. In contrast, functions that have to be provided for plants by microbes may be even more essential under stress conditions and a more complex population structure may arise, since more complex co-occurrence networks improve complementarity and efficiency (Tardy et al., 2014; Karimi et al., 2017). Thus, soil bacterial communities play a key role in stress resistance and recovery after disturbances of agroecosystems (Shade et al., 2012).

Most field studies on beneficial wheat rhizosphere microbes and their plant growth promoting properties have been performed in arid and semiarid regions of India (Rana et al., 2011; Jog et al., 2014; Kumar et al., 2014; Singh and Lal, 2016; Verma et al., 2016), Pakistan (Ilyas and Bano, 2010; Naveed et al., 2014)

and Argentina (Creus et al., 2004; Fischer et al., 2007). As these regions are strongly limited in water supply and therefore in the amount of available nutrients, which are solved in the soil water, the potential of PGPR to support wheat growth under drought conditions by different functional properties is of high interest. In contrast, studies in temperate zones with moderate precipitation are underrepresented, although climate change already causes obvious negative effects on agroecosystem productivity (Ciais et al., 2005; Trnka et al., 2014). For instance in 2018 East and Middle Germany experienced a severe drought period, which led to premature ripening and an early harvest of crops (BMEL, 2018). Drought along with nutrient limitation caused yield losses will likely increase in future as climate change models predict altered precipitation patterns with extended drought periods combined with higher temperatures in the vegetation period (Petersen and Weigel, 2015). Therefore, the role of soil bacteria and their potentials for tolerance and resilience of agroecosystems in the temperate zones is of increasing importance.

To understand the role of the rhizosphere bacterial community in providing P to wheat plants over the growing season, it is indispensable to identify P-solubilizing key species and their functionality in dependency on wheat growth stage while simultaneously considering the impacts of the two most important global change drivers, agricultural management and climate (Sala et al., 2000). In order to address this issue, we investigated P-solubilizing rhizosphere bacteria at two relevant wheat growth stages, namely stem elongation (vegetative biomass production) and grain filling stage (generative biomass production). Wheat samples were collected in the Global Change Experimental Facility (GCEF, Schädler et al., 2019). The design of the experimental field platform allows the comparison of two different farming systems (conventional vs. organic agriculture) exposed to either ambient climate, at present, or future expected climate scenario with warming and increased summer drought in the latter. Within this experimental setup we used a cultivation-dependent approach based on Pikovskaya medium (Pikovskaya, 1948) to isolate exclusively for P-solubilizing bacteria. We decided for tri-calcium phosphate (TCP) as insoluble phosphate source in the neutral Pikovskaya media since the pH in the plots of the GCEF is mainly neutral and reactive anion Ca^{2+} is predominantly binding mineral P in these soils forming stable complexes (Oehl et al., 2002, Bashan et al., 2013). Solubility of TCP increases with decreasing pH. Acidification by production and release of organic acids is thus one possibility to release phosphate from insoluble TCP source and is therefore an indicator for the potential of phosphate solubilization *in vitro* (Bashan et al., 2013). We compared the *in vitro* P solubilization potential and the drought tolerance of the predominantly isolated P-solubilizing taxa at the stem elongation stage (vegetative biomass production) with those present at the grain filling stage (generative biomass production) in different land use systems and under different climatic conditions. We aimed to distinguish the responses in terms of structural resistance and/or functional redundancy between and among cultivable P-solubilizing bacterial taxa. Under changing conditions, identical taxa and functions would suggest adaptation and resistance. In contrast, shifts in the cultivable fraction of the community would either reflect changes in functionality or reveal functional redundancy among P-solubilizing bacterial species.

We hypothesized that (i) the abundances of the predominant P-solubilizing taxa in the wheat rhizosphere vary between stages of wheat development, displaying a higher abundance, diversity and activity of P-solubilizing bacteria at the stem elongation stage due to higher resource demand by plants than at the grain filling stage (Malhi et al., 2006). Furthermore, we hypothesized that (ii) increased drought decreases diversity but induces targeted structural changes within the bacterial community, in a way that P solubilization is maintained. Finally, due to a lower availability of nutrients, we expected (iii) a lower abundance, but higher activity potentials of P-solubilizing bacteria in the organic farming system compared to the conventional farming system.

MATERIALS AND METHODS

Experimental Platform

The Global Change Experimental Facility (GCEF) (see http://www.ufz.de/index.php?en=40038 for general information and visualizations) was established in 2013 to investigate climate change effects on managed terrestrial ecosystems (Schädler et al., 2019). The GCEF is located at the field research station of the Helmholtz-Centre for Environmental Research (UFZ) in Bad Lauchstädt, Germany (51°23′35″N 11°52′55″E, 118 m a.s.l.). The site is characterized by a temperate climate with an average temperature of 9.7°C (1993–2013) and a mean annual precipitation of 525 mm (1993–2013). The soil type is a humus- and nutrient-rich Haplic Chernozem (Altermann et al., 2005). Besides three grassland management types, a conventional and an organic farming system are part of the GCEF design. In total, the facility includes 50 large plots (24 × 16 m) that allow realistic field management. The 50 plots are arranged in ten blocks, whereby each block comprises all five land use types that are randomly distributed (**Supplementary Figure S1**). Five blocks are subjected to ambient climatic conditions and the other five to future climatic conditions, resulting in a split-plot experimental design (**Supplementary Figure S1**). Ambient climate treatment refers to the actual and non-manipulated climate in terms of precipitation and temperature at the field site. According to the projected climate change within the next 50 years for Central Germany, the future climate treatment of the GCEF consists in increasing the mean daily temperature and shifting the annual precipitation pattern. Temperature is increased passively by roofing the plots during the night. Precipitation is modulated based on the actual weather conditions, with a 20% decrease of precipitation during summer (Jun-Aug) and a 10% increase of precipitation in spring (March–May) and autumn (September–November). In this study we focused on the conventional farming (CF) and organic farming (OF) systems. The CF includes a typical regional crop rotation consisting of a sequence of winter rape, winter wheat and winter barley. In two out of 3 years, the crop cycle for OF includes winter wheat and winter barley just as for the CF. In the first and fourth year of this bipartite crop sequence, the nitrogen-fixating legumes alfalfa and white clover are included in the crop cycle, respectively. Herbicides, fungicides, insecticides

and plant growth regulators are applied as usual in CF practice (Schädler et al., 2019). For OF only mechanical weed control and a restricted use of pesticides are allowed. Whereas N, P, and K in CF is applied in form of mineral fertilizers, N fertilization in OF is done by the inclusion of legumes in the crop rotation and further fertilization is restricted to the application of rock phosphate (P-Ca-Mg) and patent kali (K-Mg-S) in the first year of the crop cycle.

Sampling and Characterization of Soil Chemical Parameters

Rhizosphere from root systems of winter wheat was collected in organic (OF) and conventional farming (CF) plots of the GCEF exposed either to ambient (A) or simulated future (F) climate conditions. From each of these plots of the GCEF (20 plots) – CF-A (5 plots), CF-F (5 plots), OF-A (5 plots) and OF-F (5 plots) – three plants were taken with root system finalizing in 15 samples per treatment. To cover relevant growth stages for vegetative and generative biomass production over the growing season, sampling campaigns took place at the 26th of May 2015 (BBCH growth stages 37–39, representing stem elongation phase) and at the 8th of July 2015 (BBCH growth stages 75–77, representing the grain filling stage), respectively. Growth stages were identified according to BBCH-scale ("Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie"). Wheat plants were carefully removed from the soil, their root systems cut and shaken to discard unattached soil, and transferred in plastic bags on ice to the laboratory. Subsequently, rhizosphere soil was collected by gently, manually loosening the root-attached soil to avoid damages to the roots. To measure soil chemical properties, bulk soil samples were collected in parallel to rhizosphere samples. Six soil cores per plot were taken to a depth of 15 cm, pooled, sieved to 2 mm and manually cleaned from organic material. The obtained rhizosphere and bulk soil were stored frozen until usage.

Soil chemical properties of the bulk soil samples were analyzed as follows: gravimetric water content of fresh soil was quantified with a halogen moisture analysator (Mettler Toledo, Gießen, Deutschland). pH was measured with an electrode after shaking the soil for 1 h in 0.01 M CaCl2 (1:2.5 w/v). Amounts of total carbon and nitrogen content were determined from air-dried soil using an elemental analyzer (Elementar Vario EL III, Elementar, Hanau, Germany). For analysis of mineral nitrogen 5 g of fresh soil were suspended in 20 ml of 1 M KCl solution and measured per flow injection analysis (FlAstar 5000, Foss GmbH, Rellingen, Germany). Extraction of labile phosphorus in the soil was performed with double lactate solution (1:50 w/v) at pH 3.6 and determined using the colorimetrical molybdenum blue method (Murphy and Riley, 1962).

Selective Isolation and Phylogenetic Classification of Phosphate Solubilizing Bacteria From Wheat Rhizosphere

For cultivation and isolation of bacteria from wheat rhizosphere, 0.5 g of fresh rhizosphere soil was suspended in 50 ml of distilled water and stirred for 5 min to break up soil particles and bring bacteria in suspension. Subsequently, the samples were sonicated for 10 s and stirred for another 2 min. Mineral tri-calcium phosphate solubilizing bacteria were isolated from a 1:200 (v/v) dilution of this rhizosphere soil suspension by plating 50 µl of it in triplicate on Pikovskaya agar (Pikovskaya, 1948), supplemented with the fungicide cycloheximide (10 mg/ml). After 2 weeks of incubation at 25°C, colony forming units (CFU) were counted. Bacterial strains produce and release organic acids to solubilize P from insoluble tri-calcium phosphate source, therefore, forming a clear zone in the otherwise turbid agar around the colony - from heron referred to as halo. Up to 25 colonies (**Supplementary Table S4**) with the largest halos were collected for each sample from the Pikovskaya plates and transferred to yeast malt extract (YME) agar plates. To identify and store these isolates, polyethylene glycol (PEG) and glycerol stocks were prepared and frozen. Cells in the PEG stock were destroyed mechanically with glass beads by vortexing to release their DNA into the liquid. Polymerase chain reaction (PCR) was performed on the DNA obtained from the PEG stocks with the primers 27f (10 µM – 5′-AGAGTTTGATCMTGGCTCAG-3′: Lane, 1991) and 1492r (10 µM – 5′-GGTTACCTTGTTACGACTT-3′: Lane, 1991) and Promega Green (Promega, Madison, WI, United States). Partial *16S rRNA* sequencing was performed with the primer BAC 341f primer (10 µM – 5′-CCTACGGGAGGCAGCAG-3′: Muyzer et al., 1993) using Big Dye Termination Mix (GeneCust Europe, Dudelange, Luxemburg). Quality control of the sequences was done manually using Sequencher 5.4.5. Quality-checked sequences were compared with type strain reference sequences of the National Center of Biotechnology Information (NCBI), and subsequently clustered according to a level of 99.5% identity. Representative sequences of each cluster were aligned using neighbor-joining algorithms of the MAFFT server (Katoh and Standley, 2013). Phylogenetic trees were constructed and visualized along with the trait and abundance data using Evolview (Zhang H. et al., 2012). All *16S rRNA* gene sequences were deposited in the NCBI database with continuous accession numbers from MK637853 to MK638668 (**Supplementary Table S1**).

Estimating the Functional Properties of Isolated Bacteria

Pure cultures of the dominant P-solubilizing taxa were characterized by functional tests using agar plate bioassays. For each isolate, organic acid production to solubilize tri-calcium phosphate was quantitatively and qualitatively determined on Pikovskaya agar (Pikovskaya, 1948) by the formation of a clear zone in the otherwise turbid agar around the colony. Drought tolerance of the isolates was determined by comparing their growth on YME to surface polyethylene glycol 6000 (PEG) infiltrated YME. Thus, in this study drought tolerance potential of the strains was not related to the formation of dormant spores, but to their potential to maintain metabolisms and growth under water limiting conditions. To simulate severe drought stress, PEG concentration was set to 500 g/L, which

corresponds to an osmotic potential level of around −1.1 MPa (Verslues et al., 2006).

For both bioassays, the strains were grown to the end of the exponential growth phase in liquid YME and 1 µl of each liquid pre-culture was applied on the test media in triplicates. P solubilization was assessed after 14 and drought tolerance after 3 days of incubation in the dark at 25°C. Phosphate solubilization was quantified as P release derived from phosphate concentration per area of plate and valued using two complementary indices. On the one hand, P release was calculated based on the whole area including colony plus halo, representing the total amount of P released by the colony (Phosphate Solubilization Index 1, PSI 1). Since primarily the outer cells of a colony determine the distance between colony rim and halo, we further calculated P release (Phosphate Solubilization Index 2, PSI 2) based solely on the halo around the colony (halo diameter = twice the distance from colony rim to the end of the halo), representing cell-specific, rather than colony-specific P solubilization potential. Drought stress tolerance was quantified based on the percentage differences of colony diameters on PEG-YME agar, compared to control YME agar. For statistical analysis only data from bacterial strains growing on the functional media were used.

Statistical Analyses

All statistical analyses were performed in the open source program R version 3.4.0 (2017-04-21), GNU project (R Core Team, 2017). An analysis of variance (ANOVA) was run to evaluate, if the abundance and traits of P-solubilizing bacteria were influenced by climate, land use, wheat growth stage, or interaction of these factors. The ANOVA was followed by a multiple comparison analysis with Tukey HSD to identify respective differences in the means of groups. To test for differences in phosphate solubilization potentials and drought tolerances between dominant bacterial taxa, the factor taxa was added to the formula resulting in a four factorial ANOVA. Significance levels were defined according to $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$.

To test for homogeneity of multivariate dispersion of *Phyllobacterium*, *Pseudomonas* and *Streptomyces* phylogenetic clusters among and between test factors climate, land use and wheat growth stage, betadisper function (R, vegan package) was run based on a dissimilarity matrix using relative abundance (Bray Curtis) and incidence data (Jaccard). Biodiversity indices, i.e., species richness and Shannon-Weaver-index were calculated to test for impacts of climate, land use and wheat growth stage on structure and composition of cultured *Phyllobacterium*, *Pseudomonas* and *Streptomyces* species. ANOVA was applied to test for differences in cluster dispersion regarding structure and composition of *Phyllobacterium*, *Pseudomonas* and *Streptomyces* species between and among test factors.

RESULTS

Soil Chemical Parameters

Soil chemical properties of the studied samples are summarized in **Supplementary Table S2**. Since our study was performed only 2 years after establishment of the GCEF, basic soil parameters did not differ between treatments and showed comparable values of pH (6.7), total organic carbon (1.9%) and total nitrogen (0.16%). Available P and mineral N were higher in CF than in OF, but the difference was only significant for mineral N (**Supplementary Table S2**). The soil moisture differed between the ambient and future plots at the days of sampling and showed average values of 10.6 and 15.9% at stem elongation stage in May and at grain filling stage in July, respectively (**Supplementary Table S2**).

Effects of Wheat Growth Stage, Land Use and Climate on P-Solubilizing Rhizosphere Bacteria

Colony counts on Pikovskaya agar indicated higher numbers of P-solubilizing rhizobacteria at the stage of grain filling in July compared to the stage of stem elongation in May ($p < 0.001$). At both growth stages more bacteria were detected in CF than in OF (May: $p = 0.008$, July: $p = 0.03$) and under ambient climate than under future climate at grain filling stage in July ($p = 0.02$) (**Supplementary Figure S2**).

In total 410 bacterial isolates were obtained from samples collected at the stage of stem elongation and another 407 strains from samples at the grain filling stage (**Supplementary Table S4**). Within the isolates collected from the May samples at stem elongation stage, the dominant taxa (relative abundance ≥2%) belonged to the genera *Pseudomonas* (150 isolates = 37%), *Streptomyces* (81 isolates = 20%), *Phyllobacterium* (37 isolates = 9%), *Rhizobium* (20 isolates = 5%), *Mesorhizobium* (16 isolates = 4%), *Bacillus* (12 isolates = 3%) and *Agrobacterium* (10 isolates = 2%), while in the collection from July samples at the grain filling stage *Phyllobacterium* (147 isolates = 36%), *Streptomyces* (116 isolates = 29%), *Rhizobium* (24 isolates = 6%), *Agrobacterium* (20 isolates = 5%), *Pseudomonas* (17 isolates = 4%), *Mesorhizobium* (16 isolates = 4%), *Ensifer* (10 isolates = 2%) and *Bacillus* (9 isolates = 2%) (**Supplementary Table S5**) were the dominant genera. Thus, along both wheat growth stages, the taxa belonging to the genera *Phyllobacterium*, *Pseudomonas* and *Streptomyces* were found to dominate the bacterial collections, jointly accounting for 65% and 69% of the total isolates at stem elongation in May and grain filling stage in July, respectively. However, between the two plant growth stages, shifts in abundances of these dominant taxa were observed (**Figures 1A,B** and **Supplementary Table S5**). At the stage of stem elongation in May, *Pseudomonas* were predominant with 150 isolates accounting for more than one third of the bacterial isolates, whereas *Phyllobacterium* species only made up a small fraction with 37 isolates (9%). Conversely, at the grain filling stage in July, a much higher abundance of P-solubilizing *Phyllobacterium* species (147 isolates = 36%) than *Pseudomonas* species (17 isolates = 4%) was detected. Contrary to these, the abundance of *Streptomyces* isolates was less affected by the wheat growth stage, when 81 isolates (20%) and 116 isolates (29%) were obtained from stem elongation stage and grain filling stage, respectively.

Comparable abundances along the two growth stages were also found for the less abundant taxa of the genus

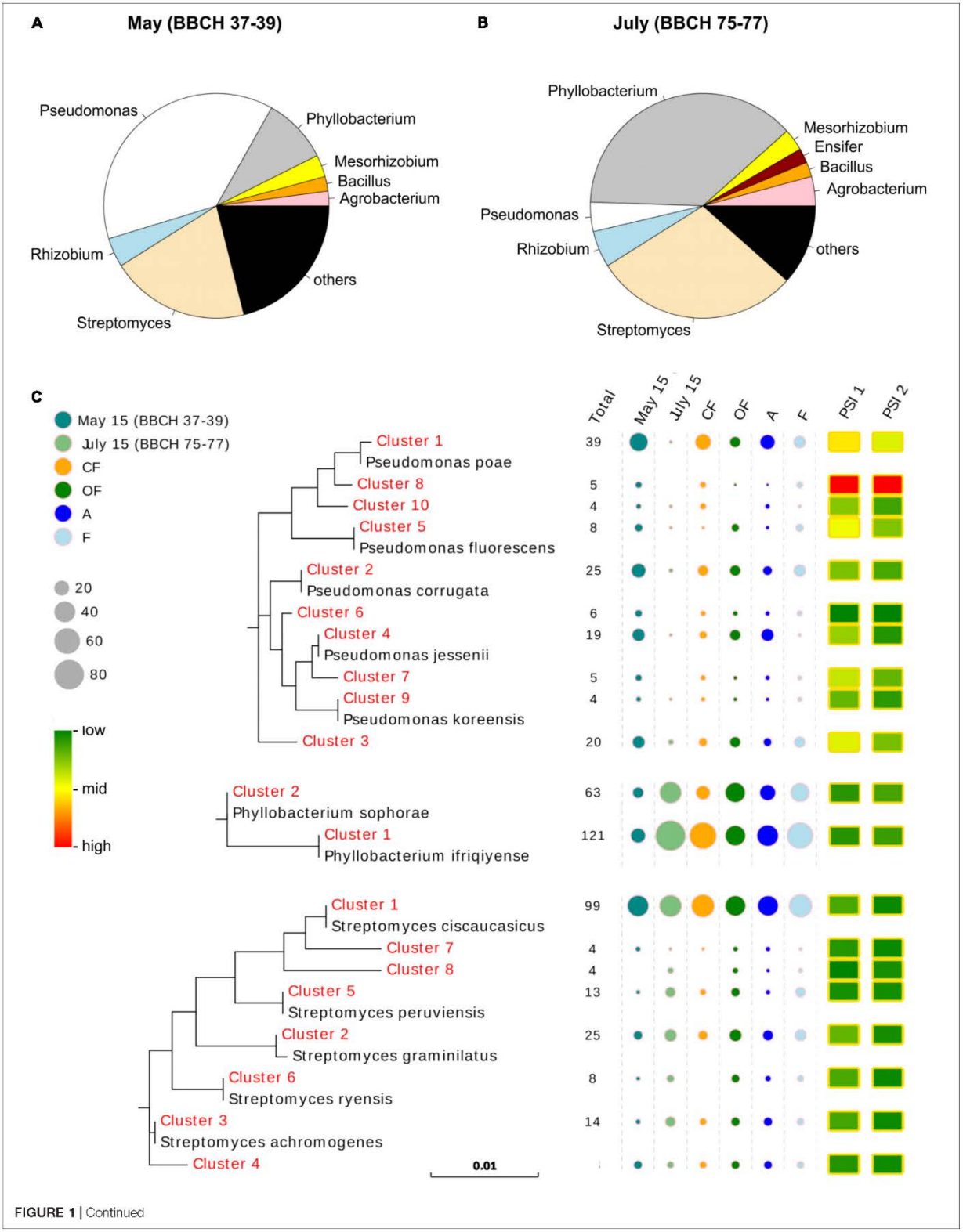


FIGURE 1 | Continued

FIGURE 1 | Identity, abundance and activity levels of cultivable mineral phosphate-solubilizing bacteria in the wheat rhizospheres. Taxa have been summarized on genus level and the most dominant ones, with a relative abundance ≥2% within the collection, are shown in the pie charts at **(A)** stem elongation stage in May 2015 and **(B)** grain filling stage in July 2015. The predominantly isolated taxa of the genus *Phyllobacterium*, *Pseudomonas* and *Streptomyces* were clustered (identity level of 99.5%) and **(C)** phylogenetic trees were created for dominant clusters (number of isolates >3) with reference sequences from NCBI. Information on abundance of the dominant clusters within treatment levels of growth stage (stem elongation in May, grain filling in July), climate (A-ambient, F-future) and land use (CF-conventional farming, OF-organic farming) are given as circles, whereas circle size is related to the number of isolates. Moreover, the average phosphate solubilizing potentials for each bacterial *16S rRNA* gene cluster were calculated for PSI 1 and PSI 2 and differences in expression levels are indicated using a heatmap.

Agrobacterium, *Bacillus* and *Rhizobium*, while the genus *Ensifer* was predominantly detected in July (**Figure 1B** and **Supplementary Table S5**). Some of the less abundant genera also showed indications for land use and climate-specific patterns. *Agrobacterium* and *Buttiauxiella* were more frequently detected in CF than in OF, whereby *Agrobacterium* spp. were mainly isolated from ambient climate plots, while *Buttiauxiella* spp. were exclusively detected in future climate plots (**Supplementary Table S5**). A comparable enrichment under future climatic conditions was also observed for *Bacillus*, and species were more often isolated from OF than from CF (**Supplementary Table S5**).

To assess species identity and trait distribution within the dominant genera, we clustered the *Phyllobacterium*, *Pseudomonas* and *Streptomyces* isolates according to their *16S rRNA* gene sequences at a similarity level of 99.5%. Phylogenetic trees were constructed with representative sequences of clusters comprising more than three isolates (**Figure 1C**), resulting in two *16S rRNA* gene clusters for *Phyllobacterium*, ten for *Pseudomonas* and eight for *Streptomyces*. The clusters of *Phyllobacterium* species were related to *Phyllobacterium ifriqiyense* (cluster 1) and *Phyllobacterium sophorae* (cluster 2). Besides the strong impact of growth stage and the fact that more isolates were assigned to cluster one than to cluster two, the abundances of the *Phyllobacterium* clusters showed divergent patterns between the two farming systems. Cluster one was more abundant in CF than in OF, while cluster two showed a contrary trend (**Figure 1C** and **Supplementary Table S6**). Phylogenetic cluster distributions of the taxa within the genus *Pseudomonas* and *Streptomyces* were more heterogeneous. *Pseudomonas* clusters grouped with *Pseudomonas poae* (clusters 1, 8, and 10), *Pseudomonas corrugate* (cluster 2), *Pseudomonas jessenii* (cluster 4 and 7), *Pseudomonas fluorescens* (cluster 5), and *Pseudomonas koreensis* (cluster 9) (**Figure 1C**). *Streptomyces* cluster 1 contained the highest number of isolates and grouped with clusters 7 and 8, and reference sequence *Streptomyces ciscaucasicus* (**Figure 1C**). The other less abundant *Streptomyces* clusters grouped with reference sequences of *Streptomyces graminilatus* (cluster 2), *Streptomyces achromogenes* (clusters 3 and 4), *Streptomyces peruviensis* (cluster 5), and *Streptomyces ryensis* (cluster 6) (**Figure 1C**). Effects of climate and land use treatments on *Streptomyces* clusters could not be detected, but for *Pseudomonas* cluster 1. Representatives of this cluster were approximately twice as often isolated from CF and plots with ambient climatic conditions compared to OF and plots with future climatic conditions (**Supplementary Table S6**).

Dispersion patterns for *Phyllobacterium*, *Pseudomonas* and *Streptomyces* clusters significantly changed over the growing season ($p = 0.001$) (**Figure 2A**). At the genus level, compositional shifts were observed among the taxa of the genus *Pseudomonas* ($p = 0.006$) and *Phyllobacterium* ($p = 0.04$), but not for *Streptomyces* (**Supplementary Figure S3**). At cluster level, a shift in the cultivable fraction of the community was detected only for clusters of genus *Pseudomonas* ($p = 0.02$). Species richness (**Figure 2B**) and Shannon-Weaver diversity index (**Figure 2C**) were found to be higher at stem elongation stage of May samples compared to grain filling stage of July samples with $p = 0.02$ and $p = 0.04$, respectively. Effects of climate or land use on dispersion patterns and diversity indices could not be observed.

P Solubilization Potentials of Key Taxa

Phosphate-solubilizing potentials of dominant taxa within the genera *Phyllobacterium*, *Pseudomonas*, and *Streptomyces* isolated from the wheat rhizospheres at the stem elongation stage in May, as well as at the grain filling stage in July are presented for PSI 1 and PSI 2 in **Figures 3A,B**, respectively. Among the taxa of the three genera, *Pseudomonas* species expressed highest P solubilization potentials irrespective of employed indices, PSI 1 (**Figure 3A**) and PSI 2 (**Figure 3B**), and at the two growth stages studied. *Phyllobacterium* species showed approximately half of the P solubilization potential of the *Pseudomonas* species at both growth stages independently of the used P solubilization index. In contrast, the assessment of the P solubilization potentials of *Streptomyces* species depended on the index used. Excluding the colony size for the assessment of P solubilization, as done in PSI 2, clearly revealed significantly lower P solubilization potentials of *Streptomyces* species compared to taxa of the two other genera ($p < 0.001$) (**Figure 3B**).

Regarding the effect of studied impact factors within the three dominant genera, neither plant growth stage (**Figure 3**), nor climate or land use affected the P solubilization potential of *Phyllobacterium* and *Streptomyces*. However, interaction effects of climate and land use were found for *Pseudomonas* species, which showed higher P solubilization potentials under future climatic conditions compared to ambient climatic conditions in CF ($p < 0.001$ for PSI 2) (**Supplementary Figure S4**). Furthermore, under future climatic conditions, P solubilization potentials of *Pseudomonas* species were significantly higher in CF compared to OF ($p < 0.001$ for both indices, **Supplementary Figure S4**).

The phylogenetic cluster-specific, mean P solubilization potentials of the genera *Phyllobacterium* and *Streptomyces* revealed consistent P solubilization potentials in either employed index (**Figure 1C** and **Supplementary Table S6**). In contrast to that, the average P solubilization potentials of the individual clusters assigned to the genus *Pseudomonas* showed a huge variance (**Figure 1C** and **Supplementary Table S6**). While, *Pseudomonas* cluster 6 showed a low P solubilization potential, the isolates assigned to cluster 8 expressed the highest average P

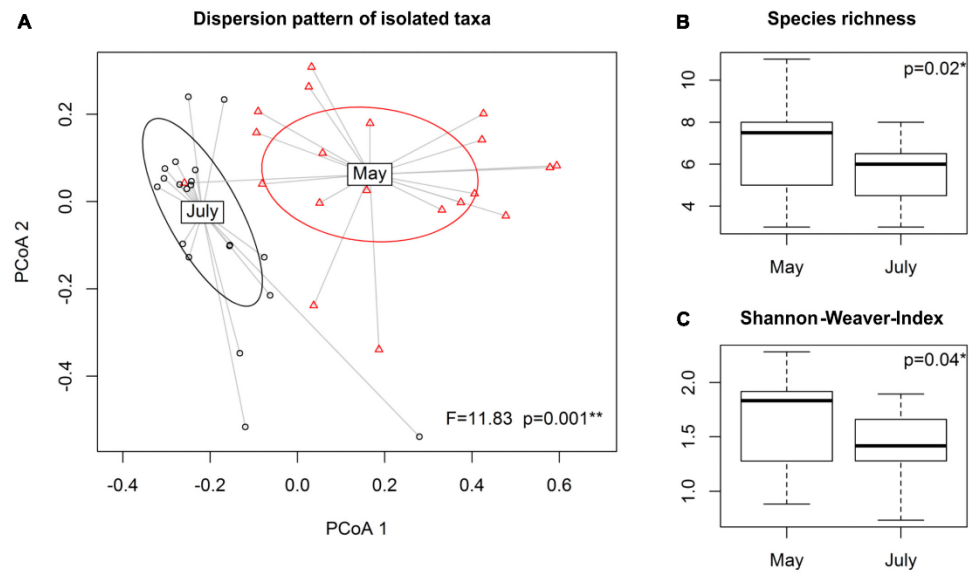


FIGURE 2 | Effect of time on composition of isolated rhizobacterial taxa among and within the dominating genera *Phyllobacterium*, *Pseudomonas* and *Streptomyces*. Running betadisper function based on Bray-Curtis dissimilarity **(A)** dispersion patterns of the isolated taxa are presented in an ordination plot. Diversity indices **(B)** species richness and **(C)** Shannon-Weaver-Index were calculated, followed by ANOVA, testing for differences in isolated species composition of the three dominant genera between the two sampling points, May (stem elongation stage, BBCH 37-39) and July (grain filling stage, BBCH 75-77) 2015, respectively.

solubilization potential among all clusters of all three dominant genera. Even more important, the most abundant *Pseudomonas* cluster (cluster 1, represented by 39 isolates = 23% of all *Pseudomonas* isolates) showed a high average P solubilization potential which exceeded mean P solubilization of all genera and clusters, besides the aforementioned *Pseudomonas* cluster 8 (**Figure 1C** and **Supplementary Table S6**).

Drought Tolerance of Key Taxa

Drought stress tolerance was quantified as relative difference in colony size on PEG medium compared to control medium. Thus, higher drought tolerance was reflected by higher maintenance of colony size (maximum 100%) and *vice versa*. *Pseudomonas* species (35.1% at stem elongation in May and 35.7% at grain filling stage in July) and *Streptomyces* species (43.3% in May and 46.7% in July) were less resistant to drought compared to species assigned to the genus *Phyllobacterium* (54.1% in May and 63.6% in July) (**Table 1**). Although the low number of isolates at stem elongation in May samples prevents from drawing significant conclusions, species drought tolerance of genus *Phyllobacterium* was improved in the course of the growing season (**Table 1**). Total drought tolerance of taxa belonging to the genera *Pseudomonas* and *Streptomyces* did not change throughout the observed growth stages, but show different behavior under ambient and future climatic conditions. Under ambient climate an increase and under future climate conditions a decrease of drought tolerance could be observed for *Pseudomonas* species, while for *Streptomyces* species the opposite pattern showed up (**Table 1**). Land use did not impact key genera's drought tolerance, but the climate treatment affected average drought tolerance of the genus *Streptomyces*. Strains isolated from plots with future climatic conditions showed higher drought tolerance (52.4%) as compared to those isolated from plots with ambient climate (38.6%) (p = 0.01). This trend was also observed at the cluster level, even though it was not significant due to the low number of isolates per cluster (**Supplementary Figure S5**). In contrast to streptomycetes, climate treatment showed no effect on the drought tolerance of taxa within the genera *Phyllobacterium* and *Pseudomonas*.

At the cluster level, a marginal higher drought tolerance of the species isolated from plots with future climatic conditions, compared to those isolated from ambient plots was observed for *Phyllobacterium* cluster 2 (**Supplementary Figure S5**). Within the genus *Pseudomonas*, we observed no increase in drought tolerance of species from future climate plots except for the low abundant cluster 10 and, to a lower extent, for cluster 3 and cluster 8 (**Supplementary Figure S5**). Overall, species of both *Phyllobacterium* clusters exhibit a significantly higher drought tolerance as compared to those of the predominant clusters of *Pseudomonas* (clusters 1 to 4) to and *Streptomyces* species (clusters 1 to 4) (**Supplementary Figure S5** and **Supplementary Table S6**).

DISCUSSION

In this study we have shown that members of the genera *Phyllobacterium, Pseudomonas* and *Streptomyces* are predominant within the cultivable fraction of the P-solubilizing

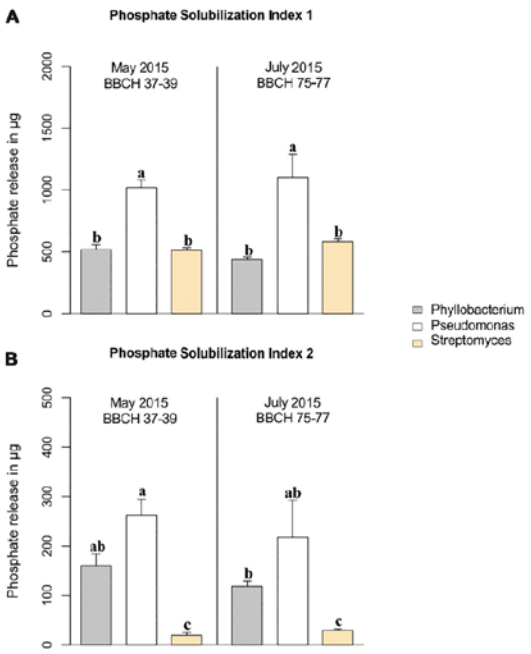


FIGURE 3 | Phosphate-solubilizing potentials of the three dominant genera. Comparison of the two indices, **(A)** PSI 1 and **(B)** PSI 2 for *Phyllobacterium*, *Pseudomonas* und *Streptomyces* isolates at stem elongation in May and at grain filling in July 2015 sampled from wheat rhizosphere. Standard errors are given in the bars. Significant differences were calculated for the both indices separately and are marked by different letters (ANOVA and Tukey HSD Test).

TABLE 1 | Drought resistance of the members of three dominant P-solubilizing genera.

	Phyllobacterium		*Pseudomonas*		*Streptomyces*	
	May	July	May	July	May	July
	BBCH 37–39	BBCH 75–77	BBCH 37–39	BBCH 75–77	BBCH 37–39	BBCH 75–77
Ambient	55.9% **ab** (6.4)	62.5% **a** (3.1)	34.2% **b** (3.9)	55.2% **ab** (15.2)	39.6% **b** (4.8)	37.8% **b** (4.1)
Future	53.2% **ab** (4.0)	64.4% **a** (2.5)	36.2% **b** (3.9)	20.2% **b** (16.3)	47.8% **ab** (6.1)	54.9% **a** (4.7)
Total	54.1% **de** (3.4)	63.6% **d** (2.0)	35.1% **f** (2.7)	35.7% **ef** (12.3)	43.3% **ef** (3.8)	46.7% **e** (3.2)

Mean levels of drought resistance exhibited by the bacterial isolates from grain filling stage (BBCH 37-39) in May and stem elongation stage (BBCH 75-77) in July under ambient and future climatic conditions. Standard error is given in parenthesis. Significant differences according to Tukey post hoc test are marked by different letters for ambient/future (a–b) and total (d–f), respectively.

community in the wheat rhizosphere along both growth stages studied, namely vegetative biomass production (BBCH 37–39, stem elongation) and generative biomass production stages (BBCH 75–77, grain filling). All taxa within the three dominant genera showed high resilience against differences in studied agricultural management practices and climatic conditions, but structure shifted with wheat growth stages. This shift was mainly driven by changes among P-solubilizing taxa of the genera *Pseudomonas* and *Phyllobacterium*, as well as on single taxa level within the genus *Pseudomonas*. The considerable *in vitro* P solubilization potential of both genera *Pseudomonas* and *Phyllobacterium* pleads for functional redundancy upon structural changes within the cultivable fraction of the P-solubilizing community. While the genera *Pseudomonas* and *Streptomyces* comprise well-known P solubilizers, *Phyllobacterium* species have rarely been associated with P solubilization. Considering their medium-borne P solubilization potential in combination with an accentuated tolerance of actively growing colonies to drought conditions, *Phyllobacterium* species may play an important role in P supply for plants under drought.

Wheat Growth Stage and Farming System Drive Abundance and Identity of Phosphate-Solubilizing Rhizobacteria

The abundance of cultivable P-solubilizing rhizobacteria (PSR), mirrored by CFU per gram dry soil, was higher at the grain filling stage of wheat in July than at the stem elongation stage in May and contradicts our assumptions made in the first hypothesis. This discrepancy might be related to higher soil moisture at the day of sampling in July, since increased water availability supports microbial growth and enhances metabolic activities (Williams and Rice, 2007). Due to the realistic scenario-based approach of the GCEF, the soil moisture is mainly dependent on the actual weather. However, the impact of the climate treatment was clearly evident for the annual soil moisture dynamics of 2015 (Schädler et al., 2019) and led to significantly reduced winter wheat yields under future climatic conditions compared to ambient ones (**Supplementary Table S3**).

According to our third hypothesis, the conventional farming (CF) showed higher PSR numbers than the organic farming (OF), which is likely related to the higher nutrient availability in CF. Physicochemical soil parameters are well known as important drivers of microbial biomass (Widmer et al., 2006) as well as of the community structure and composition (Cleveland and Liptzin, 2007; Lauber et al., 2008; Francioli et al., 2016). Nevertheless, we consistently identified *Phyllobacterium*, *Pseudomonas* and *Streptomyces* species as the predominant cultivable PSR in both farming systems. In general, plants recruit beneficial PGPR from the surrounding soil to their rhizospheres (Badri and Vivanco, 2009) by plant specific rhizodeposition patterns (Paterson et al., 2007). This selection effect may mask environmental impacts on the community structure and composition in the rhizosphere and, thus, explain the low differences in the identity of predominant PSR isolated across both farming systems.

Phosphate-Solubilizing Potential First Time Reported for *P. ifriqiyense* and *P. sophorae*

Multiple studies report *Streptomyces* (Molla et al., 1984; Jog et al., 2014) and *Pseudomonas* species to be strong P

solubilizers (e.g., Richardson et al., 2009; Marasco et al., 2012; Sharma et al., 2013) and successful colonizers of the wheat rhizosphere (Mohammadi, 2012; Jog et al., 2014). Conversely, members of the genus *Phyllobacterium*, taxonomically classified to the order Rhizobiales, are hardly described for their P solubilization potential, but rather for their biocontrol activities (Lambert et al., 1990; Aisyah et al., 2017) or the capacity to fix nitrogen (Rojas et al., 2001). Our study now provides evidence for the potential of two *Phyllobacterium* species, namely *P. ifriqiyense* and *P. sophorae*, to *in vitro* solubilize P from its bound mineral form in the rhizosphere of winter wheat in the temperate climatic zone. The two strains have first been isolated from root nodules of *Astragalus algerianus* and *Lathyrus numidicus* in South Tunisia - *P. ifriqiyense* - (Mantelin et al., 2006), and of *Sophora flavescens* in China - *P. sophorae* - (Jiao et al., 2015). Only two further previous studies indicate the potential of *Phyllobacterium myrsinacearum* for P solubilization in subtropical soils (Chen et al., 2006) and in metal-polluted soils (Ma et al., 2013). Genome shotgun sequencing of *Phyllobacterium* sp. (National Center of Biotechnology Information, NCBI) revealed quinoprotein glucose dehydrogenase coding genes. These genes are involved in gluconic acid production and, thus, suggest a potential for mineral phosphate solubilization (Rodríguez and Fraga, 1999). This coding gene has also been indicated for *Pseudomonas* species (Miller et al., 2010) and *Streptomyces* species (Jog et al., 2014) and may, therefore, serve as indicator for P solubilization potential of Phyllobacteria *in vivo*. Therefore, the contribution of *Phyllobacterium* species to phosphate solubilization in soils and rhizospheres might be still underestimated.

Complementarity Between *Phyllobacterium* and *Pseudomonas* at Different Wheat Growth Stages

Abundances of P-solubilizing *Phyllobacterium* and *Pseudomonas* isolates were complementary between the two wheat growth stages. Since an isolation-based approach was used, this complementarity mirrors different proportions of active microorganisms. Blagodatskaya and Kuzyakov (2013) suggested that more than 95% of the total microbial biomass are inactive at a given time point. Accordingly, active growth and dormancy phases of rhizosphere species are dependent on nutrient levels, interspecific competition as well as on the plant growth stage and its related rhizodeposition (Blagodatskaya and Kuzyakov, 2013). Plant nutrient uptake and root exudation were reported to be high at stem elongation stage and considerably lower at grain filling (Aulakh et al., 2001; Malhi et al., 2006). Of note, *Pseudomonas* species were predominantly isolated from the wheat rhizosphere at the stage of stem elongation in May. Members of this genus are well characterized for their rapid growth, high chemotactic activity and motility toward root exudates (Scher et al., 1988; Walsh et al., 2001), as well as the production of secondary metabolites (Haas and Defago, 2005) and secretion of siderophores (Kloepper et al., 1980), to metabolize rhizodeposits and outcompete other microorganisms (Paulsen et al., 2005). Indeed, root colonization of active *Phyllobacterium* species may be inhibited by pseudomonads competing for root exudates at the stage of high biomass production in May. Even though *Phyllobacterium* species are described to live in a large variety of habitats and to "communicate" with plant tissues, the group is also known to be non-pathogenic (Mantelin et al., 2006). When plants reach their mature state, the release of root exudates and competition between microorganisms in the rhizosphere decreases drastically (Hamlen et al., 1972), likely explaining the decline in abundance of *Pseudomonas* and the enrichment of *Phyllobacterium* species at the grain filling stage. Antagonistic effects have not been tested within this study, but may provide important information on colonization success in plant rhizospheres at different growth stages.

Resistance and Functional Redundancy in Terms of P Solubilization Between and Among Dominant Phosphate Solubilizing Genera

Besides abundance shifts, the cluster dispersion patterns of the cultivated dominant taxa varied significantly between the two time points with increased species richness at the stage of stem elongation in May samples. This partly supports our first hypothesis, since the diversity decrease in July is in line with reduced carbon and nutrient availabilities (Hamlen et al., 1972). The shifts within the cultivable fraction of the PSR community were based on two different responses: changes in the dominant PSR taxa, which are mainly driven by shifts within the genera *Phyllobacterium* and *Pseudomonas*, as well as altered species composition of *Pseudomonas* species. Changes in the structure and identity of *Pseudomonas* species at different wheat growth stages indicate that upon a change in the environmental conditions, the culture derived *Pseudomonas* community is dynamic. Concurrent changes in community structure and composition according to plants growth stages are widely observed in ecology (Dunfield and Germida, 2003; Wolsing and Priemé, 2004; Francioli et al., 2018). As we found moderate to high average P solubilization potentials of taxa classified to the genera *Pseudomonas* and *Phyllobacterium* at the two growth stages studied, the observed shifts plead for functional redundancy (Allison and Martiny, 2008) between and among genera at different wheat growth stages.

Besides the effect of different growth stages over the growing season, an effect of farming system was found. Increased P solubilization potentials for *Pseudomonas* isolates from CF, compared to OF, were detected under future climatic conditions. The mechanisms behind this observation cannot be revealed within this study, but we assume soil nutrient availability to play a role in the activity of PSR under drought. Interestingly, this finding is, however, supported by a previous study performed in grasslands, where we found higher P solubilization potentials of *Pseudomonas* species under low soil moisture conditions (Breitkreuz, unpublished). Within this study, we found comparable P solubilization potentials expressed by the predominant taxa along both time points, which is in contrast to our assumptions made in hypothesis one that P solubilization potential is higher at the stem elongation stage. Higher activities

of *Pseudomonas* species in CF under future climate contradicts hypothesis three expecting higher P solubilization potential in OF than in CF.

The abundances of isolated *Streptomyces* species, but also the structure and composition within the genus were only slightly affected by the wheat growth stage and not at all by the climate or land use treatments. Despite their high abundance, P solubilization potential expressed according to PSI 2 were significantly less pronounced compared to those of the isolated *Phyllobacterium* and *Pseudomonas* species. However, Battini et al. (2017) recently emphasized the capability of *Streptomyces* species to facilitate P uptake into fungal hyphae in root free compartments, which is subsequently transported via the mycorrhizosphere to maize plants. In fact, Tarkka et al. (in preparation) isolated phosphate solubilizing bacteria from bulk soils of conventional and organic farming plots of the GCEF and identified *Streptomyces* species among the most abundant phosphate solubilizes. Their lower phosphate solubilizing potentials may be caused by slower development rates and delayed activity expression, compared to the mainly fast growing *Phyllobacterium* and *Pseudomonas* species (Garrity et al., 2007). The slow growth of streptomycetes is related to the formation of complex structures, e.g., branching hyphal filaments and spores (Chater et al., 2010). Dormant spores in the soil are beneficial to endure severe drought events waiting for more favorable conditions (Viaene et al., 2016). In line with this, recently published studies observed accumulation of actinobacteria in drought-treated soils and rhizospheres of different plants (Bouskill et al., 2016; Taketani et al., 2017).

For estimating the strain-specific P solubilization potentials, two complementary indices were calculated. PSI 1, which is based on the total colony and halo size, is directly related to the total amount of released P, but the values are strongly biased by the respective colony size. Consequently, identical distances between halo and colony rim along with larger colony sizes may overrate the activity potential since bigger colonies have higher area values and vice versa. The same problem occurs when using the commonly published ratios, halo to colony size (e.g., Nguyen et al., 1992; Kumar and Narula, 1999; Pérez et al., 2007). However, the diffusion expanse of organic acids in the surrounding of bacterial colonies to solubilize bound P is mainly determined by the outermost cells of the colony. PSI 2 remedies the strong impact of the colony size that, in our case for *Streptomyces* species, may impact appraisal of the P solubilization potential. We thus suggest to focus on approaches based on diffusion expanse (i.e., the distance between halo and colony) for estimation of phosphate solubilizing potential when using plate bioassays.

Drought Tolerance of P-Solubilizing Bacteria

The tolerance of the predominant taxa against severe drought stress was quantified in this study by water deficit bioassays with polyethylene glycol 6000 (PEG). Using this approach, we focused on whether isolated strains remained physiologically active under low moisture conditions. In this respect, evaluating growth on nutrient agar with PEG may provide information on active PSR under drought conditions.

As assumed in our second hypothesis, we consistently observed a (by trend) increased drought tolerance of the dominant taxa isolated at stage of grain filling from July as compared to those at stem elongation from May. This indicates enhanced adaptive bacterial responses against dehydration stress in summer. While we detected no land use effect on drought tolerance, we found a higher drought tolerance for *Streptomyces* species, when isolated from plots under future climatic conditions, compared to those from ambient climate plots. Overall, we observed the highest drought tolerance for *Phyllobacterium* species, followed by *Streptomyces* and consecutively *Pseudomonas* species. Soil microorganisms tolerate water stress in a variable extent (Manzoni et al., 2012). As a most common mitigation strategy, bacteria as well as eukaryotes accumulate low molecular weight compounds, so called osmolytes, once water potentials decrease (Kempf and Bremer, 1998). These compounds do not interfere with cellular functions, but stabilize cellular structures and prevent desiccation. Their production and accumulation in the abundant clusters observed within this study is established, for *Phyllobacterium* (Fujihara, 2008; Sagot et al., 2010; Zaprasis et al., 2015), but also for *Pseudomonas* species, when isolated from arid or semi-arid regions of India (Sandhya et al., 2009). However, Lennon et al. (2012) performed a study in (MI, United States, humid region) and found with decreasing water potentials that respiration of *Pseudomonas* species drastically dropped, which supports the findings for *Pseudomonas* in our study.

Field Application Potential of P-Solubilizing Bacteria

Besides rhizosphere competence, the trait combination of high P solubilization potential and drought tolerance is a prerequisite for field applications. Inoculation with microbial species of the genera *Bacillus* and *Paenibacillus* (Zhang J. et al., 2012; Kasim et al., 2013; Kumar et al., 2014; Timmusk et al., 2014), as well as *Azospirillum* and *Pseudomonas* (Creus et al., 2004; Naiman et al., 2009; Ilyas and Bano, 2010; Kasim et al., 2013) and *Streptomyces* (Jog et al., 2014) was found to increase wheat yields in field and pot experiments. In particular, *Pseudomonas* species are well investigated, as the genus comprises species with promising plant growth promoting properties (Burr et al., 1978; Naiman et al., 2009; Rajkumar et al., 2010). In contrast, prior to this study little was known about P-solubilizing *Phyllobacterium* species, or their drought tolerance in wheat rhizosphere. They do act as PGPR e.g., in canola plants (Bertrand et al., 2001), but underlying mechanisms remain unclear. Model studies with Arabidopsis thaliana and *Phyllobacterium brassicacearum* indicated higher drought tolerance (Bresson et al., 2014). In the same context, Kechid et al. (2013) observed a delay in reproduction and lowered transpiration rate of Arabidopsis thaliana upon inoculation with *Phyllobacterium brassicacearum* accompanied by stimulated lateral root and root hair growth. Within this study we could prove *in vitro* potential for P-solubilization of two taxa in the genus *Phyllobacterium*, which has to be further justified *in vivo* under different biotic and abiotic conditions. To check applicability of these species as

phosphate delivering biofertilizers, the timing of inoculation and bacterial level has to be defined in pretests to ensure survival of the inoculated strains, but also to keep viability of the seeds. Bashan (1986) found inoculation at early growth stages of wheat with 10^5–10^6 colony forming units per ml to be optimal for *Azospirillum* and *Pseudomonas* strains to increase productivity. The success of colonization depends on different factors and is favored by high mobility of the bacteria along the growing root, rapid multiplication and the ability to colonize the rhizoplane and inner root tissues (Höflich et al., 1995). Therefore, the colonization potential for different plant root systems, the effect of different soil parameters and antagonistic effects within the native microbial community has to be quantified in inoculation experiments under natural (non-sterile) and controlled (sterile) conditions (Gaskins et al., 1985; Höflich et al., 1995). Finally, the strains have to be tested for their plant growth promoting activities exploiting various sources of insoluble P in the soil, besides tri-calcium phosphate also other insoluble inorganic and organic phosphate sources (Bashan et al., 2013), and their drought stress tolerance under natural conditions in pot and field experiments. Nevertheless, the non-pathogenic status and high plant-interaction potential of *Phyllobacterium* species, along with the considerable P solubilization potential *in vitro* and high drought tolerance revealed in this study, make this group attractive for possible future application as plant growth promoting inoculants for wheat production.

CONCLUSION

Our findings suggest that the bacterial taxa involved in the provision of P in the rhizosphere of wheat plants depend on plant's growth stage and only minor on farming system and climate under *in vitro* conditions. This observation clearly pleads for more studies at defined and yield-determining stages of plant growth under *in vitro* and *in vivo* conditions. We found that *Pseudomonas* species were dominant at the stem elongation stage, i.e., the peak of vegetative biomass production, while *Phyllobacterium* species dominated at the grain filling stage (generative stage). This is the first time that the two isolated *Phyllobacterium* species are reported for their high P solubilization potential. Drought tolerance potentials of the three dominant genera were found to be increased at the second growth stage studied in July compared to the first one in May indicating an adaptation to drought over time and a higher tolerance in summer months. *Phyllobacterium* species expressed highest drought tolerance potential among isolated dominant phosphate solubilizing rhizobacteria. Therefore, further work should concentrate on functionality and abundance of this genus in rhizospheres of plants. Especially in the context of climate change related summer drought, the observed trait combination of *Phyllobacterium* species in this study may be of particular importance to adapt agriculture to dryer conditions in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

All authors conceived and designed the experiments, obtained funding, interpreted the results, and contributed to revisions and approved submission of the manuscript. TR and CB performed the field experiments. CB performed the laboratory works and data analysis. MT, TR, and CB wrote the manuscript with input from FB.

FUNDING

The work has been done in the frame of a scholarship funded by the "Deutsche Bundesstiftung Umwelt" (DBU) with the topic "Einfluss pflanzenwachstumsfördender Rhizobakterien auf die Stressresistenz der Kulturpflanze Weizen (*Triticum aestivum L.*)" (AZ:20015/391).

ACKNOWLEDGMENTS

We appreciate the Helmholtz Association, the Federal Ministry of Education and Research, the State Ministry of Science and Economy of Saxony-Anhalt and the State Ministry for Higher Education, Research and the Arts Saxony, to fund the Global Change Experimental Facility (GCEF) project. We want to thank the staff of the Bad Lauchstädt Experimental Research Station (especially Ines Merbach and Konrad Kirsch) and Martin Schädler for their work in maintaining the plots and infrastructures of the GCEF, as well as Harald Auge, Stefan Klotz, and Martin Schädler for their role in setting up the GCEF. Special thanks go to Julia Siebert and Tom Künne, and all helpers participated in soil and rhizosphere sampling campaigns in 2015. We further want to thank Kerstin Hommel, Beatrix Schnabel, and Laura Herzig for their support in lab works. Finally we thank the Ph.D. Advisory Committee of this project: Prof. Dr. Nico Eisenhauer and Dr. Petra Högy for their input and fruitful discussions on data analysis and interpretation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.03109/full#supplementary-material

REFERENCES

Aisyah, S. N., Sulastri, S., Retmi, R., Yani, R., Syafriani, E., Syukriani, L., et al. (2017). Suppression of colletotrichum gloeosporioides by indigenous *Phyllobacterium* and its compatibility with rhizobacteria. *Asian J. Plant Pathol.* 11, 139–147. doi: 10.3923/ajppaj.2017.139.147

Allison, S. D., and Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 105, 11512–11519. doi: 10.1073/pnas.0801925105

Altermann, M., Rinklebe, J., Merbach, I., Körschens, M., Langer, U., and Hofmann, B. (2005). Chernozem-soil of the year 2005. *J. Plant Nutr. Soil Sci.* 168, 725–740. doi: 10.1002/jpln.200521814

Artursson, V., Finlay, R. D., and Jansson, J. K. (2006). Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environ. Microbiol.* 8, 1–10. doi: 10.1111/j.1462-2920.2005.00942.x

Aulakh, M. S., Wassmann, R., Bueno, C., Kreuzwieser, J., and Rennenberg, H. (2001). Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. *Plant Biol.* 3, 139–148. doi: 10.1055/s-2001-12905

Badri, D. V., and Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant Cell Environ.* 32, 666–681. doi: 10.1111/j.1365-3040.2008.01926.x

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

Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A. Y., Gattinger, A., et al. (2019). Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *ISME J.* 13, 1722–1736. doi: 10.1038/s41396-019-0383-2

Bashan, Y. (1986). Significance of timing and level of inoculation with rhizosphere bacteria on wheat plants. *Soil Biol. Biochem.* 18, 297–301. doi: 10.1016/0038-0717(86)90064-7

Bashan, Y., Kamnev, A. A., and de-Bashan, L. E. (2013). Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure. *Biol. Fertil. Soils* 49, 465–479. doi: 10.1007/s00374-012-0737-7

Battini, F., Gronlund, M., Agnolucci, M., Giovannetti, M., and Jakobsen, I. (2017). Facilitation of phosphorus uptake in maize plants by mycorrhizosphere bacteria. *Sci. Rep.* 7:4686. doi: 10.1038/s41598-017-04959-0

Bertrand, H., Nalin, R., Bally, R., and Cleyet-Marel, J.-C. (2001). Isolation and identification of the most efficient plant growth-promoting bacteria associated with canola (Brassica napus). *Biol. Fertil. Soils* 33, 152–156. doi: 10.1007/s003740000305

Blagodatskaya, E., and Kuzyakov, Y. (2013). Active microorganisms in soil: critical review of estimation criteria and approaches. *Soil Biol. Biochem.* 67, 192–211. doi: 10.1016/j.soilbio.2013.08.024

BMEL (2018). *Erntebericht 2018*. Bonn: BMEL.

Bouskill, N. J., Wood, T. E., and Baran, R. (2016). Belowground response to drought in a tropical forest soil. I. changes in microbial functional potential and metabolism. *Front. Microbiol.* 7:525. doi: 10.3389/fmicb.2016.00525

Bresson, J., Vasseur, F., Dauzat, M., Labadie, M., Varoquaux, F., Touraine, B., et al. (2014). Interact to survive: *Phyllobacterium brassicacearum* improves *Arabidopsis* tolerance to severe water deficit and growth recovery. *PLoS One* 9:e107607. doi: 10.1371/journal.pone.0107607

Burr, T., Schroth, M., and Suslow, T. (1978). Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas* fluorescens and P. *putida*. *Phytopathology* 68, 1377–1383.

Chater, K. F., Biro, S., Lee, K. J., Palmer, T., and Schrempf, H. (2010). The complex extracellular biology of *Streptomyces*. *FEMS Microbiol. Rev.* 34, 171–198.

Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F. T., Lai, W. A., and Young, C. C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.* 34, 33–41. doi: 10.1016/j.apsoil.2005.12.002

Ciais, P., Reichstein, M., and Viovy, N. (2005). Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 437:529. doi: 10.1038/nature03972

Cleveland, C. C., and Liptzin, D. (2007). C: N: p stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry* 85, 235–252. doi: 10.1007/s10533-007-9132-0

Cordell, D., Drangert, J. O., and White, S. (2009). The story of phosphorus: global food security and food for thought. *Glob. Environ. Change* 19, 292–305. doi: 10.1016/j.gloenvcha.2008.10.009

Creus, C. M., Sueldo, R. J., and Barassi, C. A. (2004). Water relations and yield in *Azospirillum*-inoculated wheat exposed to drought in the field. *Can. J. Botany* 82, 273–281. doi: 10.1139/b03-119

de Vries, F. T., and Shade, A. (2013). Controls on soil microbial community stability under climate change. *Front. Microbiol.* 4:265. doi: 10.3389/fmicb.2013.00265

Dunfield, K. E., and Germida, J. J. (2003). Seasonal changes in the rhizosphere microbial communities associated with field-grown genetically modified canola (Brassica napus). *Appl. Environ. Microbiol.* 69, 7310–7318. doi: 10.1128/aem.69.12.7310-7318.2003

Elliott, E. T., Hunt, H. W., and Walter, D. E. (1988). Detrital foodweb interactions in North American grassland ecosystems. *Agric. Ecosyst. Environm.* 24, 41–56. doi: 10.1016/0167-8809(88)90055-2

Fischer, C. (2003). *Geographie Infothek: Weizen. Klettverlag. Leipzig*. Available at: https://www.klett.de/alias/1010150 (accessed February 28, 2019).

Fischer, S. E., Fischer, S. I., Magris, S., and Mori, G. B. (2007). Isolation and characterization of bacteria from the rhizosphere of wheat. *World J. Microbiol. Biotechnol.* 23, 895–903.

Francioli, D., Schulz, E., Buscot, F., and Reitz, T. (2018). Dynamics of soil bacterial communities over a vegetation season relate to both soil nutrient status and plant growth phenology. *Microb. Ecol.* 75, 216–227. doi: 10.1007/s00248-017-1012-0

Francioli, D., Schulz, E., Purahong, W., Buscot, F., and Reitz, T. (2016). Reinoculation elucidates mechanisms of bacterial community assembly in soil and reveals undetected microbes. *Biol. Fertil. Soils* 52, 1073–1083. doi: 10.1007/s00374-016-1141-5

Frey, S. D., Elliott, E. T., and Paustian, K. (1999). Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. *Soil Biol. Biochem.* 31, 573–585. doi: 10.1016/s0038-0717(98)00161-8

Fujihara, S. (2008). Biogenic amines in rhizobia and legume root nodules. *Microb. Environ.* 24, 1–13. doi: 10.1264/jsme2.me08557

Garrity, G., Brenner, D. J., Krieg, N. R., and Staley, J. R. (2007). *Bergey's Manual® of Systematic Bacteriology: Volume 2: The Proteobacteria, Part B: The Gammaproteobacteria*. Berlin: Springer.

Gaskins, M. H., Albrecht, S. L., and Hubbell, D. H. (1985). Rhizosphere bacteria and their use to increase plant productivity: a review. *Agric. Ecosyst. Environ.* 12, 99–116. doi: 10.1016/0167-8809(85)90071-4

Haas, D., and Defago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol.* 3, 307–319. doi: 10.1038/nrmicro1129

Hamlen, R. A., Lukezic, F. L., and Bloom, J. R. (1972). Influence of age and stage of development on the neutral carbohydrate components in root exudates from alfalfa plants grown in a gnotobiotic environment. *Can. J. Plant Sci.* 52, 633–642. doi: 10.4141/cjps72-097

Hendrix, P. F., Parmelee, R. W., Crossley, D. A. Jr., Coleman, D. C., Odum, E. P., and Groffman, P. M. (1986). Detritus food webs in conventional and no-tillage agroecosystems. *Bioscience* 36, 374–380. doi: 10.2307/1310259

Höflich, G., Wiehe, W., and Hecht-Buchholz, C. (1995). Rhizosphere colonization of different crops with growth promoting *Pseudomonas* and Rhizobium bacteria. *Microbiol. Res.* 150, 139–147. doi: 10.1016/s0944-5013(11)80048-0

Ilyas, N., and Bano, A. (2010). *Azospirillum* strains isolated from roots and rhizosphere soil of wheat (*Triticum aestivum* L.) grown under different soil moisture conditions. *Biol. Fertil. Soil* 46, 393–406. doi: 10.1007/s00374-009-0438-z

Jiao, Y. S., Yan, H., Ji, Z. J., Liu, Y. H., Sui, X. H., Zhang, X. X., et al. (2015). *Phyllobacterium sophorae* sp. nov., a symbiotic bacterium isolated from root nodules of *Sophora flavescens*. *Int. J. Syst. Evol. Microbiol.* 65, 399–406. doi: 10.1099/ijs.0.067017-0

Jog, R., Pandya, M., Nareshkumar, G., and Rajkumar, S. (2014). Mechanism of phosphate solubilization and antifungal activity of *Streptomyces spp.* isolated from wheat roots and rhizosphere and their application in improving plant growth. *Microbiology* 160, 778–788. doi: 10.1099/mic.0.074146-0

Juhnke, M. E., Mathre, D. E., and Sands, D. C. (1987). Identification and characterization of Rhizosphere-competent bacteria of wheat. *Appl. Environ. Microbiol.* 53, 2793–2799.

Karimi, B., Maron, P. A., Chemidlin-Prevost Boure, N., Bernard, N., Gilbert, D., and Ranjard, L. (2017). Microbial diversity and ecological networks as indicators of environmental quality. *Environ. Chem. Lett.* 15, 265–281. doi: 10.1007/s10311-017-0614-6

Kasim, W. A., Osman, M. E., Omar, M. N., Abd El-Daim, I. A., Bejai, S., and Meijer, J. (2013). Control of drought stress in wheat using plant-growth-promoting bacteria. *J. Plant Growth Regul.* 32, 122–130. doi: 10.1007/s00344-012-9283-7

Katoh, K., and Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780. doi: 10.1093/molbev/mst010

Kechid, M., Desbrosses, G., Rokhsi, W., Varoquaux, F., Djekoun, A., and Touraine, B. (2013). The NRT2. 5 and NRT2. 6 genes are involved in growth promotion of Arabidopsis by the plant growth-promoting rhizobacterium (PGPR) strain *Phyllobacterium brassicacearum* STM196. *New Phytol.* 198, 514–524. doi: 10.1111/nph.12158

Kempf, B., and Bremer, E. (1998). Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Arch. Microbiol.* 170, 319–330. doi: 10.1007/s002030050649

Kennedy, A. C. (1999). Bacterial diversity in agroecosystems. *Agric. Ecosyst. Environ.* 74, 65–76. doi: 10.1016/b978-0-444-50019-9.50007-8

Kladivko, E. J. (2001). Tillage systems and soil ecology. *Soil Tillage Res.* 61, 61–76. doi: 10.1016/s0167-1987(01)00179-9

Kloepper, J. W., Leong, J., Teintze, M., and Schroth, M. N. (1980). *Pseudomonas* siderophores - a mechanism explaining disease-suppressive soils. *Curr. Microbiol.* 4, 317–320. doi: 10.1007/bf02602840

Kumar, A., Maurya, B. R., and Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). *Biocataly. Agric. Biotechnol.* 3, 121–128. doi: 10.1016/j.bcab.2014.08.003

Kumar, V., and Narula, N. (1999). *Solubilization* of inorganic phosphates and growth emergence of wheat as affected by *Azotobacter chroococcum* mutants. *Biol. Fertil. Soils* 28, 301–305. doi: 10.1007/s003740050497

Lambert, B., Joos, H., Dierickx, S., Vantomme, R., Swings, J., Kersters, K., et al. (1990). Identification and plant interaction of a *Phyllobacterium* sp., a predominant rhizobacterium of young sugar beet plants. *Appl. Environ. Microbiol.* 56, 1093–1102.

Lane, D. J. (1991). "16S/23S rRNA sequencing," in *Nucleic Acid Techniques in Bacterial Systematics*, eds E. Stackebrandt, and M. Goodfellow (New York, NY: John Wiley and Sons), 115–175.

Lauber, C. L., Strickland, M. S., Bradford, M. A., and Fierer, N. (2008). The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol. Biochem.* 40, 2407–2415. doi: 10.1016/j.soilbio.2008.05.021

Lennon, J. T., Aanderud, Z. T., Lehmkuhl, B. K., and Schoolmaster, D. R. (2012). Mapping the niche space of soil microorganisms using taxonomy and traits. *Ecology* 93, 1867–1879. doi: 10.1890/11-1745.1

Lucas García, J. A., Barbas, C., Probanza, A., Barrientos, M. L., and Gutierrez Mañero, F. J. (2001). Low molecular weight organic acids and fatty acids in root exudates of two Lupinus cultivars at flowering and fruiting stages. *Phytochem. Anal.* 12, 305–311. doi: 10.1002/pca.596

Ma, Y., Rajkumar, M., Luo, Y., and Freitas, H. (2013). Phytoextraction of heavy metal polluted soils using sedum plumbizincicola inoculated with metal mobilizing *Phyllobacterium myrsinacearum* RC6b. *Chemosphere* 93, 1386–1392. doi: 10.1016/j.chemosphere.2013.06.077

Malhi, S. S., Johnston, A. M., Schoenau, J. J., Wang, Z. L., and Vera, C. L. (2006). Seasonal biomass accumulation and nutrient uptake of wheat, barley and oat on a Black Chernozem Soil in Saskatchewan. *Can. J. Plant Sci.* 86, 1005–1014. doi: 10.4141/p05-116

Mantelin, S., Saux, M. F., Zakhia, F., Bena, G., Bonneau, S., Jeder, H., et al. (2006). Emended description of the genus *Phyllobacterium* and description of four novel species associated with plant roots: *Phyllobacterium bourgognense* sp. nov., *Phyllobacterium ifriqiyense* sp. nov., *Phyllobacterium leguminum* sp. nov. and *Phyllobacterium brassicacearum* sp. nov. *Int. J. Syst. Evol. Microbiol.* 56, 827–839. doi: 10.1099/ijs.0.63911-0

Manzoni, S., Schimel, J. P., and Porporato, A. (2012). Responses of soil microbial communities to water stress: results from a meta-analysis. *Ecology* 93, 930–938. doi: 10.1890/11-0026.1

Marasco, R., Rolli, E., and Ettoumi, B. (2012). A drought resistance-promoting microbiome is selected by root system under desert farming. *PLoS One* 7:e48479. doi: 10.1371/journal.pone.0048479

Miller, S. H., Browne, P., Prigent-Combaret, C., Combes-Meynet, E., Morrissey, J. P., and O'Gara, F. (2010). Biochemical and genomic comparison of inorganic phosphate solubilization in *Pseudomonas* species. *Environ. Microbiol. Rep.* 2, 403–411. doi: 10.1111/j.1758-2229.2009.00105.x

Mogollón, J. M., Beusen, A. H. W., Van Grinsven, H. J. M., Westhoek, H., and Bouwman, A. F. (2018). Future agricultural phosphorus demand according to the shared socioeconomic pathways. *Glob. Environ. Change* 50, 149–163. doi: 10.1016/j.gloenvcha.2018.03.007

Mohammadi, K. (2012). Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *Resour. Environ.* 2, 80–85.

Molla, M. A. Z., Chowdhury, A. A., Islam, A., and Hoque, S. (1984). Microbial mineralization of organic phosphate in soil. *Plant Soil* 78, 393–399. doi: 10.1007/bf02450372

Murphy, J., and Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analy. Chim. Acta* 27, 31–36. doi: 10.1016/s0003-2670(00)88444-5

Muyzer, G., de Waal, E. C., and Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59, 695–700.

Naiman, A. D., Latrónico, A., and García de Salamone, I. E. (2009). Inoculation of wheat with *Azospirillum brasilense* and *Pseudomonas* fluorescens: impact on the production and culturable rhizosphere microflora. *Eur. J. Soil Bio.* 45, 44–51. doi: 10.1016/j.ejsobi.2008.11.001

Naveed, M., Hussain, M. B., Zahir, Z. A., Mitter, B., and Sessitsch, A. (2014). Drought stress amelioration in wheat through inoculation with Burkholderia phytofirmans strain PsJN. *Plant Growth Regul.* 73, 121–131. doi: 10.1007/s10725-013-9874-8

Nguyen, C., Yan, W., Le Tacon, F., and Lapeyrie, F. (1992). Genetic variability of phosphate solubilizing activity by monocaryotic and dicaryotic mycelia of the ectomycorrhizal fungus Laccaria bicolor (Maire) PD Orton. *Plant Soil* 143, 193–199. doi: 10.1007/bf00007873

Oehl, F., Oberson, A., Tagmann, H. U., Besson, J. M., Dubois, D., Mäder, P., et al. (2002). Phosphorus budget and phosphorus availability in soils under organic and conventional farming. *Nutr. Cycl. Agroecosyst.* 62, 25–35.

Paterson, E., Gebbing, T., Abel, C., Sim, A., and Telfer, G. (2007). Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytol.* 173, 600–610. doi: 10.1111/j.1469-8137.2006.01931.x

Paulsen, I. T., Press, C. M., and Ravel, J. (2005). Complete genome sequence of the plant commensal *Pseudomonas* fluorescens Pf-5. *Nat. Biotechnol.* 23:873.

Pérez, E., Sulbarán, M., Ball, M. M., and Yarzábal, L. A. (2007). Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biol. Biochem.* 39, 2905–2914. doi: 10.1016/j.soilbio.2007.06.017

Petersen, U., and Weigel, H.-J. (2015). *Klimaresilienz Durch Agrobiodiversität?*. Thünen-Report 25. Waldsieversdorf: Thünen.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologiya* 17, 362–370.

R Core Team (2017). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rajkumar, M., Ae, N., Prasad, M. N., and Freitas, H. (2010). Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends Biotechnol.* 28, 142–149. doi: 10.1016/j.tibtech.2009.12.002

Rana, A., Saharan, B., Joshi, M., Prasanna, R., Kumar, K., and Nain, L. (2011). Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. *Ann. Microbiol.* 61, 893–900. doi: 10.1007/s13213-011-0211-z

Richardson, A. E., Barea, J.-M., McNeill, A. M., and Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* 321, 305–339. doi: 10.1007/s11104-009-9895-2

Rodríguez, H., and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* 17, 319–339. doi: 10.1016/s0734-9750(99)00014-2

Rojas, A., Holguin, G., Glick, B. R., and Bashan, Y. (2001). Synergism between *Phyllobacterium* sp. (*N*2-fixer) and *Bacillus licheniformis* (P-solubilizer), both from a semiarid mangrove rhizosphere. *FEMS Microbiol. Ecol.* 35, 181–187. doi: 10.1016/s0168-6496(01)00091-5

Sagot, B., Gaysinski, M., Mehiri, M., Guigonis, J.-M., Le Rudulier, D., and Alloing, G. (2010). Osmotically induced synthesis of the dipeptide N-acetylglutaminylglutamine amide is mediated by a new pathway conserved among bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 107, 12652–12657. doi: 10.1073/pnas.1003063107

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., et al. (2000). Global biodiversity scenarios for the year 2100. *Science* 287, 1770–1774. doi: 10.1126/science.287.5459.1770

Sandhya, V., Grover, M., Reddy, G., and Venkateswarlu, B. (2009). Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas* putida strain GAP-P45. *Biol. Fertil. Soils* 46, 17–26. doi: 10.1007/s00374-009-0401-z

Schädler, M., Buscot, F., Klotz, S., Reitz, T., Durka, W., Bumberger, J., et al. (2019). Investigating the consequences of climate change under different land-use regimes - a novel experimental infrastructure. *Ecosphere* 10:e02635. doi: 10.1002/ecs2.2635

Scher, F. M., Kloepper, J. W., Singleton, C., Zaleska, I., and Laliberte, M. (1988). Colonization of sybean roots by *Pseudomonas* and Serratia species: relationship to bacterial motility, chemotaxis, and generation time. *Phytopathology* 78, 1055–1059.

Schmeling, D. (2017). *Auf 54% der Getreideanbaufläche steht 2017 Winterweizen*. Available at: https://www.destatis.de/DE/PresseService/Presse/Pressemitteilungen/2017/05/PD17_163_412pdf.pdf?__blob=publicationFile (accessed March 18, 2019).

Shade, A., Peter, H., Allison, S. D., Baho, D. L., Berga, M., Bürgmann, H., et al. (2012). Fundamentals of microbial community resistance and resilience. *Front. Microbiol.* 3:417. doi: 10.3389/fmicb.2012.00417

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., and Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springerplus* 2:587. doi: 10.1186/2193-1801-2-587

Sheik, C. S., Beasley, W. H., Elshahed, M. S., Zhou, X., Luo, Y., and Krumholz, L. R. (2011). Effect of warming and drought on grassland microbial communities. *ISME J.* 5, 1692–1700. doi: 10.1038/ismej.2011.32

Singh, Y., and Lal, N. (2016). Isolation and Characterization of PGPR from Wheat (Triticum aestivum) Rhizosphere and Their Plant Growth Promoting Traits in Vitro. *Front. Microbiol.* 6:198.

Smith, S. E., and Read, D. J. (2010). *Mycorrhizal Symbiosis*. Cambridge, MA: Academic press.

Statista, (2018). *Worldwide Production of Grain in 2017/18*. Hamburg: Statista.

Taketani, R. G., Lançoni, M. D., Kavamura, V. N., Durrer, A., Andreote, F. D., and Melo, I. S. (2017). Dry season constrains bacterial phylogenetic diversity in a semi-arid rhizosphere system. *Microb. Ecol.* 73, 153–161. doi: 10.1007/s00248-016-0835-4

Tardy, V., Mathieu, O., Lévêque, J., Terrat, S., Chabbi, A., Lemanceau, P., et al. (2014). Stability of soil microbial structure and activity depends on microbial diversity. *Environ. Microbiol. Rep.* 6, 173–183. doi: 10.1111/1758-2229.12126

Tiemann, L. K., Grandy, A. S., Atkinson, E. E., Marin-Spiotta, E., and McDaniel, M. D. (2015). Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecology Lett.* 18, 761–771. doi: 10.1111/ele.12453

Timmusk, S., Abd El-Daim, I. A., and Copolovici, L. (2014). Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. *PLoS One* 9:e96086. doi: 10.1371/journal.pone.0096086

Trnka, M., Rötter, R. P., Ruiz-Ramos, M., Kersebaum, K. C., Olesen, J. E., Žalud, Z., et al. (2014). Adverse weather conditions for European wheat production will become more frequent with climate change. *Nat. Clim. Change* 4:637. doi: 10.1038/nclimate2242

Verbruggen, E., Röling, W. F., Gamper, H. A., Kowalchuk, G. A., Verhoef, H. A., and van der Heijden, M. G. (2010). Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytol.* 186, 968–979. doi: 10.1111/j.1469-8137.2010.03230.x

Verma, P., Yadav, A. N., Khannam, K. S., Kumar, S., Saxena, A. K., and Suman, A. (2016). Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. *J. Basic Microbiol.* 56, 44–58. doi: 10.1002/jobm.201500459

Verslues, P. E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J., and Zhu, J.-K. (2006). Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant J.* 45, 523–539. doi: 10.1111/j.1365-313x.2005.02593.x

Viaene, T., Langendries, S., Beirinckx, S., Maes, M., and Goormachtig, S. (2016). *Streptomyces* as a plant's best friend? *FEMS Microbiol. Ecol.* 92:fiw119. doi: 10.1093/femsec/fiw119

Walsh, U. F., Morrissey, J. P., and O'Gara, F. (2001). *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. *Curr. Opin. Biotechnol.* 12, 289–295. doi: 10.1016/s0958-1669(00)00212-3

Widmer, F., Rasche, F., Hartmann, M., and Fliessbach, A. (2006). Community structures and substrate utilization of bacteria in soils from organic and conventional farming systems of the DOK long-term field experiment. *Appl. Soil Ecol.* 33, 294–307. doi: 10.1016/j.apsoil.2005.09.007

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

Wolsing, M., and Priemé, A. (2004). Observation of high seasonal variation in community structure of denitrifying bacteria in arable soil receiving artificial fertilizer and cattle manure by determining T-RFLP of nir gene fragments. *FEMS Microbiol. Ecol.* 48, 261–271. doi: 10.1016/j.femsec.2004.02.002

Zaprasis, A., Bleisteiner, M., Kerres, A., Hoffmann, T., and Bremer, E. (2015). Uptake of amino acids and their metabolic conversion into the compatible solute proline confers osmoprotection to Bacillus subtilis. *Appl. Environ. Microbiol.* 81, 250–259. doi: 10.1128/AEM.02797-14

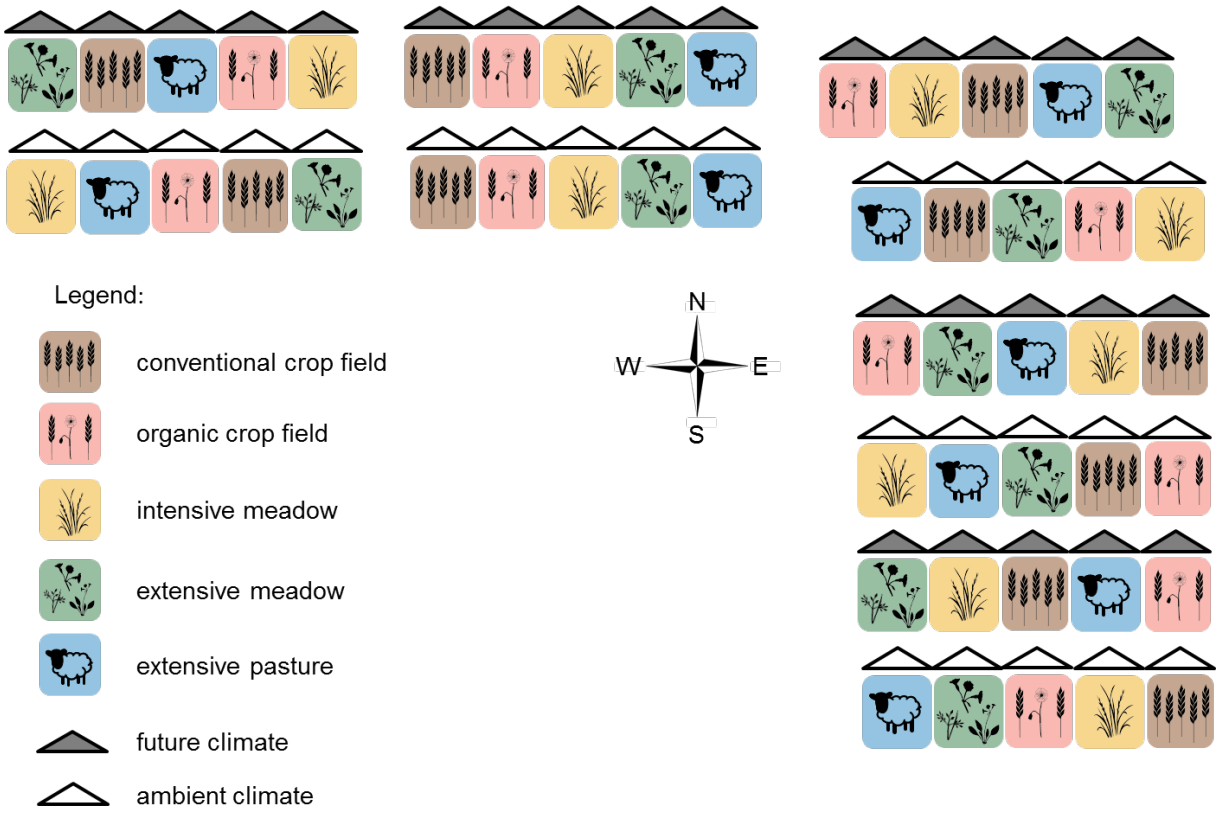
Zhang, H., Gao, S., Lercher, M. J., Hu, S., and Chen, W. H. (2012). EvolView, an online tool for visualizing, annotating and managing phylogenetic trees. *Nucleic Acids Res.* 40, W569–W572. doi: 10.1093/nar/gks576

Zhang, J., Liu, J., Meng, L., Ma, Z., Tang, X., Cao, Y., et al. (2012). Isolation and characterization of plant growth-promoting rhizobacteria from wheat roots by wheat germ agglutinin labeled with *Fluorescein isothiocyanate*. *J. Microbiol.* 50, 191–198. doi: 10.1007/s12275-012-1472-3

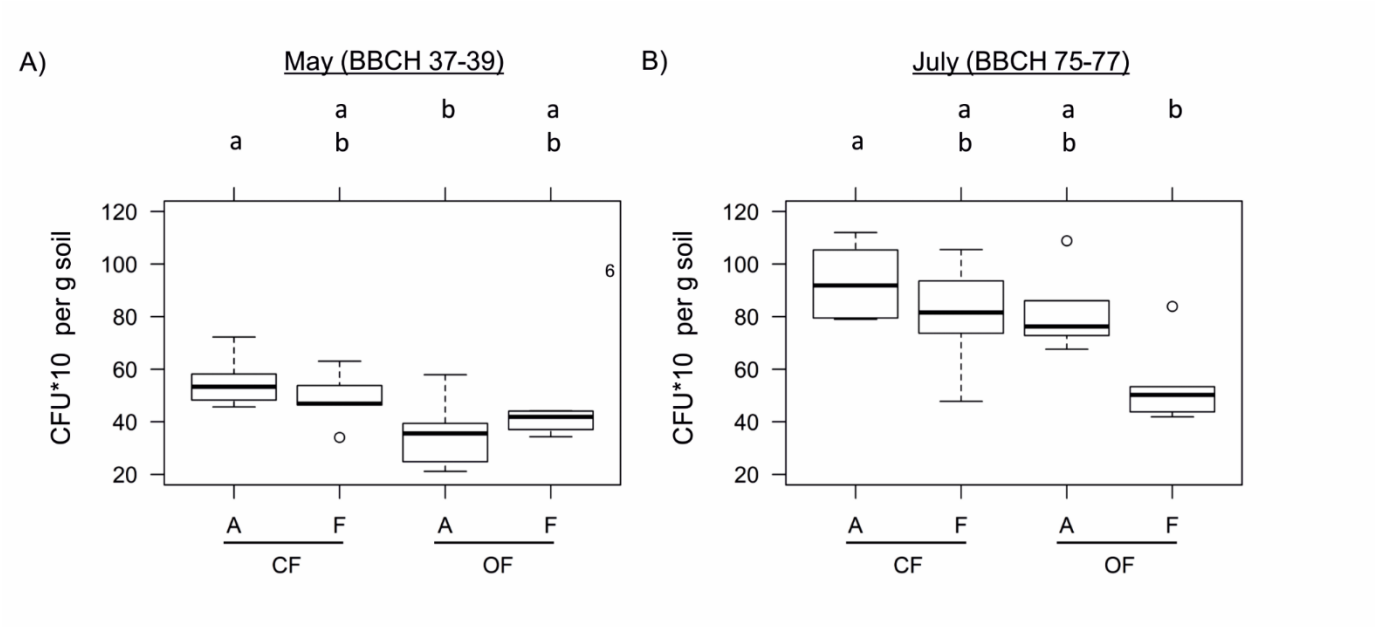
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material:

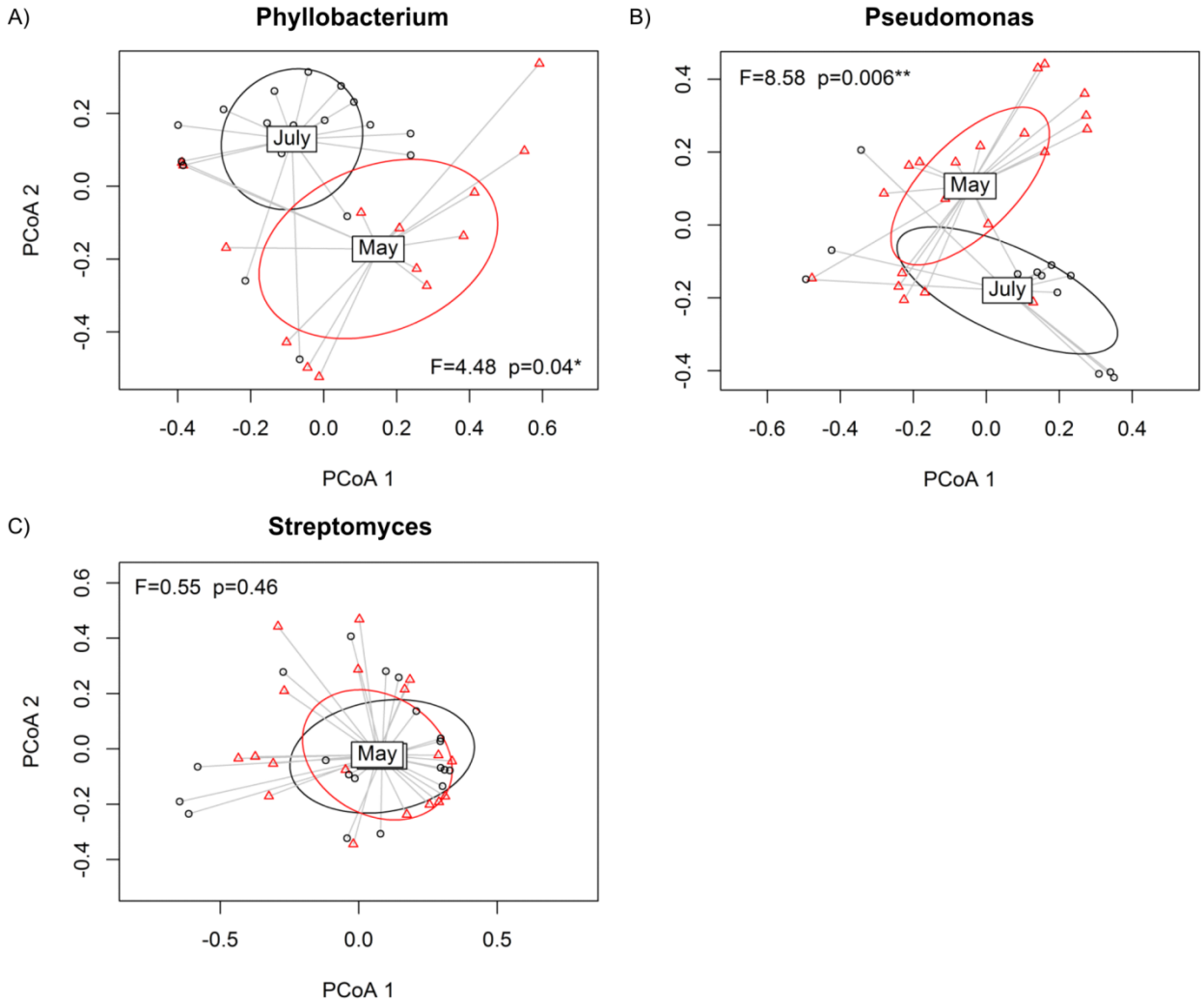
Supplementary Figures



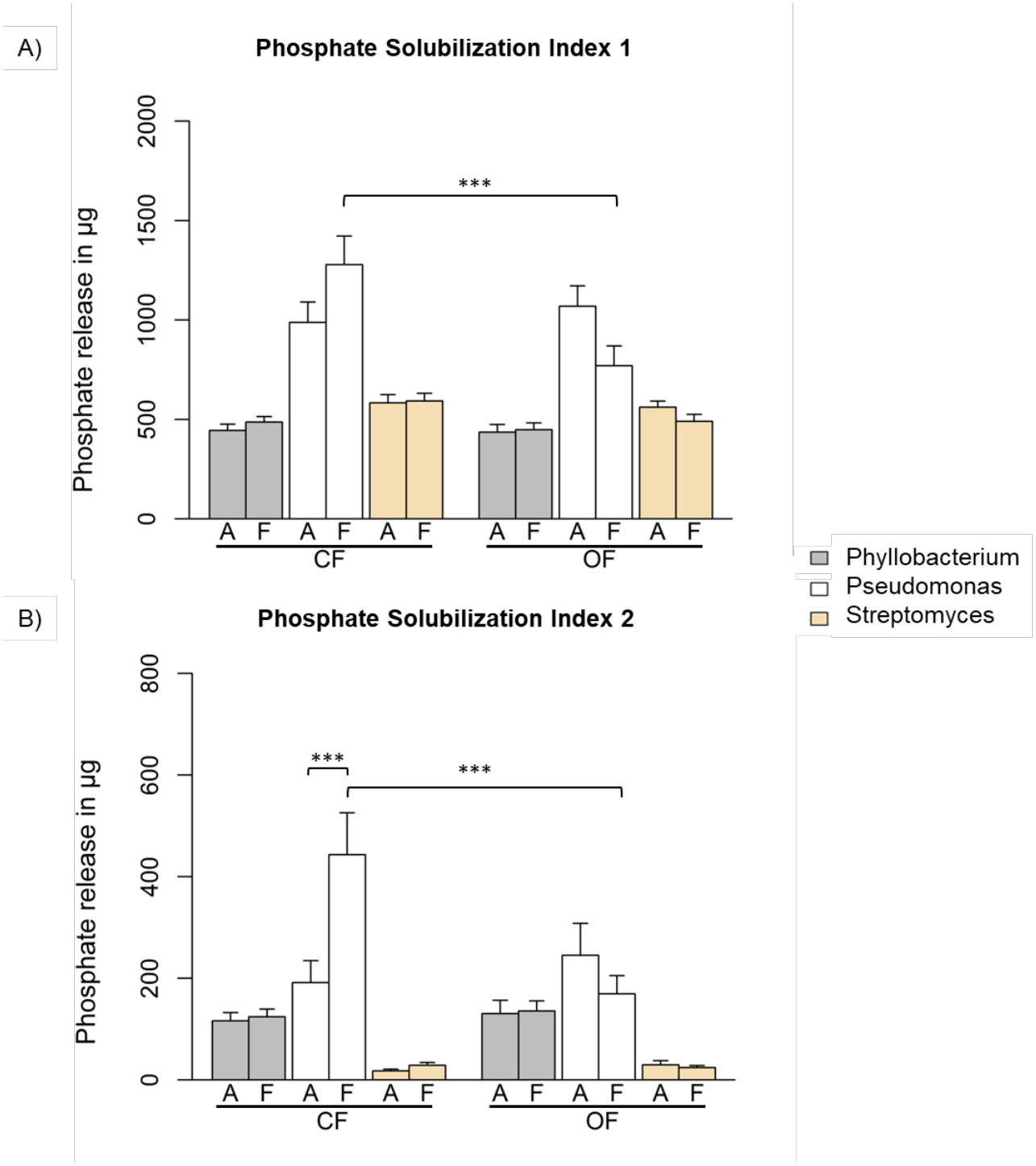
Supplementary Figure 1: Experimental design of the Global Change Experimental Facility (GCEF). The facility includes 50 plots (subplots) arranged in ten blocks (mainplots). Five blocks are attributed to ambient and future climate, respectively, whereby each block comprises all five land use types. Copyrights of land use icons are held by Gottschall/Siebert (Schädler et al. 2019).



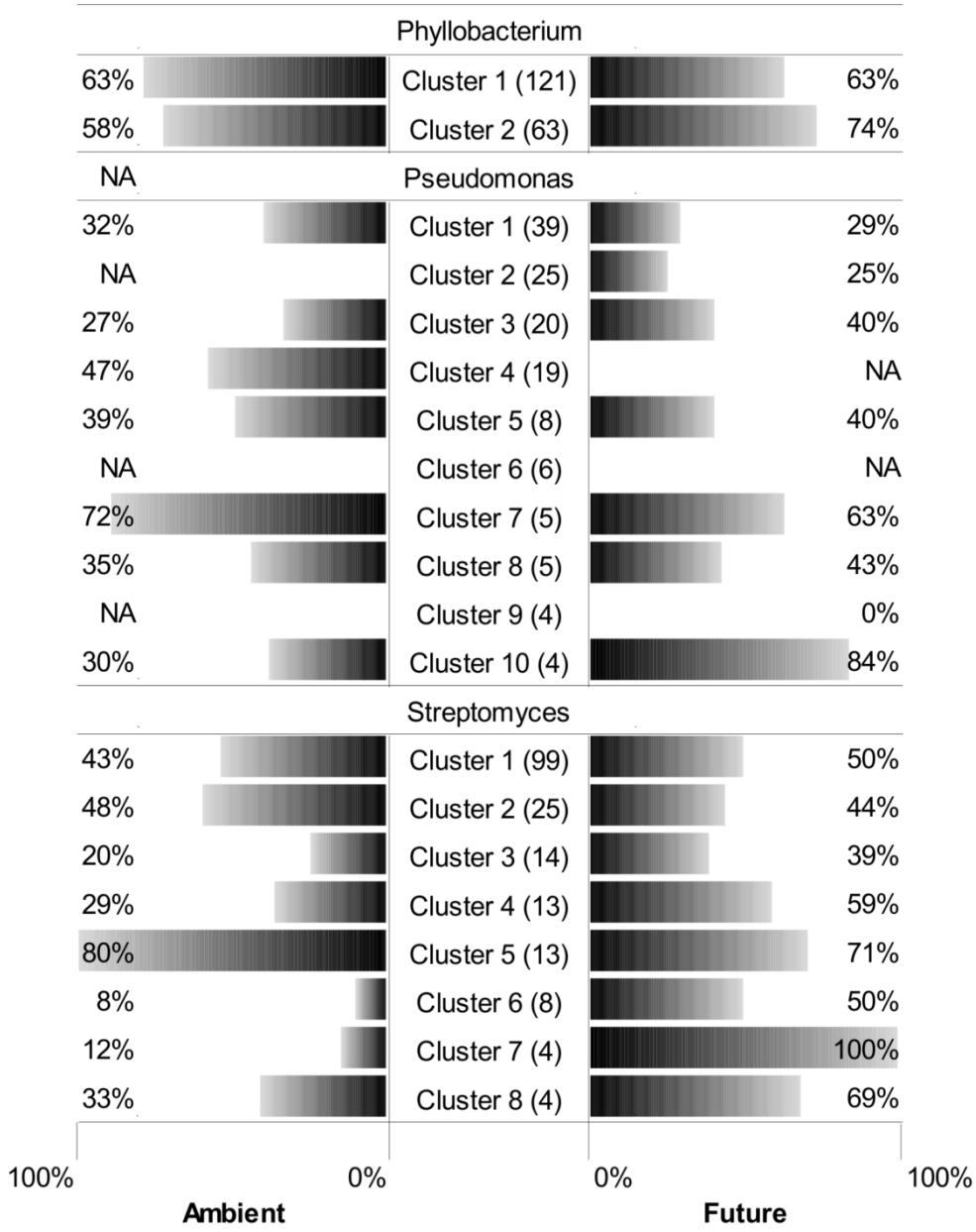
Supplementary Figure 2: Abundance of phosphate solubilizing rhizobacteria. Colony forming units of wheat rhizobacteria per g of soil, grown on Pikovskaya agar plates, in A) May 2015 sampling at BBCH stage 37-39 and in B) July 2015 sampling, at BBCH stage 75-77. Significant differences between treatments - ambient (A) and future (F) climate; conventional (CF) and organic (OF) farming system - for each time point are marked by different small letters.



Supplementary Figure 3: Effect of wheat growth stage on A) *Phyllobacterium*, B) *Pseudomonas* and C) *Streptomyces* isolates structure. Dispersion patterns running betadisper function were calculated on distance matrix using method of Bray Curtis, followed by ANOVA testing for differences in composition of isolated species between the two sampling points, May (stem elongation, BBCH 37-39, red triangles) and July (grain filling, BBCH 75-77, black circles) respectively.



Supplementary Figure 4: Impact of climate and farming system on phosphate solubilization potentials of the three most dominant genera. Potentials are given for *Phyllobacterium*, *Pseudomonas* and *Streptomyces* calculated with A) PSI 1 and B) PSI 2, and isolated from conventional farming (CF) and organic farming (OF) under ambient (A) and future (F) climate conditions. Significant interactions within each genus are marked with $p<0.001$***.



Supplementary Figure 5: Drought resistance under ambient and future climate conditions among the predominant phylogenetic clusters of *Phyllobacterium*, *Pseudomonas* and *Streptomyces*. Number of isolates for each cluster is given in parenthesis. Respective clusters marked with NA contain isolates that did not grow, neither on control (YME) nor on test media (YME+PEG).

Supplementary Tables

Supplementary Table 1: Total number, origin and description of isolates cultivated on Pikovskaya medium. Isolates were gained at two different sampling points (May and July 2015), from two different farming systems (CF-conventional farming, OF-organic farming) and climate treatments (A-ambient climate, F-future climate). Number of base pairs (bp), assignment to genus and NCBI (National Center for Biotechnology Information-gene data bank) number is given.

Isolates	Sampling	Genus	Size (bp)	Land use	Climate	Acc. Number (NCBI)
PN2-B01P2-1	May 2015	*Pseudomonas sp.*	1086	CF	A	MK637864
PN2-B01P2-2	May 2015	*Pseudomonas sp.*	1057	CF	A	MK637859
PN2-B01P2-3	May 2015	*Pseudomonas sp.*	1057	CF	A	MK637861
PN2-B01P2-4	May 2015	*Pseudomonas sp.*	1050	CF	A	MK637884
PN2-B01P2-5	May 2015	*Streptomyces sp.*	1053	CF	A	MK638553
PN2-B01P2-6	May 2015	*Pseudomonas sp.*	1045	CF	A	MK637871
PN2-B01P2-7	May 2015	*Variovorax sp.*	1051	CF	A	MK638652
PN2-B01P2-8	May 2015	*Agrobacterium sp.*	1078	CF	A	MK638040
PN2-B01P2-9	May 2015	*Pseudomonas sp.*	1057	CF	A	MK637870
PN2-B01P2-10	May 2015	*Pseudomonas sp.*	1057	CF	A	MK637882
PN2-B01P2-11	May 2015	*Pseudomonas sp.*	1057	CF	A	MK637867
PN2-B01P2-12	May 2015	*Pseudomonas sp.*	1057	CF	A	MK637869
PN2-B01P2-13	May 2015	*Dyella sp.*	1063	CF	A	MK638116
PN2-B01P2-14	May 2015	*Dyella sp.*	1059	CF	A	MK638115
PN2-B01P2-15	May 2015	*Mesorhizobium sp.*	1011	CF	A	MK638184
PN2-B01P2-16	May 2015	*Pseudomonas sp.*	1057	CF	A	MK637865
PN2-B01P2-17	May 2015	*Rhizobium sp.*	1036	CF	A	MK638386
PN2-B01P2-18	May 2015	*Mesorhizobium sp.*	1037	CF	A	MK638154
PN2-B01P2-19	May 2015	*Streptomyces sp.*	1023	CF	A	MK638528
PN2-B01P2-20	May 2015	*Mesorhizobium sp.*	1036	CF	A	MK638159
PN2-B01P4-1	May 2015	*Pseudomonas sp.*	1057	OF	A	MK637877
PN2-B01P4-2	May 2015	*Massilia sp.*	1062	OF	A	MK638153
PN2-B01P4-3	May 2015	*Pseudomonas sp.*	1056	OF	A	MK637855
PN2-B01P4-4	May 2015	*Rhizobium sp.*	1033	OF	A	MK638383
PN2-B01P4-5	May 2015	*Pseudomonas sp.*	1057	OF	A	MK637858
PN2-B01P4-6	May 2015	*Streptomyces sp.*	1018	OF	A	MK638619
PN2-B01P4-7	May 2015	*Streptomyces sp.*	1039	OF	A	MK638454

PN2-B01P4-8	May 2015	*Streptomyces sp.*	1019	OF	A	MK638482
PN2-B01P4-9	May 2015	*Streptomyces sp.*	1086	OF	A	MK638574
PN2-B01P4-10	May 2015	*Streptomyces sp.*	997	OF	A	MK638597
PN2-B01P4-12	May 2015	*Streptomyces sp.*	1018	OF	A	MK638618
PN2-B01P4-13	May 2015	*Mesorhizobium sp.*	1037	OF	A	MK638157
PN2-B01P4-14	May 2015	*Streptomyces sp.*	1049	OF	A	MK638545
PN2-B01P4-15	May 2015	*Mesorhizobium sp.*	1037	OF	A	MK638158
PN2-B01P4-16	May 2015	*Burkholderia sp.*	1043	OF	A	MK638086
PN2-B01P4-17	May 2015	*Streptomyces sp.*	924	OF	A	MK638646
PN2-B01P4-18	May 2015	*Bradyrhizobium sp.*	600	OF	A	MK638085
PN2-B01P4-19	May 2015	*Mesorhizobium sp.*	662	OF	A	MK638185
PN2-B01P4-20	May 2015	*Phyllobacterium sp.*	746	OF	A	MK638374
PN2-B01P4-21	May 2015	*Rhizobium sp.*	1033	OF	A	MK638385
PN2-B01P4-22	May 2015	*Streptomyces sp.*	1080	OF	A	MK638468
PN2-B01P4-24	May 2015	*Phyllobacterium sp.*	1037	OF	A	MK638203
PN2-B02P3-1	May 2015	*Pseudomonas sp.*	1061	OF	F	MK637881
PN2-B02P3-2	May 2015	*Stenotrophomonas sp.*	1034	OF	F	MK638444
PN2-B02P3-3	May 2015	*Pseudomonas sp.*	1049	OF	F	MK637868
PN2-B02P3-4	May 2015	*Pseudomonas sp.*	1060	OF	F	MK637872
PN2-B02P3-5	May 2015	*Pseudomonas sp.*	1053	OF	F	MK637862
PN2-B02P3-6	May 2015	*Streptomyces sp.*	1085	OF	F	MK638470
PN2-B02P3-7	May 2015	*Pseudomonas sp.*	1057	OF	F	MK637874
PN2-B02P3-8	May 2015	*Streptomyces sp.*	1049	OF	F	MK638476
PN2-B02P3-9	May 2015	*Arthrobacter sp.*	1044	OF	F	MK638053
PN2-B02P3-10	May 2015	*Pseudomonas sp.*	1057	OF	F	MK637879
PN2-B02P3-11	May 2015	*Tardiphaga sp.*	1068	OF	F	MK638648
PN2-B02P3-12	May 2015	*Stenotrophomonas sp.*	1087	OF	F	MK638445
PN2-B02P3-13	May 2015	*Pseudomonas sp.*	1069	OF	F	MK637856
PN2-B02P3-14	May 2015	*Rhizobium sp.*	1077	OF	F	MK638384

PN2-B02P3-15	May 2015	*Mesorhizobium sp.*	1037	OF	F	MK638156
PN2-B02P3-16	May 2015	*Pseudomonas sp.*	1057	OF	F	MK637873
PN2-B02P3-17	May 2015	*Streptomyces sp.*	1042	OF	F	MK638575
PN2-B02P3-18	May 2015	*Dyadobacter sp.*	1015	OF	F	MK638112
PN2-B02P3-19	May 2015	*Streptomyces sp.*	1049	OF	F	MK638452
PN2-B02P3-20	May 2015	*Phyllobacterium sp.*	1037	OF	F	MK638202
PN2-B02P3-22	May 2015	*Mesorhizobium sp.*	1037	OF	F	MK638155
PN2-B02P4-1	May 2015	*Pseudomonas sp.*	1069	CF	F	MK637863
PN2-B02P4-2	May 2015	*Pseudomonas sp.*	1057	CF	F	MK637875
PN2-B02P4-3	May 2015	*Pseudomonas sp.*	1057	CF	F	MK637880
PN2-B02P4-4	May 2015	*Buttiauxella sp.*	1060	CF	F	MK638100
PN2-B02P4-5	May 2015	*Buttiauxella sp.*	1060	CF	F	MK638101
PN2-B02P4-6	May 2015	*Pseudomonas sp.*	1077	CF	F	MK637853
PN2-B02P4-7	May 2015	*Buttiauxella sp.*	614	CF	F	MK638102
PN2-B02P4-8	May 2015	*Pseudomonas sp.*	1057	CF	F	MK637876
PN2-B02P4-9	May 2015	*Pseudomonas sp.*	1078	CF	F	MK637854
PN2-B02P4-10	May 2015	*Pseudomonas sp.*	1057	CF	F	MK637860
PN2-B02P4-11	May 2015	*Pseudomonas sp.*	1063	CF	F	MK637878
PN2-B02P4-12	May 2015	*Buttiauxella sp.*	1083	CF	F	MK638096
PN2-B02P4-13	May 2015	*Pseudomonas sp.*	1060	CF	F	MK638009
PN2-B02P4-14	May 2015	*Buttiauxella sp.*	1068	CF	F	MK638097
PN2-B02P4-15	May 2015	*Pantoea sp.*	1080	CF	F	MK638201
PN2-B02P4-16	May 2015	*Buttiauxella sp.*	1068	CF	F	MK638098
PN2-B02P4-17	May 2015	*Pseudomonas sp.*	1057	CF	F	MK637866
PN2-B02P4-18	May 2015	*Pseudomonas sp.*	1069	CF	F	MK637857
PN2-B02P4-19	May 2015	*Buttiauxella sp.*	1073	CF	F	MK638099
PN2-B02P4-20	May 2015	*Buttiauxella sp.*	1038	CF	F	MK638103
PN2-B03P1-1	May 2015	*Pseudomonas sp.*	1057	CF	A	MK637883
PN2-B03P1-2	May 2015	*Sphingomonas sp.*	1034	CF	A	MK638443
PN2-B03P1-3	May 2015	*Agrobacterium sp.*	604	CF	A	MK638050
PN2-B03P1-4	May 2015	*Agrobacterium sp.*	1032	CF	A	MK638045

PN2-B03P1-5	May 2015	*Bacillus sp.*	1068	CF	A	MK638061
PN2-B03P1-6	May 2015	*Bacillus sp.*	883	CF	A	MK638063
PN2-B03P1-7	May 2015	*Streptomyces sp.*	1063	CF	A	MK638469
PN2-B03P1-10	May 2015	*Pedobacter sp.*	1013	CF	A	MK638129
PN2-B03P1-11	May 2015	*Rugamonas sp.*	1059	CF	A	MK638095
PN2-B03P1-12	May 2015	*Streptomyces sp.*	1080	CF	A	MK638453
PN2-B03P1-13	May 2015	*Rhodococcus sp.*	1037	CF	A	MK638438
PN2-B03P1-14	May 2015	*Mesorhizobium sp.*	1077	CF	A	MK638160
PN2-B03P1-15	May 2015	*Phyllobacterium sp.*	841	CF	A	MK638207
PN2-B03P1-16	May 2015	*Caulobacter sp.*	1069	CF	A	MK638104
PN2-B03P1-17	May 2015	*Phyllobacterium sp.*	1070	CF	A	MK638208
PN2-B03P1-18	May 2015	*Streptomyces sp.*	1072	CF	A	MK638530
PN2-B03P1-19	May 2015	*Streptomyces sp.*	1087	CF	A	MK638596
PN2-B03P1-20	May 2015	*Rhizobium sp.*	1076	CF	A	MK638389
PN2-B03P1-21	May 2015	*Rhizobium sp.*	1066	CF	A	MK638391
PN2-B03P1-22	May 2015	*Streptomyces sp.*	1018	CF	A	MK638620
PN2-B03P1-23	May 2015	*Agrobacterium sp.*	1054	CF	A	MK638046
PN2-B03P1-24	May 2015	*Rhizobium sp.*	1066	CF	A	MK638394
PN2-B03P1-25	May 2015	*Streptomyces sp.*	1066	CF	A	MK638529
PN2-B03P2-1	May 2015	*Pseudomonas sp.*	1072	OF	A	MK637910
PN2-B03P2-2	May 2015	*Pseudomonas sp.*	1045	OF	A	MK637908
PN2-B03P2-3	May 2015	*Variovorax sp.*	1079	OF	A	MK638653
PN2-B03P2-4	May 2015	*Arthrobacter sp.*	1071	OF	A	MK638057
PN2-B03P2-5	May 2015	*Pseudomonas sp.*	1056	OF	A	MK637897
PN2-B03P2-6	May 2015	*Arthrobacter sp.*	1060	OF	A	MK638054
PN2-B03P2-7	May 2015	*Arthrobacter sp.*	1071	OF	A	MK638058
PN2-B03P2-8	May 2015	*Pseudomonas sp.*	1057	OF	A	MK637902
PN2-B03P2-9	May 2015	*Bacillus sp.*	1068	OF	A	MK638067
PN2-B03P2-10	May 2015	*Bacillus sp.*	1082	OF	A	MK638066
PN2-B03P2-11	May 2015	*Streptomyces sp.*	1084	OF	A	MK638539

PN2-B03P2-12	May 2015	*Pseudomonas sp.*	1069	OF	A	MK637904
PN2-B03P2-13	May 2015	*Streptomyces sp.*	1090	OF	A	MK638570
PN2-B03P2-14	May 2015	*Streptomyces sp.*	1090	OF	A	MK638611
PN2-B03P2-15	May 2015	*Streptomyces sp.*	853	OF	A	MK638531
PN2-B03P2-16	May 2015	*Mesorhizobium sp.*	1070	OF	A	MK638162
PN2-B03P2-17	May 2015	*Streptomyces sp.*	1066	OF	A	MK638475
PN2-B03P2-18	May 2015	*Inquilinus sp.*	1043	OF	A	MK638134
PN2-B03P2-19	May 2015	*Mesorhizobium sp.*	596	OF	A	MK638182
PN2-B03P2-20	May 2015	*Streptomyces sp.*	1066	OF	A	MK638474
PN2-B03P2-21	May 2015	*Mesorhizobium sp.*	1083	OF	A	MK638161
PN2-B03P2-22	May 2015	*Streptomyces sp.*	1084	OF	A	MK638536
PN2-B03P2-23	May 2015	*Mesorhizobium sp.*	1070	OF	A	MK638163
PN2-B04P1-1	May 2015	*Pseudomonas sp.*	1072	CF	F	MK637891
PN2-B04P1-2	May 2015	*Mucilaginibacter sp.*	1083	CF	F	MK638193
PN2-B04P1-3	May 2015	*Streptomyces sp.*	1072	CF	F	MK638557
PN2-B04P1-4	May 2015	*Streptomyces sp.*	1085	CF	F	MK638472
PN2-B04P1-5	May 2015	*Rugamonas sp.*	1061	CF	F	MK638090
PN2-B04P1-6	May 2015	*Chitinophaga sp.*	1087	CF	F	MK638106
PN2-B04P1-7	May 2015	*Rhizobium sp.*	1079	CF	F	MK638388
PN2-B04P1-8	May 2015	*Arthrobacter sp.*	1057	CF	F	MK638055
PN2-B04P1-9	May 2015	*Chitinophaga sp.*	1088	CF	F	MK638107
PN2-B04P1-10	May 2015	*Arthrobacter sp.*	1084	CF	F	MK638060
PN2-B04P1-11	May 2015	*Streptomyces sp.*	1018	CF	F	MK638621
PN2-B04P1-12	May 2015	*Rhizobium sp.*	1061	CF	F	MK638387
PN2-B04P1-13	May 2015	*Phyllobacterium sp.*	1079	CF	F	MK638205
PN2-B04P1-14	May 2015	*Streptomyces sp.*	1090	CF	F	MK638555
PN2-B04P1-15	May 2015	*Bradyrhizobium sp.*	1071	CF	F	MK638082
PN2-B04P1-16	May 2015	*Rhizobium sp.*	1066	CF	F	MK638393

PN2-B04P1-17	May 2015	*Phyllobacterium sp.*	1043	CF	F	MK638206
PN2-B04P1-18	May 2015	*Streptomyces sp.*	1055	CF	F	MK638569
PN2-B04P1-19	May 2015	*Chryseobacterium sp.*	1049	CF	F	MK638108
PN2-B04P1-20	May 2015	*Rhizobium sp.*	1073	CF	F	MK638392
PN2-B04P2-1	May 2015	*Pseudomonas sp.*	1072	OF	F	MK637894
PN2-B04P2-2	May 2015	*Pseudomonas sp.*	1085	OF	F	MK637888
PN2-B04P2-3	May 2015	*Bacillus sp.*	1076	OF	F	MK638065
PN2-B04P2-4	May 2015	*Burkholderia sp.*	1051	OF	F	MK638094
PN2-B04P2-5	May 2015	*Bacillus sp.*	1089	OF	F	MK638064
PN2-B04P2-6	May 2015	*Streptomyces sp.*	1018	OF	F	MK638622
PN2-B04P2-7	May 2015	*Pseudomonas sp.*	1066	OF	F	MK637901
PN2-B04P2-8	May 2015	*Streptomyces sp.*	1018	OF	F	MK638623
PN2-B04P2-9	May 2015	*Streptomyces sp.*	962	OF	F	MK638537
PN2-B04P2-10	May 2015	*Streptomyces sp.*	1055	OF	F	MK638573
PN2-B04P2-11	May 2015	*Pseudomonas sp.*	1083	OF	F	MK637893
PN2-B04P2-12	May 2015	*Pseudomonas sp.*	1057	OF	F	MK637892
PN2-B04P2-13	May 2015	*Arthrobacter sp.*	1078	OF	F	MK638059
PN2-B04P2-14	May 2015	*Mesorhizobium sp.*	1054	OF	F	MK638164
PN2-B04P2-15	May 2015	*Streptomyces sp.*	1055	OF	F	MK638571
PN2-B04P2-16	May 2015	*Streptomyces sp.*	1055	OF	F	MK638572
PN2-B04P2-17	May 2015	*Ochrobactrum sp.*	1062	OF	F	MK638197
PN2-B04P2-18	May 2015	*Streptomyces sp.*	1076	OF	F	MK638471
PN2-B04P2-19	May 2015	*Phyllobacterium sp.*	1078	OF	F	MK638204
PN2-B04P2-20	May 2015	*Rhizobium sp.*	1074	OF	F	MK638390
PN2-B04P2-21	May 2015	*Paraburkholderia sp.*	1061	OF	F	MK638087
PN2-B04P2-22	May 2015	*Ochrobactrum sp.*	1053	OF	F	MK638198
PN2-B05P1-1	May 2015	*Pseudomonas sp.*	1057	OF	A	MK637896
PN2-B05P1-2	May 2015	*Pseudomonas sp.*	1056	OF	A	MK637900
PN2-B05P1-3	May 2015	*Pseudomonas sp.*	1086	OF	A	MK637889
PN2-B05P1-4	May 2015	*Pseudomonas sp.*	1085	OF	A	MK637886
PN2-B05P1-5	May 2015	*Pseudomonas sp.*	1086	OF	A	MK637887
PN2-B05P1-6	May 2015	*Pseudomonas sp.*	1057	OF	A	MK637905

PN2-B05P1-7	May 2015	*Pseudomonas sp.*	1064	OF	A	MK637906
PN2-B05P1-8	May 2015	*Pseudomonas sp.*	1069	OF	A	MK637911
PN2-B05P1-9	May 2015	*Pseudomonas sp.*	1069	OF	A	MK637907
PN2-B05P1-10	May 2015	*Pseudomonas sp.*	1075	OF	A	MK637898
PN2-B05P1-11	May 2015	*Pseudomonas sp.*	1055	OF	A	MK637890
PN2-B05P1-12	May 2015	*Pseudomonas sp.*	1071	OF	A	MK637885
PN2-B05P1-13	May 2015	*Pseudomonas sp.*	1073	OF	A	MK637899
PN2-B05P1-14	May 2015	*Pseudomonas sp.*	1086	OF	A	MK637903
PN2-B05P1-15	May 2015	*Pseudomonas sp.*	1072	OF	A	MK637895
PN2-B05P1-16	May 2015	*Pseudomonas sp.*	1066	OF	A	MK637909
PN2-B05P1-17	May 2015	*Streptomyces sp.*	1090	OF	A	MK638556
PN2-B05P1-18	May 2015	*Plantibacter sp.*	1084	OF	A	MK638377
PN2-B05P1-20	May 2015	*Rhizobium sp.*	1066	OF	A	MK638395
PN2-B05P3-1	May 2015	*Pseudomonas sp.*	1085	CF	A	MK637917
PN2-B05P3-2	May 2015	*Pseudomonas sp.*	1086	CF	A	MK637924
PN2-B05P3-3	May 2015	*Pseudomonas sp.*	1076	CF	A	MK637952
PN2-B05P3-4	May 2015	*Pseudomonas sp.*	1085	CF	A	MK637937
PN2-B05P3-5	May 2015	*Pseudomonas sp.*	1072	CF	A	MK637945
PN2-B05P3-6	May 2015	*Pseudomonas sp.*	1081	CF	A	MK637923
PN2-B05P3-7	May 2015	*Pseudomonas sp.*	1085	CF	A	MK637935
PN2-B05P3-8	May 2015	*Pseudomonas sp.*	1077	CF	A	MK637936
PN2-B05P3-9	May 2015	*Pseudomonas sp.*	1085	CF	A	MK637944
PN2-B05P3-10	May 2015	*Agrobacterium sp.*	1068	CF	A	MK638047
PN2-B05P3-11	May 2015	*Streptomyces sp.*	1012	CF	A	MK638463
PN2-B05P3-12	May 2015	*Streptomyces sp.*	1085	CF	A	MK638458
PN2-B05P3-13	May 2015	*Phyllobacterium sp.*	1071	CF	A	MK638211
PN2-B05P3-14	May 2015	*Phyllobacterium sp.*	1070	CF	A	MK638213
PN2-B05P3-15	May 2015	*Pseudomonas sp.*	1076	CF	A	MK637919
PN2-B05P3-16	May 2015	*Rugamonas sp.*	1047	CF	A	MK638091
PN2-B05P3-17	May 2015	*Achromobacter sp.*	1017	CF	A	MK638022

PN2-B05P3-18	May 2015	*Phyllobacterium sp.*	1068	CF	A	MK638399
PN2-B05P3-19	May 2015	*Streptomyces sp.*	1087	CF	A	MK638568
PN2-B05P3-20	May 2015	*Phyllobacterium sp.*	1021	CF	A	MK638216
PN2-B05P3-21	May 2015	*Agromyces sp.*	1072	CF	A	MK638052
PN2-B06P2-1	May 2015	*Pseudomonas sp.*	1076	CF	F	MK637941
PN2-B06P2-2	May 2015	*Pseudomonas sp.*	1066	CF	F	MK637939
PN2-B06P2-3	May 2015	*Pseudomonas sp.*	1072	CF	F	MK637930
PN2-B06P2-4	May 2015	*Pseudomonas sp.*	1077	CF	F	MK637942
PN2-B06P2-5	May 2015	*Pseudomonas sp.*	1053	CF	F	MK637940
PN2-B06P2-6	May 2015	*Pseudomonas sp.*	1085	CF	F	MK637926
PN2-B06P2-7	May 2015	*Pseudomonas sp.*	1085	CF	F	MK637949
PN2-B06P2-8	May 2015	*Bacillus sp.*	280	CF	F	MK638081
PN2-B06P2-9	May 2015	*Pseudomonas sp.*	1085	CF	F	MK637914
PN2-B06P2-10	May 2015	*Pseudomonas sp.*	1082	CF	F	MK637951
PN2-B06P2-11	May 2015	*Pseudomonas sp.*	1073	CF	F	MK637913
PN2-B06P2-12	May 2015	*Pseudomonas sp.*	1081	CF	F	MK637922
PN2-B06P2-14	May 2015	*Pseudomonas sp.*	1077	CF	F	MK637928
PN2-B06P2-15	May 2015	*Pseudomonas sp.*	1085	CF	F	MK637931
PN2-B06P2-16	May 2015	*Phyllobacterium sp.*	1070	CF	F	MK638212
PN2-B06P2-17	May 2015	*Streptomyces sp.*	1086	CF	F	MK638567
PN2-B06P2-19	May 2015	*Pseudomonas sp.*	1080	CF	F	MK637920
PN2-B06P2-20	May 2015	*Phyllobacterium sp.*	1079	CF	F	MK638210
PN2-B06P4-1	May 2015	*Pseudomonas sp.*	1072	OF	F	MK637925
PN2-B06P4-2	May 2015	*Pseudomonas sp.*	1084	OF	F	MK637933
PN2-B06P4-3	May 2015	*Pseudomonas sp.*	1077	OF	F	MK637929
PN2-B06P4-4	May 2015	*Pseudomonas sp.*	1057	OF	F	MK637934
PN2-B06P4-5	May 2015	*Serratia sp.*	1039	OF	F	MK638441
PN2-B06P4-6	May 2015	*Pseudomonas sp.*	1072	OF	F	MK637921
PN2-B06P4-7	May 2015	*Streptomyces sp.*	1070	OF	F	MK638459
PN2-B06P4-8	May 2015	*Streptomyces sp.*	1080	OF	F	MK638461
PN2-B06P4-9	May 2015	*Bacillus sp.*	1094	OF	F	MK638068
PN2-B06P4-10	May 2015	*Streptomyces sp.*	1070	OF	F	MK638595
PN2-B06P4-11	May 2015	*Pseudomonas sp.*	1072	OF	F	MK637918

PN2-B06P4-12	May 2015	*Streptomyces sp.*	1085	OF	F	MK638457
PN2-B06P4-13	May 2015	*Pseudomonas sp.*	1085	OF	F	MK637915
PN2-B06P4-14	May 2015	*Phyllobacterium sp.*	1066	OF	F	MK638396
PN2-B06P4-16	May 2015	*Phyllobacterium sp.*	1070	OF	F	MK638214
PN2-B06P4-17	May 2015	*Streptomyces sp.*	1085	OF	F	MK638473
PN2-B06P4-18	May 2015	*Streptomyces sp.*	1064	OF	F	MK638593
PN2-B06P4-19	May 2015	*Streptomyces sp.*	1079	OF	F	MK638612
PN2-B06P4-20	May 2015	*Streptomyces sp.*	1010	OF	F	MK638462
PN2-B06P4-22	May 2015	*Phyllobacterium sp.*	1068	OF	F	MK638397
PN2-B07P1-1	May 2015	*Pseudomonas sp.*	1067	OF	F	MK637954
PN2-B07P1-2	May 2015	*Pseudomonas sp.*	1070	OF	F	MK637953
PN2-B07P1-3	May 2015	*Pseudomonas sp.*	1076	OF	F	MK637956
PN2-B07P1-4	May 2015	*Pseudomonas sp.*	1029	OF	F	MK637946
PN2-B07P1-5	May 2015	*Pseudomonas sp.*	1070	OF	F	MK637943
PN2-B07P1-6	May 2015	*Pseudomonas sp.*	1069	OF	F	MK637947
PN2-B07P1-7	May 2015	*Rugamonas sp.*	1068	OF	F	MK638092
PN2-B07P1-8	May 2015	*Pseudomonas sp.*	1073	OF	F	MK637912
PN2-B07P1-9	May 2015	*Rhizobium sp.*	1025	OF	F	MK638400
PN2-B07P1-10	May 2015	*Rhodococcus sp.*	1078	OF	F	MK638436
PN2-B07P1-11	May 2015	*Streptomyces sp.*	1085	OF	F	MK638565
PN2-B07P1-12	May 2015	*Pseudomonas sp.*	1072	OF	F	MK637916
PN2-B07P1-13	May 2015	*Streptomyces sp.*	1085	OF	F	MK638566
PN2-B07P1-14	May 2015	*Pseudomonas sp.*	466	OF	F	MK638010
PN2-B07P1-15	May 2015	*Ochrobactrum sp.*	1049	OF	F	MK638199
PN2-B07P1-16	May 2015	*Pseudomonas sp.*	1077	OF	F	MK637927
PN2-B07P1-17	May 2015	*Rhizobium sp.*	1062	OF	F	MK638401
PN2-B07P1-18	May 2015	*Plantibacter sp.*	1076	OF	F	MK638378
PN2-B07P1-19	May 2015	*Streptomyces sp.*	1060	OF	F	MK638460
PN2-B07P1-20	May 2015	*Variovorax sp.*	1031	OF	F	MK638654

PN2-B07P5-1	May 2015	*Tsukamurella sp.*	1058	CF	F	MK638650
PN2-B07P5-3	May 2015	*Streptomyces sp.*	1085	CF	F	MK638456
PN2-B07P5-4	May 2015	*Pseudomonas sp.*	1067	CF	F	MK637938
PN2-B07P5-5	May 2015	*Pseudomonas sp.*	1085	CF	F	MK637950
PN2-B07P5-6	May 2015	*Tsukamurella sp.*	1076	CF	F	MK638651
PN2-B07P5-7	May 2015	*Pseudomonas sp.*	1077	CF	F	MK637932
PN2-B07P5-8	May 2015	*Pseudomonas sp.*	1078	CF	F	MK637948
PN2-B07P5-9	May 2015	*Streptomyces sp.*	1085	CF	F	MK638467
PN2-B07P5-10	May 2015	*Dyadobacter sp.*	1071	CF	F	MK638114
PN2-B07P5-11	May 2015	*Pseudomonas sp.*	1076	CF	F	MK637955
PN2-B07P5-12	May 2015	*Dyadobacter sp.*	1040	CF	F	MK638113
PN2-B07P5-13	May 2015	*Phyllobacterium sp.*	1065	CF	F	MK638215
PN2-B07P5-14	May 2015	*Streptomyces sp.*	1018	CF	F	MK638624
PN2-B07P5-15	May 2015	*Phyllobacterium sp.*	1070	CF	F	MK638219
PN2-B07P5-16	May 2015	*Mesorhizobium sp.*	1080	CF	F	MK638165
PN2-B07P5-17	May 2015	*Streptomyces sp.*	673	CF	F	MK638647
PN2-B07P5-18	May 2015	*Phyllobacterium sp.*	1077	CF	F	MK638224
PN2-B07P5-20	May 2015	*Phyllobacterium sp.*	1066	CF	F	MK638225
PN2-B07P5-21	May 2015	*Streptomyces sp.*	1076	CF	F	MK638532
PN2-B07P5-22	May 2015	*Phyllobacterium sp.*	1070	CF	F	MK638221
PN2-B07P5-23	May 2015	*Phyllobacterium sp.*	1070	CF	F	MK638223
PN2-B07P5-24	May 2015	*Phyllobacterium sp.*	1077	CF	F	MK638217
PN2-B08P4-1	May 2015	*Pseudomonas sp.*	1074	CF	A	MK637962
PN2-B08P4-2	May 2015	*Pseudomonas sp.*	1072	CF	A	MK637967
PN2-B08P4-3	May 2015	*Pseudomonas sp.*	1070	CF	A	MK637976
PN2-B08P4-4	May 2015	*Pseudomonas sp.*	1076	CF	A	MK637960
PN2-B08P4-5	May 2015	*Pseudomonas sp.*	1073	CF	A	MK637963
PN2-B08P4-6	May 2015	*Pseudomonas sp.*	1077	CF	A	MK637971
PN2-B08P4-7	May 2015	*Pseudomonas sp.*	1077	CF	A	MK637973
PN2-B08P4-8	May 2015	*Pseudomonas sp.*	1072	CF	A	MK637984
PN2-B08P4-9	May 2015	*Pseudomonas sp.*	766	CF	A	MK637990
PN2-B08P4-10	May 2015	*Pseudomonas sp.*	1074	CF	A	MK637977
PN2-B08P4-11	May 2015	*Pseudomonas sp.*	1072	CF	A	MK637969

PN2-B08P4-12	May 2015	*Pseudomonas sp.*	1072	CF	A	MK637983
PN2-B08P4-13	May 2015	*Pseudomonas sp.*	1077	CF	A	MK637975
PN2-B08P4-14	May 2015	*Pseudomonas sp.*	1073	CF	A	MK637961
PN2-B08P4-15	May 2015	*Serratia sp.*	1081	CF	A	MK638442
PN2-B08P4-16	May 2015	*Pseudomonas sp.*	1073	CF	A	MK637982
PN2-B08P4-17	May 2015	*Pseudomonas sp.*	1074	CF	A	MK637957
PN2-B08P4-18	May 2015	*Microbacterium sp.*	303	CF	A	MK638190
PN2-B08P4-19	May 2015	*Pseudomonas sp.*	1085	CF	A	MK637972
PN2-B08P4-20	May 2015	*Pseudomonas sp.*	1077	CF	A	MK637965
PN2-B08P5-1	May 2015	*Pseudomonas sp.*	1072	OF	A	MK637986
PN2-B08P5-2	May 2015	*Pseudomonas sp.*	1086	OF	A	MK637970
PN2-B08P5-3	May 2015	*Pseudomonas sp.*	1079	OF	A	MK637966
PN2-B08P5-4	May 2015	*Variovorax sp.*	1067	OF	A	MK638655
PN2-B08P5-5	May 2015	*Pseudomonas sp.*	1074	OF	A	MK637974
PN2-B08P5-6	May 2015	*Pseudomonas sp.*	1072	OF	A	MK637985
PN2-B08P5-7	May 2015	*Pseudomonas sp.*	1072	OF	A	MK637980
PN2-B08P5-8	May 2015	*Pseudomonas sp.*	1072	OF	A	MK637981
PN2-B08P5-9	May 2015	*Rhodococcus sp.*	721	OF	A	MK638440
PN2-B08P5-10	May 2015	*Pseudomonas sp.*	1072	OF	A	MK637979
PN2-B08P5-11	May 2015	*Rhizobium sp.*	1062	OF	A	MK638404
PN2-B08P5-12	May 2015	*Rhodococcus sp.*	1030	OF	A	MK638437
PN2-B08P5-13	May 2015	*Flavobacterium sp.*	1053	OF	A	MK638130
PN2-B08P5-14	May 2015	*Phyllobacterium sp.*	1070	OF	A	MK638232
PN2-B08P5-15	May 2015	*Streptomyces sp.*	1073	OF	A	MK638466
PN2-B08P5-16	May 2015	*Ensifer sp.*	1070	OF	A	MK638118
PN2-B08P5-17	May 2015	*Phyllobacterium sp.*	1070	OF	A	MK638218
PN2-B08P5-18	May 2015	*Phyllobacterium sp.*	1066	OF	A	MK638402
PN2-B08P5-19	May 2015	*Rhizobium sp.*	1066	OF	A	MK638403
PN2-B08P5-20	May 2015	*Streptomyces sp.*	1056	OF	A	MK638576

PN2-B08P5-21	May 2015	*Phyllobacterium sp.*	1070	OF	A	MK638230
PN2-B09P3-1	May 2015	*Streptomyces sp.*	1076	CF	F	MK638479
PN2-B09P3-2	May 2015	*Pseudomonas sp.*	1072	CF	F	MK637964
PN2-B09P3-3	May 2015	*Streptomyces sp.*	1066	CF	F	MK638522
PN2-B09P3-4	May 2015	*Rugamonas sp.*	1071	CF	F	MK638093
PN2-B09P3-5	May 2015	*Streptomyces sp.*	1079	CF	F	MK638465
PN2-B09P3-7	May 2015	*Arthrobacter sp.*	1093	CF	F	MK638056
PN2-B09P3-8	May 2015	*Streptomyces sp.*	1070	CF	F	MK638480
PN2-B09P3-9	May 2015	*Rhodococcus sp.*	1069	CF	F	MK638439
PN2-B09P3-10	May 2015	*Streptomyces sp.*	1056	CF	F	MK638592
PN2-B09P3-11	May 2015	*Streptomyces sp.*	1073	CF	F	MK638598
PN2-B09P3-13	May 2015	*Flavobacterium sp.*	1040	CF	F	MK638131
PN2-B09P3-14	May 2015	*Phyllobacterium sp.*	1070	CF	F	MK638227
PN2-B09P3-15	May 2015	*Streptomyces sp.*	1070	CF	F	MK638521
PN2-B09P3-16	May 2015	*Phyllobacterium sp.*	1068	CF	F	MK638228
PN2-B09P3-17	May 2015	*Phyllobacterium sp.*	1068	CF	F	MK638229
PN2-B09P3-18	May 2015	*Phyllobacterium sp.*	1068	CF	F	MK638226
PN2-B09P3-19	May 2015	*Microbacterium sp.*	1077	CF	F	MK638186
PN2-B09P3-21	May 2015	*Phyllobacterium sp.*	1065	CF	F	MK638231
PN2-B09P3-22	May 2015	*Streptomyces sp.*	1076	CF	F	MK638481
PN2-B09P3-24	May 2015	*Phyllobacterium sp.*	1070	CF	F	MK638222
PN2-B09P5-1	May 2015	*Pseudomonas sp.*	1073	OF	F	MK637978
PN2-B09P5-2	May 2015	*Plantibacter sp.*	1071	OF	F	MK638380
PN2-B09P5-3	May 2015	*Pseudomonas sp.*	1074	OF	F	MK637968
PN2-B09P5-4	May 2015	*Pseudomonas sp.*	1074	OF	F	MK637958
PN2-B09P5-5	May 2015	*Agrobacterium sp.*	1073	OF	F	MK638044
PN2-B09P5-6	May 2015	*Streptomyces sp.*	1087	OF	F	MK638464
PN2-B09P5-7	May 2015	*Bacillus sp.*	1073	OF	F	MK638071
PN2-B09P5-8	May 2015	*Pseudomonas sp.*	1049	OF	F	MK637989
PN2-B09P5-9	May 2015	*Flavobacterium sp.*	907	OF	F	MK638132
PN2-B09P5-10	May 2015	*Pseudomonas sp.*	1045	OF	F	MK637988
PN2-B09P5-11	May 2015	*Bacillus sp.*	1072	OF	F	MK638070
PN2-B09P5-12	May 2015	*Agrobacterium sp.*	1029	OF	F	MK638043

PN2-B09P5-13	May 2015	*Pseudomonas sp.*	806	OF	F	MK637987
PN2-B09P5-14	May 2015	*Streptomyces sp.*	1085	OF	F	MK638519
PN2-B09P5-15	May 2015	*Phyllobacterium sp.*	1066	OF	F	MK638220
PN2-B09P5-16	May 2015	*Streptomyces sp.*	1066	OF	F	MK638477
PN2-B09P5-17	May 2015	*Plantibacter sp.*	1057	OF	F	MK638379
PN2-B09P5-18	May 2015	*Bacillus sp.*	1076	OF	F	MK638069
PN2-B09P5-21	May 2015	*Plantibacter sp.*	1015	OF	F	MK638381
PN2-B09P5-22	May 2015	*Phyllobacterium sp.*	1026	OF	F	MK638359
PN2-B10P3-1	May 2015	*Pseudomonas sp.*	1043	OF	A	MK638012
PN2-B10P3-2	May 2015	*Stenotrophomonas sp.*	1032	OF	A	MK638450
PN2-B10P3-3	May 2015	*Stenotrophomonas sp.*	1034	OF	A	MK638451
PN2-B10P3-5	May 2015	*Pseudomonas sp.*	1032	OF	A	MK638013
PN2-B10P3-6	May 2015	*Pseudomonas sp.*	1032	OF	A	MK638014
PN2-B10P3-7	May 2015	*Pseudomonas sp.*	1032	OF	A	MK638015
PN2-B10P3-8	May 2015	*Pseudomonas sp.*	1032	OF	A	MK638016
PN2-B10P3-9	May 2015	*Pseudomonas sp.*	1032	OF	A	MK638017
PN2-B10P3-10	May 2015	*Variovorax sp.*	464	OF	A	MK638661
PN2-B10P3-11	May 2015	*Variovorax sp.*	470	OF	A	MK638662
PN2-B10P3-12	May 2015	*Variovorax sp.*	751	OF	A	MK638663
PN2-B10P3-13	May 2015	*Stenotrophomonas sp.*	1034	OF	A	MK638448
PN2-B10P3-14	May 2015	*Variovorax sp.*	506	OF	A	MK638664
PN2-B10P3-15	May 2015	*Mesorhizobium sp.*	1037	OF	A	MK638183
PN2-B10P3-17	May 2015	*Streptomyces sp.*	1025	OF	A	MK638613
PN2-B10P3-18	May 2015	*Streptomyces sp.*	957	OF	A	MK638614
PN2-B10P3-20	May 2015	*Rhizobium sp.*	1004	OF	A	MK638432
PN2-B10P3-21	May 2015	*Stenotrophomonas sp.*	1024	OF	A	MK638449
PN2-B10P5-1	May 2015	*Pseudomonas sp.*	1032	CF	A	MK638018
PN2-B10P5-2	May 2015	*Flavobacterium sp.*	1027	CF	A	MK638133
PN2-B10P5-3	May 2015	*Rhizobium sp.*	1017	CF	A	MK638433

PN2-B10P5-4	May 2015	*Pseudomonas sp.*	1032	CF	A	MK638021
PN2-B10P5-5	May 2015	*Streptomyces sp.*	1071	CF	A	MK638515
PN2-B10P5-6	May 2015	*Clavibacter sp.*	1003	CF	A	MK638110
PN2-B10P5-8	May 2015	*Agrobacterium sp.*	1008	CF	A	MK638048
PN2-B10P5-10	May 2015	*Pseudomonas sp.*	1032	CF	A	MK638019
PN2-B10P5-11	May 2015	*Pseudomonas sp.*	1032	CF	A	MK638020
PN2-B10P5-12	May 2015	*Bacillus sp.*	1027	CF	A	MK638062
PN2-B10P5-13	May 2015	*Streptomyces sp.*	1066	CF	A	MK638455
PN2-B10P5-14	May 2015	*Pseudomonas sp.*	1077	CF	A	MK637959
PN2-B10P5-15	May 2015	*Streptomyces sp.*	1001	CF	A	MK638594
PN2-B10P5-16	May 2015	*Agrobacterium sp.*	1068	CF	A	MK638042
PN2-B10P5-17	May 2015	*Agrobacterium sp.*	1070	CF	A	MK638041
PN2-B10P5-18	May 2015	*Streptomyces sp.*	1073	CF	A	MK638478
PN2-B10P5-19	May 2015	*Streptomyces sp.*	1069	CF	A	MK638560
PN2-B10P5-20	May 2015	*Rhizobium sp.*	1075	CF	A	MK638398
PN2-B10P5-21	May 2015	*Phyllobacterium sp.*	1070	CF	A	MK638209
PN2-B10P5-22	May 2015	*Leifsonia sp.*	1082	CF	A	MK638143
PN3-B01P2-1	July 2015	*Phyllobacterium sp.*	998	CF	A	MK638236
PN3-B01P2-2	July 2015	*Streptomyces sp.*	1018	CF	A	MK638483
PN3-B01P2-3	July 2015	*Rhizobium sp.*	1023	CF	A	MK638410
PN3-B01P2-4	July 2015	*Mesorhizobium sp.*	1025	CF	A	MK638169
PN3-B01P2-5	July 2015	*Streptomyces sp.*	1047	CF	A	MK638559
PN3-B01P2-6	July 2015	*Rhizobium sp.*	1026	CF	A	MK638412
PN3-B01P2-7	July 2015	*Streptomyces sp.*	1008	CF	A	MK638514
PN3-B01P2-9	July 2015	*Rhizobium sp.*	1009	CF	A	MK638408
PN3-B01P2-10	July 2015	*Mesorhizobium sp.*	1034	CF	A	MK638167
PN3-B01P2-12	July 2015	*Rhizobium sp.*	1029	CF	A	MK638411
PN3-B01P2-14	July 2015	*Mesorhizobium sp.*	1026	CF	A	MK638168
PN3-B01P2-15	July 2015	*Leifsonia sp.*	1043	CF	A	MK638144
PN3-B01P2-17	July 2015	*Rhizobium sp.*	1020	CF	A	MK638407

PN3-B01P2-18	July 2015	*Inquilinus sp.*	952	CF	A	MK638135
PN3-B01P2-19	July 2015	*Streptomyces sp.*	1018	CF	A	MK638512
PN3-B01P2-20	July 2015	*Inquilinus sp.*	999	CF	A	MK638136
PN3-B01P2-21	July 2015	*Phyllobacterium sp.*	997	CF	A	MK638241
PN3-B01P2-22	July 2015	*Phyllobacterium sp.*	1025	CF	A	MK638239
PN3-B01P2-23	July 2015	*Leifsonia sp.*	1032	CF	A	MK638145
PN3-B01P4-1	July 2015	*Streptomyces sp.*	1026	OF	A	MK638563
PN3-B01P4-3	July 2015	*Streptomyces sp.*	1010	OF	A	MK638558
PN3-B01P4-5	July 2015	*Streptomyces sp.*	1035	OF	A	MK638562
PN3-B01P4-6	July 2015	*Burkholderia sp.*	926	OF	A	MK638088
PN3-B01P4-7	July 2015	*Streptomyces sp.*	1015	OF	A	MK638540
PN3-B01P4-8	July 2015	*Phyllobacterium sp.*	1026	OF	A	MK638242
PN3-B01P4-9	July 2015	*Agrobacterium sp.*	943	OF	A	MK638023
PN3-B01P4-10	July 2015	*Ensifer sp.*	1016	OF	A	MK638119
PN3-B01P4-11	July 2015	*Streptomyces sp.*	1041	OF	A	MK638518
PN3-B01P4-12	July 2015	*Streptomyces sp.*	1064	OF	A	MK638509
PN3-B01P4-13	July 2015	*Agrobacterium sp.*	1042	OF	A	MK638039
PN3-B01P4-14	July 2015	*Streptomyces sp.*	1038	OF	A	MK638534
PN3-B01P4-15	July 2015	*Phyllobacterium sp.*	1026	OF	A	MK638237
PN3-B01P4-16	July 2015	*Streptomyces sp.*	1028	OF	A	MK638516
PN3-B01P4-17	July 2015	*Agrobacterium sp.*	948	OF	A	MK638024
PN3-B01P4-18	July 2015	*Rhizobium sp.*	1028	OF	A	MK638409
PN3-B01P4-19	July 2015	*Rhizobium sp.*	818	OF	A	MK638413
PN3-B01P4-20	July 2015	*Rhizobium sp.*	895	OF	A	MK638414
PN3-B01P4-21	July 2015	*Ensifer sp.*	1029	OF	A	MK638120
PN3-B01P4-22	July 2015	*Ensifer sp.*	1023	OF	A	MK638122
PN3-B01P4-23	July 2015	*Rhizobium sp.*	1039	OF	A	MK638405

PN3-B01P4-24	July 2015	*Streptomyces sp.*	1025	OF	A	MK638517
PN3-B02P3-1	July 2015	*Phyllobacterium sp.*	1026	OF	F	MK638246
PN3-B02P3-2	July 2015	*Variovorax sp.*	266	OF	F	MK638660
PN3-B02P3-3	July 2015	*Pseudomonas sp.*	951	OF	F	MK637991
PN3-B02P3-4	July 2015	*Phyllobacterium sp.*	950	OF	F	MK638238
PN3-B02P3-5	July 2015	*Streptomyces sp.*	1013	OF	F	MK638600
PN3-B02P3-6	July 2015	*Bacillus sp.*	841	OF	F	MK638072
PN3-B02P3-7	July 2015	*Bacillus sp.*	982	OF	F	MK638074
PN3-B02P3-8	July 2015	*Bacillus sp.*	1024	OF	F	MK638075
PN3-B02P3-9	July 2015	*Bacillus sp.*	1009	OF	F	MK638079
PN3-B02P3-10	July 2015	*Bacillus sp.*	1011	OF	F	MK638077
PN3-B02P3-11	July 2015	*Bacillus sp.*	967	OF	F	MK638073
PN3-B02P3-12	July 2015	*Bacillus sp.*	1024	OF	F	MK638076
PN3-B02P3-13	July 2015	*Streptomyces sp.*	1010	OF	F	MK638526
PN3-B02P3-14	July 2015	*Streptomyces sp.*	1031	OF	F	MK638510
PN3-B02P3-15	July 2015	*Bacillus sp.*	977	OF	F	MK638078
PN3-B02P3-17	July 2015	*Streptomyces sp.*	1018	OF	F	MK638523
PN3-B02P3-18	July 2015	*Phyllobacterium sp.*	999	OF	F	MK638243
PN3-B02P3-19	July 2015	*Phyllobacterium sp.*	1026	OF	F	MK638244
PN3-B02P3-20	July 2015	*Phyllobacterium sp.*	1024	OF	F	MK638245
PN3-B02P3-21	July 2015	*Streptomyces sp.*	1018	OF	F	MK638535
PN3-B02P3-22	July 2015	*Bradyrhizobium sp.*	1027	OF	F	MK638083
PN3-B02P4-1	July 2015	*Phyllobacterium sp.*	1034	CF	F	MK638235
PN3-B02P4-3	July 2015	*Mesorhizobium sp.*	1026	CF	F	MK638166
PN3-B02P4-4	July 2015	*Phyllobacterium sp.*	907	CF	F	MK638247
PN3-B02P4-5	July 2015	*Herbiconiux sp.*	293	CF	F	MK638668
PN3-B02P4-6	July 2015	*Phyllobacterium sp.*	663	CF	F	MK638248
PN3-B02P4-7	July 2015	*Phyllobacterium sp.*	1030	CF	F	MK638240
PN3-B02P4-15	July 2015	*Streptomyces sp.*	1057	CF	F	MK638561

PN3-B02P4-17	July 2015	*Streptomyces sp.*	1012	CF	F	MK638601
PN3-B02P4-18	July 2015	*Streptomyces sp.*	914	CF	F	MK638603
PN3-B02P4-19	July 2015	*Phyllobacterium sp.*	1024	CF	F	MK638234
PN3-B02P4-20	July 2015	*Pseudaminobacter sp.*	1012	CF	F	MK638382
PN3-B02P4-21	July 2015	*Streptomyces sp.*	1018	CF	F	MK638513
PN3-B02P4-22	July 2015	*Rhizobium sp.*	1023	CF	F	MK638406
PN3-B03P1-1	July 2015	*Agrobacterium sp.*	1029	CF	A	MK638038
PN3-B03P1-2	July 2015	*Variovorax sp.*	624	CF	A	MK638659
PN3-B03P1-3	July 2015	*Mesorhizobium sp.*	1001	CF	A	MK638171
PN3-B03P1-4	July 2015	*Streptomyces sp.*	814	CF	A	MK638602
PN3-B03P1-5	July 2015	*Mesorhizobium sp.*	1052	CF	A	MK638170
PN3-B03P1-6	July 2015	*Ensifer sp.*	1021	CF	A	MK638121
PN3-B03P1-7	July 2015	*Streptomyces sp.*	1022	CF	A	MK638564
PN3-B03P1-10	July 2015	*Streptomyces sp.*	908	CF	A	MK638604
PN3-B03P1-12	July 2015	*Inquilinus sp.*	1019	CF	A	MK638137
PN3-B03P1-13	July 2015	*Phyllobacterium sp.*	1011	CF	A	MK638233
PN3-B03P1-14	July 2015	*Leifsonia sp.*	1019	CF	A	MK638146
PN3-B03P1-16	July 2015	*Streptomyces sp.*	1010	CF	A	MK638599
PN3-B03P1-17	July 2015	*Streptomyces sp.*	1025	CF	A	MK638511
PN3-B03P1-18	July 2015	*Dyella sp.*	962	CF	A	MK638117
PN3-B03P1-19	July 2015	*Streptomyces sp.*	1010	CF	A	MK638533
PN3-B03P1-20	July 2015	*Variovorax sp.*	991	CF	A	MK638656
PN3-B03P1-21	July 2015	*Streptomyces sp.*	1060	CF	A	MK638550
PN3-B03P1-22	July 2015	*Streptomyces sp.*	1034	CF	A	MK638520
PN3-B03P1-23	July 2015	*Streptomyces sp.*	1024	CF	A	MK638525
PN3-B03P2-1	July 2015	*Dyella sp.*	1051	OF	A	MK638665
PN3-B03P2-2	July 2015	*Dyella sp.*	1039	OF	A	MK638666
PN3-B03P2-3	July 2015	*Streptomyces sp.*	1070	OF	A	MK638581
PN3-B03P2-4	July 2015	*Streptomyces sp.*	1019	OF	A	MK638548
PN3-B03P2-5	July 2015	*Streptomyces sp.*	1059	OF	A	MK638547
PN3-B03P2-6	July 2015	*Rhizobium sp.*	1050	OF	A	MK638420

PN3-B03P2-7	July 2015	*Streptomyces sp.*	1070	OF	A	MK638546
PN3-B03P2-8	July 2015	*Rhizobium sp.*	1062	OF	A	MK638415
PN3-B03P2-9	July 2015	*Streptomyces sp.*	1066	OF	A	MK638544
PN3-B03P2-10	July 2015	*Rhizobium sp.*	1058	OF	A	MK638418
PN3-B03P2-12	July 2015	*Leifsonia sp.*	1058	OF	A	MK638147
PN3-B03P2-13	July 2015	*Streptomyces sp.*	1041	OF	A	MK638579
PN3-B03P2-14	July 2015	*Mesorhizobium sp.*	1061	OF	A	MK638172
PN3-B03P2-15	July 2015	*Mesorhizobium sp.*	1073	OF	A	MK638173
PN3-B03P2-16	July 2015	*Streptomyces sp.*	1002	OF	A	MK638586
PN3-B03P2-17	July 2015	*Streptomyces sp.*	1022	OF	A	MK638543
PN3-B03P2-18	July 2015	*Streptomyces sp.*	1018	OF	A	MK638625
PN3-B03P2-19	July 2015	*Pseudomonas sp.*	1069	OF	A	MK637995
PN3-B03P2-20	July 2015	*Luteibacter sp.*	1039	OF	A	MK638152
PN3-B03P2-21	July 2015	*Streptomyces sp.*	1070	OF	A	MK638552
PN3-B03P2-22	July 2015	*Streptomyces sp.*	1024	OF	A	MK638609
PN3-B04P1-1	July 2015	*Pseudomonas sp.*	1067	CF	F	MK637996
PN3-B04P1-2	July 2015	*Streptomyces sp.*	1055	CF	F	MK638577
PN3-B04P1-3	July 2015	*Rhizobium sp.*	1069	CF	F	MK638421
PN3-B04P1-4	July 2015	*Phyllobacterium sp.*	1052	CF	F	MK638259
PN3-B04P1-6	July 2015	*Rhizobium sp.*	1024	CF	F	MK638422
PN3-B04P1-7	July 2015	*Phyllobacterium sp.*	1061	CF	F	MK638249
PN3-B04P1-8	July 2015	*Microbacterium sp.*	1048	CF	F	MK638188
PN3-B04P1-9	July 2015	*Stenotrophomonas sp.*	1041	CF	F	MK638446
PN3-B04P1-10	July 2015	*Mesorhizobium sp.*	1042	CF	F	MK638175
PN3-B04P1-11	July 2015	*Streptomyces sp.*	1055	CF	F	MK638578
PN3-B04P1-12	July 2015	*Phyllobacterium sp.*	1049	CF	F	MK638250
PN3-B04P1-14	July 2015	*Phyllobacterium sp.*	1068	CF	F	MK638256
PN3-B04P1-15	July 2015	*Streptomyces sp.*	1073	CF	F	MK638551

PN3-B04P1-17	July 2015	*Microbacterium sp.*	1047	CF	F	MK638187
PN3-B04P1-18	July 2015	*Tardiphaga sp.*	1035	CF	F	MK638649
PN3-B04P1-20	July 2015	*Caulobacter sp.*	541	CF	F	MK638105
PN3-B04P1-21	July 2015	*Streptomyces sp.*	1024	CF	F	MK638589
PN3-B04P1-22	July 2015	*Streptomyces sp.*	1007	CF	F	MK638554
PN3-B04P2-1	July 2015	*Luteibacter sp.*	1029	OF	F	MK638151
PN3-B04P2-2	July 2015	*Pseudomonas sp.*	1055	OF	F	MK637993
PN3-B04P2-3	July 2015	*Streptomyces sp.*	1061	OF	F	MK638580
PN3-B04P2-4	July 2015	*Pseudomonas sp.*	1055	OF	F	MK637997
PN3-B04P2-5	July 2015	*Streptomyces sp.*	1061	OF	F	MK638582
PN3-B04P2-6	July 2015	*Rhizobium sp.*	1026	OF	F	MK638419
PN3-B04P2-7	July 2015	*Phyllobacterium sp.*	1038	OF	F	MK638260
PN3-B04P2-8	July 2015	*Ensifer sp.*	1029	OF	F	MK638123
PN3-B04P2-9	July 2015	*Mycobacterium sp.*	1067	OF	F	MK638195
PN3-B04P2-10	July 2015	*Luteibacter sp.*	1051	OF	F	MK638150
PN3-B04P2-11	July 2015	*Agrobacterium sp.*	1065	OF	F	MK637994
PN3-B04P2-12	July 2015	*Mycobacterium sp.*	1047	OF	F	MK638194
PN3-B04P2-13	July 2015	*Phyllobacterium sp.*	541	OF	F	MK638367
PN3-B04P2-14	July 2015	*Streptomyces sp.*	1018	OF	F	MK638626
PN3-B04P2-15	July 2015	*Streptomyces sp.*	955	OF	F	MK638607
PN3-B04P2-16	July 2015	*Rhizobium sp.*	1064	OF	F	MK638416
PN3-B04P2-17	July 2015	*Mesorhizobium sp.*	1057	OF	F	MK638174
PN3-B04P2-19	July 2015	*Mesorhizobium sp.*	1068	OF	F	MK638177
PN3-B04P2-20	July 2015	*Streptomyces sp.*	1055	OF	F	MK638605
PN3-B04P2-21	July 2015	*Rhizobium sp.*	1062	OF	F	MK638417
PN3-B04P2-22	July 2015	*Streptomyces sp.*	1067	OF	F	MK638584
PN3-B04P2-23	July 2015	*Phyllobacterium sp.*	541	OF	F	MK638366
PN3-B05P1-2	July 2015	*Phyllobacterium sp.*	1051	OF	A	MK638255
PN3-B05P1-3	July 2015	*Inquilinus sp.*	1053	OF	A	MK638138

PN3-B05P1-4	July 2015	*Microbacterium sp.*	507	OF	A	MK638191
PN3-B05P1-5	July 2015	*Phyllobacterium sp.*	1073	OF	A	MK638251
PN3-B05P1-6	July 2015	*Streptomyces sp.*	1025	OF	A	MK638588
PN3-B05P1-7	July 2015	*Paraburkholderia sp.*	1036	OF	A	MK638089
PN3-B05P1-8	July 2015	*Inquilinus sp.*	1047	OF	A	MK638139
PN3-B05P1-9	July 2015	*Streptomyces sp.*	1073	OF	A	MK638583
PN3-B05P1-10	July 2015	*Streptomyces sp.*	891	OF	A	MK638608
PN3-B05P1-11	July 2015	*Phyllobacterium sp.*	1029	OF	A	MK638258
PN3-B05P1-12	July 2015	*Phyllobacterium sp.*	1070	OF	A	MK638257
PN3-B05P1-13	July 2015	*Pseudomonas sp.*	1030	OF	A	MK637998
PN3-B05P1-14	July 2015	*Streptomyces sp.*	1022	OF	A	MK638585
PN3-B05P1-15	July 2015	*Mesorhizobium sp.*	1074	OF	A	MK638176
PN3-B05P1-16	July 2015	*Streptomyces sp.*	864	OF	A	MK638606
PN3-B05P1-17	July 2015	*Phyllobacterium sp.*	1043	OF	A	MK638254
PN3-B05P1-18	July 2015	*Streptomyces sp.*	1055	OF	A	MK638587
PN3-B05P1-19	July 2015	*Rhizobium sp.*	1026	OF	A	MK638423
PN3-B05P1-21	July 2015	*Agrobacterium sp.*	1062	OF	A	MK638025
PN3-B05P3-1	July 2015	*Phyllobacterium sp.*	1052	CF	A	MK638253
PN3-B05P3-2	July 2015	*Streptomyces sp.*	1060	CF	A	MK638524
PN3-B05P3-3	July 2015	*Agromyces sp.*	1023	CF	A	MK638051
PN3-B05P3-4	July 2015	*Streptomyces sp.*	1054	CF	A	MK638527
PN3-B05P3-5	July 2015	*Pseudomonas sp.*	1067	CF	A	MK637992
PN3-B05P3-6	July 2015	*Phyllobacterium sp.*	1065	CF	A	MK638252
PN3-B05P3-7	July 2015	*Phyllobacterium sp.*	1031	CF	A	MK638261
PN3-B05P3-8	July 2015	*Phyllobacterium sp.*	1055	CF	A	MK638262
PN3-B05P3-9	July 2015	*Phyllobacterium sp.*	1051	CF	A	MK638272
PN3-B05P3-10	July 2015	*Streptomyces sp.*	931	CF	A	MK638484
PN3-B05P3-11	July 2015	*Inquilinus sp.*	1054	CF	A	MK638140

PN3-B05P3-12	July 2015	*Phyllobacterium sp.*	1053	CF	A	MK638285
PN3-B05P3-13	July 2015	*Pseudomonas sp.*	1074	CF	A	MK638000
PN3-B05P3-14	July 2015	*Phyllobacterium sp.*	1052	CF	A	MK638264
PN3-B05P3-15	July 2015	*Phyllobacterium sp.*	1053	CF	A	MK638288
PN3-B05P3-17	July 2015	*Streptomyces sp.*	1060	CF	A	MK638490
PN3-B05P3-18	July 2015	*Phyllobacterium sp.*	1062	CF	A	MK638269
PN3-B05P3-19	July 2015	*Phyllobacterium sp.*	1034	CF	A	MK638277
PN3-B05P3-20	July 2015	*Phyllobacterium sp.*	1045	CF	A	MK638270
PN3-B05P3-21	July 2015	*Streptomyces sp.*	1064	CF	A	MK638491
PN3-B05P3-23	July 2015	*Streptomyces sp.*	1060	CF	A	MK638508
PN3-B05P3-24	July 2015	*Streptomyces sp.*	1060	CF	A	MK638610
PN3-B06P2-1	July 2015	*Phyllobacterium sp.*	588	CF	F	MK638336
PN3-B06P2-2	July 2015	*Phyllobacterium sp.*	1029	CF	F	MK638296
PN3-B06P2-3	July 2015	*Phyllobacterium sp.*	1004	CF	F	MK638309
PN3-B06P2-4	July 2015	*Phyllobacterium sp.*	1034	CF	F	MK638298
PN3-B06P2-5	July 2015	*Phyllobacterium sp.*	1065	CF	F	MK638276
PN3-B06P2-6	July 2015	*Phyllobacterium sp.*	1035	CF	F	MK638291
PN3-B06P2-7	July 2015	*Devosia sp.*	1030	CF	F	MK638111
PN3-B06P2-8	July 2015	*Phyllobacterium sp.*	1062	CF	F	MK638275
PN3-B06P2-9	July 2015	*Phyllobacterium sp.*	1062	CF	F	MK638273
PN3-B06P2-10	July 2015	*Streptomyces sp.*	1060	CF	F	MK638505
PN3-B06P2-11	July 2015	*Phyllobacterium sp.*	1053	CF	F	MK638265
PN3-B06P2-12	July 2015	*Phyllobacterium sp.*	837	CF	F	MK638297
PN3-B06P2-13	July 2015	*Bacillus sp.*	527	CF	F	MK638080
PN3-B06P2-14	July 2015	*Phyllobacterium sp.*	1065	CF	F	MK638284

PN3-B06P2-15	July 2015	*Phyllobacterium sp.*	1053	CF	F	MK638266
PN3-B06P2-16	July 2015	*Streptomyces sp.*	1068	CF	F	MK638507
PN3-B06P2-17	July 2015	*Streptomyces sp.*	605	CF	F	MK638627
PN3-B06P2-18	July 2015	*Rhizobium sp.*	939	CF	F	MK638424
PN3-B06P2-19	July 2015	*Phyllobacterium sp.*	1030	CF	F	MK638274
PN3-B06P2-20	July 2015	*Streptomyces sp.*	1064	CF	F	MK638489
PN3-B06P2-21	July 2015	*Phyllobacterium sp.*	1073	CF	F	MK638271
PN3-B06P2-22	July 2015	*Chryseobacterium sp.*	1045	CF	F	MK638109
PN3-B06P2-23	July 2015	*Streptomyces sp.*	1062	CF	F	MK638492
PN3-B06P4-1	July 2015	*Pseudomonas sp.*	1030	OF	F	MK638002
PN3-B06P4-2	July 2015	*Pseudomonas sp.*	1068	OF	F	MK637999
PN3-B06P4-3	July 2015	*Phyllobacterium sp.*	1055	OF	F	MK638279
PN3-B06P4-4	July 2015	*Variovorax sp.*	1031	OF	F	MK638657
PN3-B06P4-5	July 2015	*Streptomyces sp.*	1060	OF	F	MK638538
PN3-B06P4-6	July 2015	*Phyllobacterium sp.*	1070	OF	F	MK638263
PN3-B06P4-7	July 2015	*Phyllobacterium sp.*	1050	OF	F	MK638287
PN3-B06P4-8	July 2015	*Phyllobacterium sp.*	1065	OF	F	MK638282
PN3-B06P4-9	July 2015	*Phyllobacterium sp.*	1029	OF	F	MK638278
PN3-B06P4-10	July 2015	*Streptomyces sp.*	1060	OF	F	MK638496
PN3-B06P4-11	July 2015	*Streptomyces sp.*	1059	OF	F	MK638506
PN3-B06P4-12	July 2015	*Phyllobacterium sp.*	1029	OF	F	MK638302
PN3-B06P4-13	July 2015	*Phyllobacterium sp.*	1031	OF	F	MK638294
PN3-B06P4-14	July 2015	*Streptomyces sp.*	1064	OF	F	MK638493
PN3-B06P4-15	July 2015	*Streptomyces sp.*	1055	OF	F	MK638494
PN3-B06P4-16	July 2015	*Phyllobacterium sp.*	1055	OF	F	MK638268
PN3-B06P4-17	July 2015	*Streptomyces sp.*	1056	OF	F	MK638590

PN3-B06P4-18	July 2015	*Streptomyces sp.*	1025	OF	F	MK638498
PN3-B06P4-19	July 2015	*Streptomyces sp.*	1062	OF	F	MK638499
PN3-B06P4-20	July 2015	*Phyllobacterium sp.*	1055	OF	F	MK638290
PN3-B06P4-21	July 2015	*Phyllobacterium sp.*	1061	OF	F	MK638267
PN3-B06P4-22	July 2015	*Phyllobacterium sp.*	1037	OF	F	MK638303
PN3-B06P4-23	July 2015	*Streptomyces sp.*	1056	OF	F	MK638500
PN3-B07P1-1	July 2015	*Streptomyces sp.*	1073	OF	F	MK638497
PN3-B07P1-3	July 2015	*Phyllobacterium sp.*	1061	OF	F	MK638280
PN3-B07P1-4	July 2015	*Phyllobacterium sp.*	1027	OF	F	MK638293
PN3-B07P1-5	July 2015	*Phyllobacterium sp.*	1034	OF	F	MK638306
PN3-B07P1-6	July 2015	*Phyllobacterium sp.*	1062	OF	F	MK638286
PN3-B07P1-7	July 2015	*Phyllobacterium sp.*	1006	OF	F	MK638310
PN3-B07P1-8	July 2015	*Phyllobacterium sp.*	1033	OF	F	MK638301
PN3-B07P1-9	July 2015	*Streptomyces sp.*	1064	OF	F	MK638549
PN3-B07P1-11	July 2015	*Pseudomonas sp.*	900	OF	F	MK638011
PN3-B07P1-12	July 2015	*Streptomyces sp.*	1056	OF	F	MK638591
PN3-B07P1-13	July 2015	*Phyllobacterium sp.*	1066	OF	F	MK638289
PN3-B07P1-14	July 2015	*Phyllobacterium sp.*	1035	OF	F	MK638307
PN3-B07P1-15	July 2015	*Streptomyces sp.*	1053	OF	F	MK638542
PN3-B07P1-16	July 2015	*Streptomyces sp.*	1046	OF	F	MK638487
PN3-B07P1-17	July 2015	*Phyllobacterium sp.*	1046	OF	F	MK638292
PN3-B07P1-18	July 2015	*Streptomyces sp.*	1056	OF	F	MK638541
PN3-B07P1-19	July 2015	*Phyllobacterium sp.*	999	OF	F	MK638295
PN3-B07P1-20	July 2015	*Phyllobacterium sp.*	1054	OF	F	MK638281
PN3-B07P1-21	July 2015	*Phyllobacterium sp.*	999	OF	F	MK638300

PN3-B07P1-22	July 2015	*Phyllobacterium sp.*	1035	OF	F	MK638299
PN3-B07P5-1	July 2015	*Phyllobacterium sp.*	1030	CF	F	MK638283
PN3-B07P5-2	July 2015	*Phyllobacterium sp.*	1021	CF	F	MK638376
PN3-B07P5-3	July 2015	*Phyllobacterium sp.*	1006	CF	F	MK638311
PN3-B07P5-4	July 2015	*Phyllobacterium sp.*	1010	CF	F	MK638305
PN3-B07P5-5	July 2015	*Agrobacterium sp.*	946	CF	F	MK638037
PN3-B07P5-6	July 2015	*Mesorhizobium sp.*	1027	CF	F	MK638178
PN3-B07P5-7	July 2015	*Mesorhizobium sp.*	1030	CF	F	MK638179
PN3-B07P5-8	July 2015	*Streptomyces sp.*	1064	CF	F	MK638495
PN3-B07P5-9	July 2015	*Streptomyces sp.*	1018	CF	F	MK638630
PN3-B07P5-10	July 2015	*Agrobacterium sp.*	1065	CF	F	MK638001
PN3-B07P5-11	July 2015	*Streptomyces sp.*	670	CF	F	MK638628
PN3-B07P5-12	July 2015	*Streptomyces sp.*	1059	CF	F	MK638488
PN3-B07P5-13	July 2015	*Streptomyces sp.*	1018	CF	F	MK638629
PN3-B07P5-14	July 2015	*Streptomyces sp.*	1070	CF	F	MK638501
PN3-B07P5-15	July 2015	*Ensifer sp.*	1072	CF	F	MK638124
PN3-B07P5-16	July 2015	*Mesorhizobium sp.*	1070	CF	F	MK638180
PN3-B07P5-18	July 2015	*Inquilinus sp.*	1035	CF	F	MK638141
PN3-B07P5-19	July 2015	*Agrobacterium sp.*	1030	CF	F	MK638036
PN3-B07P5-20	July 2015	*Phyllobacterium sp.*	925	CF	F	MK638314
PN3-B07P5-22	July 2015	*Mesorhizobium sp.*	1072	CF	F	MK638181
PN3-B07P5-23	July 2015	*Pseudomonas sp.*	1030	CF	F	MK638006
PN3-B08P4-1	July 2015	*Leifsonia sp.*	1059	CF	A	MK638148
PN3-B08P4-2	July 2015	*Agrobacterium sp.*	1051	CF	A	MK638032
PN3-B08P4-3	July 2015	*Agrobacterium sp.*	1070	CF	A	MK638030
PN3-B08P4-4	July 2015	*Agrobacterium sp.*	1032	CF	A	MK638035
PN3-B08P4-5	July 2015	*Agrobacterium sp.*	1071	CF	A	MK638031
PN3-B08P4-6	July 2015	*Agrobacterium sp.*	1029	CF	A	MK638033
PN3-B08P4-7	July 2015	*Phyllobacterium sp.*	1070	CF	A	MK638327
PN3-B08P4-9	July 2015	*Agrobacterium sp.*	1068	CF	A	MK638027

PN3-B08P4-10	July 2015	*Agrobacterium sp.*	1029	CF	A	MK638029
PN3-B08P4-11	July 2015	*Agrobacterium sp.*	1022	CF	A	MK638034
PN3-B08P4-12	July 2015	*Phyllobacterium sp.*	1042	CF	A	MK638338
PN3-B08P4-14	July 2015	*Pseudomonas sp.*	1057	CF	A	MK638004
PN3-B08P4-15	July 2015	*Phyllobacterium sp.*	1027	CF	A	MK638370
PN3-B08P4-16	July 2015	*Agrobacterium sp.*	1061	CF	A	MK638028
PN3-B08P4-18	July 2015	*Pseudomonas sp.*	1085	CF	A	MK638003
PN3-B08P4-19	July 2015	*Phyllobacterium sp.*	1068	CF	A	MK638319
PN3-B08P4-21	July 2015	*Phyllobacterium sp.*	1072	CF	A	MK638347
PN3-B08P4-22	July 2015	*Leifsonia sp.*	1046	CF	A	MK638149
PN3-B08P5-1	July 2015	*Pseudomonas sp.*	1043	OF	A	MK638008
PN3-B08P5-2	July 2015	*Phyllobacterium sp.*	1030	OF	A	MK638334
PN3-B08P5-3	July 2015	*Pseudomonas sp.*	1056	OF	A	MK638005
PN3-B08P5-4	July 2015	*Phyllobacterium sp.*	1073	OF	A	MK638340
PN3-B08P5-5	July 2015	*Phyllobacterium sp.*	1068	OF	A	MK638320
PN3-B08P5-6	July 2015	*Streptomyces sp.*	1070	OF	A	MK638502
PN3-B08P5-7	July 2015	*Pseudomonas sp.*	1043	OF	A	MK638007
PN3-B08P5-8	July 2015	*Phyllobacterium sp.*	1068	OF	A	MK638329
PN3-B08P5-9	July 2015	*Agrobacterium sp.*	1072	OF	A	MK638026
PN3-B08P5-10	July 2015	*Phyllobacterium sp.*	1034	OF	A	MK638352
PN3-B08P5-11	July 2015	*Microbacterium sp.*	855	OF	A	MK638192
PN3-B08P5-12	July 2015	*Streptomyces sp.*	1018	OF	A	MK638631
PN3-B08P5-13	July 2015	*Phyllobacterium sp.*	1054	OF	A	MK638331
PN3-B08P5-14	July 2015	*Streptomyces sp.*	955	OF	A	MK638632
PN3-B08P5-15	July 2015	*Phyllobacterium sp.*	1070	OF	A	MK638426
PN3-B08P5-16	July 2015	*Streptomyces sp.*	561	OF	A	MK638633
PN3-B08P5-18	July 2015	*Phyllobacterium sp.*	1042	OF	A	MK638346

PN3-B08P5-19	July 2015	*Phyllobacterium sp.*	1070	OF	A	MK638428
PN3-B08P5-20	July 2015	*Rhizobium sp.*	1070	OF	A	MK638430
PN3-B08P5-21	July 2015	*Phyllobacterium sp.*	1068	OF	A	MK638321
PN3-B08P5-22	July 2015	*Streptomyces sp.*	1071	OF	A	MK638504
PN3-B08P5-23	July 2015	*Stenotrophomonas sp.*	1039	OF	A	MK638447
PN3-B08P5-24	July 2015	*Streptomyces sp.*	955	OF	A	MK638634
PN3-B09P3-2	July 2015	*Ensifer sp.*	1070	CF	F	MK638127
PN3-B09P3-3	July 2015	*Afipia sp.*	834	CF	F	MK638667
PN3-B09P3-4	July 2015	*Streptomyces sp.*	1070	CF	F	MK638503
PN3-B09P3-5	July 2015	*Streptomyces sp.*	1019	CF	F	MK638638
PN3-B09P3-6	July 2015	*Streptomyces sp.*	1018	CF	F	MK638639
PN3-B09P3-8	July 2015	*Phyllobacterium sp.*	1031	CF	F	MK638317
PN3-B09P3-9	July 2015	*Bosea sp.*	1072	CF	F	MK638084
PN3-B09P3-10	July 2015	*Phyllobacterium sp.*	1073	CF	F	MK638425
PN3-B09P3-11	July 2015	*Ensifer sp.*	1070	CF	F	MK638126
PN3-B09P3-12	July 2015	*Streptomyces sp.*	660	CF	F	MK638635
PN3-B09P3-14	July 2015	*Phyllobacterium sp.*	1070	CF	F	MK638335
PN3-B09P3-15	July 2015	*Phyllobacterium sp.*	1029	CF	F	MK638343
PN3-B09P3-16	July 2015	*Phyllobacterium sp.*	1070	CF	F	MK638322
PN3-B09P3-17	July 2015	*Ensifer sp.*	1029	CF	F	MK638128
PN3-B09P3-18	July 2015	*Phyllobacterium sp.*	1052	CF	F	MK638351
PN3-B09P3-19	July 2015	*Streptomyces sp.*	1018	CF	F	MK638636
PN3-B09P3-20	July 2015	*Streptomyces sp.*	1018	CF	F	MK638637
PN3-B09P3-21	July 2015	*Phyllobacterium sp.*	1055	CF	F	MK638353
PN3-B09P3-22	July 2015	*Phyllobacterium sp.*	1052	CF	F	MK638350
PN3-B09P5-1	July 2015	*Phyllobacterium sp.*	1068	OF	F	MK638318
PN3-B09P5-2	July 2015	*Phyllobacterium sp.*	1070	OF	F	MK638330

PN3-B09P5-3	July 2015	*Phyllobacterium sp.*	1062	OF	F	MK638341
PN3-B09P5-4	July 2015	*Phyllobacterium sp.*	1071	OF	F	MK638315
PN3-B09P5-5	July 2015	*Phyllobacterium sp.*	1073	OF	F	MK638324
PN3-B09P5-6	July 2015	*Phyllobacterium sp.*	1025	OF	F	MK638371
PN3-B09P5-7	July 2015	*Phyllobacterium sp.*	1011	OF	F	MK638373
PN3-B09P5-8	July 2015	*Streptomyces sp.*	393	OF	F	MK638643
PN3-B09P5-9	July 2015	*Streptomyces sp.*	1018	OF	F	MK638644
PN3-B09P5-10	July 2015	*Paenarthrobacter sp.*	1040	OF	F	MK638200
PN3-B09P5-11	July 2015	*Phyllobacterium sp.*	944	OF	F	MK638357
PN3-B09P5-12	July 2015	*Phyllobacterium sp.*	1070	OF	F	MK638328
PN3-B09P5-13	July 2015	*Phyllobacterium sp.*	1070	OF	F	MK638427
PN3-B09P5-14	July 2015	*Streptomyces sp.*	1018	OF	F	MK638640
PN3-B09P5-15	July 2015	*Streptomyces sp.*	1018	OF	F	MK638641
PN3-B09P5-16	July 2015	*Phyllobacterium sp.*	1034	OF	F	MK638356
PN3-B09P5-17	July 2015	*Phyllobacterium sp.*	1070	OF	F	MK638431
PN3-B09P5-18	July 2015	*Streptomyces sp.*	601	OF	F	MK638642
PN3-B09P5-19	July 2015	*Phyllobacterium sp.*	1034	OF	F	MK638354
PN3-B09P5-20	July 2015	*Phyllobacterium sp.*	1026	OF	F	MK638358
PN3-B09P5-22	July 2015	*Phyllobacterium sp.*	1052	OF	F	MK638349
PN3-B10P3-1	July 2015	*Microbacterium sp.*	1020	OF	A	MK638189
PN3-B10P3-2	July 2015	*Phyllobacterium sp.*	1060	OF	A	MK638344
PN3-B10P3-4	July 2015	*Phyllobacterium sp.*	1070	OF	A	MK638316
PN3-B10P3-5	July 2015	*Agrobacterium sp.*	1008	OF	A	MK638049
PN3-B10P3-6	July 2015	*Streptomyces sp.*	813	OF	A	MK638617
PN3-B10P3-7	July 2015	*Phyllobacterium sp.*	1023	OF	A	MK638372
PN3-B10P3-8	July 2015	*Phyllobacterium sp.*	1008	OF	A	MK638365
PN3-B10P3-9	July 2015	*Rhizobium sp.*	718	OF	A	MK638434

PN3-B10P3-10	July 2015	*Phyllobacterium sp.*	1025	OF	A	MK638369
PN3-B10P3-12	July 2015	*Kaistia sp.*	1008	OF	A	MK638142
PN3-B10P3-13	July 2015	*Phyllobacterium sp.*	1008	OF	A	MK638360
PN3-B10P3-14	July 2015	*Streptomyces sp.*	1018	OF	A	MK638615
PN3-B10P3-15	July 2015	*Phyllobacterium sp.*	1008	OF	A	MK638361
PN3-B10P3-16	July 2015	*Phyllobacterium sp.*	1008	OF	A	MK638362
PN3-B10P3-17	July 2015	*Phyllobacterium sp.*	1008	OF	A	MK638363
PN3-B10P3-18	July 2015	*Streptomyces sp.*	750	OF	A	MK638616
PN3-B10P3-19	July 2015	*Phyllobacterium sp.*	1008	OF	A	MK638364
PN3-B10P3-20	July 2015	*Phyllobacterium sp.*	1053	OF	A	MK638355
PN3-B10P3-21	July 2015	*Rhizobium sp.*	1028	OF	A	MK638429
PN3-B10P3-22	July 2015	*Rhizobium sp.*	1021	OF	A	MK638435
PN3-B10P5-1	July 2015	*Phyllobacterium sp.*	686	CF	A	MK638313
PN3-B10P5-2	July 2015	*Phyllobacterium sp.*	1073	CF	A	MK638339
PN3-B10P5-3	July 2015	*Phyllobacterium sp.*	1062	CF	A	MK638345
PN3-B10P5-4	July 2015	*Mycobacterium sp.*	442	CF	A	MK638196
PN3-B10P5-5	July 2015	*Phyllobacterium sp.*	1052	CF	A	MK638337
PN3-B10P5-7	July 2015	*Phyllobacterium sp.*	1048	CF	A	MK638333
PN3-B10P5-8	July 2015	*Phyllobacterium sp.*	1037	CF	A	MK638368
PN3-B10P5-9	July 2015	*Phyllobacterium sp.*	1067	CF	A	MK638325
PN3-B10P5-10	July 2015	*Phyllobacterium sp.*	1071	CF	A	MK638326
PN3-B10P5-11	July 2015	*Phyllobacterium sp.*	1055	CF	A	MK638342
PN3-B10P5-12	July 2015	*Phyllobacterium sp.*	1071	CF	A	MK638323
PN3-B10P5-13	July 2015	*Variovorax sp.*	1050	CF	A	MK638658
PN3-B10P5-14	July 2015	*Phyllobacterium sp.*	1061	CF	A	MK638348

PN3-B10P5-15	July 2015	*Phyllobacterium sp.*	1027	CF	A	MK638375
PN3-B10P5-16	July 2015	*Ensifer sp.*	1073	CF	A	MK638125
PN3-B10P5-17	July 2015	*Phyllobacterium sp.*	1070	CF	A	MK638332
PN3-B10P5-18	July 2015	*Streptomyces sp.*	1018	CF	A	MK638645
PN3-B10P5-19	July 2015	*Streptomyces sp.*	970	CF	A	MK638486
PN3-B10P5-20	July 2015	*Phyllobacterium sp.*	1029	CF	A	MK638308
PN3-B10P5-21	July 2015	*Streptomyces sp.*	955	CF	A	MK638485
PN3-B10P5-22	July 2015	*Phyllobacterium sp.*	1053	CF	A	MK638304
PN3-B10P5-23	July 2015	*Phyllobacterium sp.*	708	CF	A	MK638312

Supplementary Table 2: Properties of the studied soils in the treatments of the GCEF. Values represent means and standard deviations (in parenthesis) for soil moisture, pH, total carbon content (TOC), total nitrogen content (TN), mineral nitrogen (N_{min}) and available phosphorus (P_{DL}) of five replicates for each treatment. Treatments include conventional (CF) and organic farming (OF) under ambient (A) and future (F) climatic conditions implemented in the GCEF. Samples were taken in May and July 2015.

	May 2015 (BBCH 37-39)				**July 2015 (BBCH 75-77)**			
Treatment	**CF-A**	**CF-F**	**OF-A**	**OF-F**	**CF-A**	**CF-F**	**OF-A**	**OF-F**
Moisture	10.9a	10.1a	10.6a	10.6a	15.9a	15.9a	16.0a	15.8a
[% w/w]	(0.6)	(0.8)	(0.6)	(0.4)	(0.2)	(0.5)	(0.2)	(0.5)
pH	6.8a (0.4)	6.8a (0.4)	6.5a (0.6)	6.6a (0.5)	6.8a (0.4)	6.8a (0.5)	6.6a (0.6)	6.6a (0.5)
TOC	2.0a	2.0a	2.0a	1.9a	1.9a	1.9a	1.9a	1.9a
[% w/w]	(0.1)	(0.1)	(0.1)	(0.1)	(0.2)	(0.2)	(0.1)	(0.2)
TN	0.17a	0.17a	0.16a	0.16a	0.16a	0.15a	0.15a	0.15a
[% w/w]	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.01)	(0.02)
N_{min}	13.4ab	9.5b	5.0c	4.8c	12.3ab	13.7a	9.6ab	7.7bc
[mg/kg]	(5.1)	(2.6)	(1.4)	(1.0)	(2.1)	(1.6)	(4.4)	(1.2)
P_{DL}	7.6a	8.4a	6.8a	6.0a	7.3a	8.9a	7.2a	6.0a
[mg/100g]	(4.1)	(3.7)	(3.7)	(2.5)	(3.2)	(4.3)	(4.2)	(2.2)

Supplementary Table 3: Winter wheat yields in the GCEF in 2015. Corn and straw yields are presented for conventional and organic farming systems under ambient and future climatic conditions. Significant differences in yield between the four treatments are marked by different letters according to analysis of variance and Tukey post hoc test.

Land use	Climate	Crop	Corn yield in dt/ha	Straw yield in dt/ha	Ratio corn/straw
Conventional Farming	Ambient	Winter wheat	83.4 **a** (3.2)	69.0 (4.3)	0.82 (0.04)
Conventional Farming	Future	Winter wheat	69.0 **b** (10.4)	58.3 (9.7)	0.85 (0.02)
Organic Farming	Ambient	Winter wheat	82.9 **a** (4.9)	71.0 (4.5)	0.86 (0.06)
Organic Farming	Future	Winter wheat	74.4 **b** (5.0)	69.2 (6.1)	0.93 (0.03)

Supplementary Table 4: Number of isolated colonies cultivated on Pikovskaya medium. Plot code refers to the coding of the GCEF plots, and land use and climate treatment of each plot is indicated. The land use treatment includes conventional and organic farming, and the climate ambient and future climatic conditions. Samples were taken in May and July 2015.

Plot	Land use	Climate	May 2015 (BBCH 37-39)	July 2015 (BBCH 75-77)
B01P2	Conventional Farming	Ambient	20	19
B01P4	Organic Farming	Ambient	22	22
B02P3	Organic Farming	Future	21	21
B02P4	Conventional Farming	Future	20	14
B03P1	Conventional Farming	Ambient	23	19
B03P2	Organic Farming	Ambient	23	21
B04P1	Conventional Farming	Future	20	18
B04P2	Organic Farming	Future	22	22
B05P1	Organic Farming	Ambient	19	19
B05P3	Conventional Farming	Ambient	21	22
B06P2	Conventional Farming	Future	18	23
B06P4	Organic Farming	Future	20	23
B07P1	Organic Farming	Future	20	20
B07P5	Conventional Farming	Future	22	21
B08P4	Conventional Farming	Ambient	20	18
B08P5	Organic Farming	Ambient	21	23
B09P3	Conventional Farming	Future	20	19
B09P5	Organic Farming	Future	20	21
B10P3	Organic Farming	Ambient	18	20
B10P5	Conventional Farming	Ambient	20	22

Supplementary Table 5: Bacteria cultivated on Pikovskaya medium from rhizosphere soil of winter wheat. Bacteria were identified by partial *16S rRNA* sequencing. Distribution of genera among conventional (CF) and organic farming system (OF), ambient (A) and future climate conditions (F), as well as sampling time, May and July 2015.

Genus	Total	May 2015 (BBCH 37-39)	July 2015 (BBCH 75-77)	CF	OF	A	F
Achromobacter	1	1	0	1	0	1	0
Afipia	1	0	1	1	0	0	1
Agrobacterium	30	10	20	21	9	24	6
Agromyces	2	1	1	2	0	2	0
Arthrobacter	8	8	0	3	5	3	5
Bacillus	21	12	9	5	16	5	16
Bosea	1	0	1	1	0	0	1
Bradyrhizobium	3	2	1	1	2	1	2
Burkholderia	3	2	1	0	3	2	1
Buttiauxella	8	8	0	8	0	0	8
Caulobacter	2	1	1	2	0	1	1
Chitinophaga	2	2	0	2	0	0	2
Chryseobacterium	2	1	1	2	0	0	2
Clavibacter	1	1	0	1	0	1	0
Devosia	1	0	1	1	0	0	1
Dyadobacter	3	3	0	2	1	0	3
Dyella	5	2	3	3	2	5	0
Ensifer	11	1	10	6	5	6	5
Flavobacterium	4	4	0	2	2	2	2
Herbiconiux	1	0	1	1	0	0	1
Inquilinus	8	1	7	5	3	7	1
Kaistia	1	0	1	0	1	1	0
Leifsonia	7	1	6	6	1	7	0
Luteibacter	3	0	3	0	3	1	2
Massilia	1	1	0	0	1	1	0
Mesorhizobium	32	16	16	16	16	20	12
Microbacterium	7	2	5	4	3	4	3
Mucilaginibacter	1	1	0	1	0	0	1
Mycobacterium	3	0	3	1	2	1	2
Ochrobactrum	3	3	0	0	3	0	3
Paenarthrobacter	1	0	1	0	1	0	1
Pantoea	1	1	0	1	0	0	1
Paraburkholderia	2	1	1	0	2	1	1
Pedobacter	1	1	0	1	0	1	0
Phyllobacterium	184	37	147	96	88	77	107
Plantibacter	5	5	0	0	5	1	4
Pseudaminobacter	1	0	1	1	0	0	1
Pseudomonas	167	150	17	82	85	91	76
Rhizobium	44	20	24	19	25	29	15
Rhodococcus	5	5	0	2	3	3	2

Rugamonas	5	5	0	4	1	2	3
Serratia	2	2	0	1	1	1	1
Sphingomonas	1	1	0	1	0	1	0
Stenotrophomonas	8	6	2	1	7	5	3
Streptomyces	197	81	116	85	112	95	102
Tardiphaga	2	1	1	1	1	0	2
Tsukamurella	2	2	0	2	0	0	2
Variovorax	13	8	5	4	9	10	3
Total	**817**	**410**	**407**	**399**	**418**	**412**	**405**

Supplementary Table 6: Abundances and activity levels of dominant genera and their respective phylogenetic clusters. Numbers of isolates of each cluster are listed for May (BBCH 37-39) and July 2015 (BBCH 75-77) samples, in conventional (CF) and organic farming systems (OF), as well as under ambient (A) and future (F) climatic conditions. Mean activities ± standard deviations for phosphate solubilization, PSI 1 and PSI 2, and mean drought resistance are given for each cluster. Significant differences in phosphate solubilizing and drought resistance, between clusters are marked by different letters according to analysis of variance and Tukey post hoc test.

Cluster	May	July	CF	OF	A	F	PSI 1 in µg PO_4^{3-}	PSI 2 in µg PO_4^{3-}	Resistance in %
Phyllobacterium									
	33	**142**	**94**	**81**	**73**	**102**	**456.1 ± 16.7**	**126.6 ± 9.7**	**61.7 ± 1.8**
C 1	24	97	76	45	50	71	452.3 ± 20.2^{c}	117.8 ± 10.8^{b}	58.8 ± 1.7^{ac}
C 2	9	45	18	36	23	31	463.3 ± 27.9^{c}	143.5 ± 18.4^{b}	67.1 ± 3.7^{a}
Pseudomonas									
	150	**19**	**83**	**86**	**91**	**78**	**1027.3 ± 51.2**	**258.8 ± 26.8**	**37.3 ± 2.6**
C 1	38	1	26	13	24	15	1378.7 ± 104.5^{a}	433.8 ± 69.5^{a}	31.0 ± 2.5^{b}
C 2	23	2	12	13	10	15	637.81 ± 95.6^{bc}	142.3 ± 32.7^{bc}	25.0 ± 0^{bc}
C 3	17	3	7	13	8	12	1076.2 ± 149.7^{ab}	236.7 ± 41.5^{ab}	35.6 ± 5^{abc}
C 4	18	1	6	13	18	1	758.89 ± 72^{b}	68.1 ± 37.8^{bc}	39.5 ± 9.9^{bc}
C 5	7	1	1	7	2	6	1151.5 ± 178.8	251.0 ± 91.1	40.1 ± 2.7
C 6	6	0	3	3	3	3	100.87 ± 56.4	0.7 ± 0.5	NA
C 7	5	0	3	2	3	2	951.77 ± 284.4	196.7 ± 109.6	56.7 ± 19.7
C 8	5	0	4	1	1	4	2386.8 ± 75.4	1028.4 ± 113	41.4 ± 7.1
C 9	3	1	2	2	2	2	538.43 ± 110.9	88.2 ± 19.2	0.0
C 10	3	1	4	0	3	1	688.32 ± 218.2	126.5 ± 126	43.9 ± 14.2
C 11	0	3	0	3	0	3	290.92 ± 105.9	0.0 ± 0	0.0
C 12	1	2	1	2	3	0	1450.8 ± 0	0.0 ± 0	100.0 ± 0
C 13	3	0	1	2	1	2	692.48 ± 402.9	71.5 ± 39.8	30.0 ± 0
C 14	2	0	2	0	1	1	1276.7 ± 174.1	105.4 ± 10	46.5 ± 20.2
C 15	2	0	0	2	2	0	431.25 ± 30.4	1.5 ± 0.6	75.4 ± 6.7
C 16	1	1	2	0	1	1	1682.5 ± 0	504.6 ± 0	0.0
C 17	2	0	0	2	1	1	508.39 ± 87.8	11.9 ± 11.9	28.6 ± 0
C 18	2	0	0	2	2	0	1451.7 ± 163.6	483.1 ± 21.5	25.4 ± 16.3
C 19	1	1	1	1	1	1	584.22 ± 0	85.8 ± 51.5	16.7 ± 0
C 20	1	0	1	0	0	1	344.33 ± 0	61.0 ± 0	33.3 ± 0
C 21	1	0	1	0	1	0	NA	NA	NA
C 22	1	0	1	0	1	0	NA	NA	NA
C 23	1	0	0	1	0	1	1236.2 ± 0	420.64 ± 0	NA
C 24	1	0	1	0	0	1	1763.6 ± 0	420.6 ± 0	30.0 ± 0
C 25	1	0	0	1	0	1	NA	NA	NA
C 26	1	0	0	1	0	1	1038.7 ± 0	105.16 ± 0	42.9 ± 0
C 27	1	0	1	0	1	0	802.16 ± 0	15.3 ± 0	0.0
C 28	1	0	1	0	1	0	NA	NA	NA
C 29	1	0	0	1	0	1	86.082 ± 0	0.2385 ± 0	46.7 ± 0
C 30	1	0	1	0	1	0	137.35 ± 0	34.3 ± 0	NA
Streptomyces									
	81	**116**	**85**	**112**	**95**	**102**	**553.7 ± 16.9**	**25.3 ± 2.8**	**45.4 ± 0.1**
C 1	48	51	55	44	45	54	580.4 ± 30.6^{bc}	20.8 ± 4.5^{c}	46.3 ± 3^{b}

C 2	9	16	9	16	12	13	687.9 ± 62.9^{b}	41.8 ± 5.5^{bc}	45.8 ± 6.1^{bc}
C 3	3	11	5	9	9	5	562.8 ± 70.9^{bc}	24.6 ± 6.7^{bc}	26.4 ± 9.3^{b}
C 4	8	5	5	8	8	5	431.9 ± 55^{bc}	31.2 ± 8.8^{bc}	41.1 ± 9.7^{ab}
C 5	2	11	4	9	3	10	384.8 ± 86.5^{bc}	40.3 ± 11.8^{bc}	71.6 ± 11.8^{ac}
C 6	2	6	0	8	4	4	586.9 ± 77.6	22.0 ± 8.3	31.8 ± 11.8
C 7	3	1	1	3	3	1	441.2 ± 158	19.7 ± 19.2	33.7 ± 23.6
C 8	0	4	0	4	2	2	291.5 ± 65.2	44.4 ± 25.6	50.7 ± 15.9
C 9	0	3	2	1	1	2	118.4 ± 10.8	3.2 ± 2.2	59.5 ± 29.8
C 10	1	1	0	2	1	1	858.4 ± 0	27.7 ± 19.1	46.5 ± 9
C 11	1	0	1	0	1	0	236.6 ± 0	0.5 ± 0	66.7 ± 0
C 12	1	0	0	1	1	0	NA	NA	NA
C 13	1	0	0	1	1	0	504.6 ± 0	0.0 ± 0	50.0 ± 0
C 14	1	0	1	0	1	0	34.3 ± 0	0.0 ± 0	0.0
C 15	0	1	0	1	0	1	292.1 ± 0	2.1 ± 0	81.8 ± 0
C 16	1	0	0	1	1	0	596.1 ± 0	3.8 ± 0	61.1 ± 0
C 17	0	1	0	1	1	0	774.7 ± 0	0.2 ± 0	65.6 ± 0
C 18	0	1	0	1	1	0	309.04 ± 0	8.6 ± 0	100.0 ± 0
C 19	0	1	1	0	0	1	504.6 ± 0	8.6 ± 0	0.0
C 20	0	1	0	1	0	1	68.9 ± 0	6.0 ± 0	66.7 ± 0
C 21	0	1	1	0	0	1	596.1 ± 0	46.7 ± 0	44.1 ± 0
C 22	0	1	0	1	0	1	381.5 ± 0	95.4 ± 0	30.3 ± 0

SYNOPSIS

The present thesis aims to contribute unravelling structural and functional adaptation processes in the wheat rhizobacterial communities to drought, and how these changes induced by drought are further influenced by abiotic and biotic factors. The three studies that constitute the thesis use cultivation-based and -independent methods to gain a broad perspective on the complex interplay between plants and soil in agricultural systems under semi-controlled conditions in the cold greenhouse and under natural field conditions (Global Change Experimental Facility, Schädler et al. 2019). In particular, the impact of farming system and water availability/climate (all experiments, Chapter 1 to 3), soil type and wheat cultivar (pot experiment, Chapter 1), as well as plant growth stage (field experiment, Chapter 2 and 3) were investigated. The main findings of single and joint effects of the three studies are summarized under the following bullet points:

- The drought treatment in the pot experiment dramatically shaped rhizobacterial community composition of wheat, with a strong potential for degradation of complex carbon compounds within the obtained drought-adapted community (Chapter 1). In contrast, the climate treatment in the field experiment had only a minor effect on community composition, but led to an overall decrease in extracellular enzyme activities (Chapter 2). Since the GCEF was established in 2013 and samples were taken in May and July 2015, the moderate climate treatment will probably only result in changes in soil conditions that will accumulate over time. Therefore, the specific adaptations are likely to become more evident in subsequent years. However, an effect was detected in the fraction of cultivated bacteria already indicating a higher drought-stress resistance potential among dominant bacterial species under future climate conditions (Chapter 3). This result suggest adaption of single species to drought, which are drowning in the community.
- When screening for effective phosphate solubilizing bacteria in the rhizosphere of wheat grown under field conditions, we found, besides already known representatives of the phyla *Pseudomonas* and *Streptomyces*, *Phyllobacterium* species to be dominant

(Chapter 3). Since our findings proved for the first time *Phyllobacterium* species as effective phosphate-solubilzers in the rhizosphere of wheat grown in temperate climate zone, and since they further expressed a strong resistance to drought, species of this phylum might be potential candidates for future application as biofertilizer.

- Apart from the drought treatment, the cultivated soil type was the strongest driver of community composition and function in the pot experiment, followed by agricultural practice and no significant effect of wheat cultivar was detected (Chapter 1), which is in concordance with previous results (Schlatter et al 2020). The effect of wheat cultivar was specific to increased production of carbon cycling enzymes under drought in dependency of soil type and farming system in the rhizosphere of the demanding cultivar (Chapter 1). In the field experiment, growth stage mainly shaped rhizobacterial community composition, whereby extracellular enzyme production was mainly affected by agricultural practice (Chapter 2). These results demonstrate the complexity of the studied topic and plead for more studies on the interconnecting effects of multiple experimental factors to unravel common and more specific adaptations of bacterial communities in structure and function, especially with respect to the impact of climate change.
- The evaluation of computational prediction tools Tax4Fun and PanFP in relation to the different treatments applied, i.e. farming system, climate and plant growth stages in Chapter 2, proved qualitative concordances between predicted abundances of functional genes and the respective corresponding measured enzyme activities. This suggests that we can use this method to conduct time- and cost-saving research by pre-analyzing soil bacterial communities from various environments for differences in their functional profiles. Building on that more advanced analysis with a higher resolution may be applied specifically to further quantitatively assess functions of interest.

Multidisciplinary approaches combine advantages of cultivation-based and high throughput community-based methods

For a long time, methodical approaches to study diversity and functions of soil bacterial communities were limited to cultivation-based approaches. Microbes from environmental samples were isolated by plating, purified, classified and characterized (reviewed in Hugerth and Andersson, 2017). By the use of this approach, detailed physiological studies could be performed and a comprehensive functional profile of a given organism obtained, which is a prerequisite for the identification of potential plant growth promoting rhizobacteria (PGPR) (term first introduced by Kloepper and Schroth, 1978). In Chapter 3, we applied this method in a modified form. The traditional approach was to cultivate bacterial colonies on different media to obtain diverse collections with potentially wide range of functions from the environmental sample. Whereas such cultivation strategy may yield a diverse collection of bacteria, it does not necessarily lead to the isolation of potentially plant beneficial ones (reviewed in Alaylar et al 2020). Avoiding the random selection we steered the composition of the culture collection towards a specific functional trait by screening for phosphate-solubilizing bacteria on Pikovskaya medium (Pikovskaya 1948), and subsequently tested for further functional traits (Chapter 3). To capture both fast and slowly growing bacterial species we prepared soil solutions with a high dilution factor (1:200) and let the colonies grow for two weeks. The large culture collection we obtained in that way comprised around 800 functionally classified bacterial isolates from different environmental samples.

Since one gram of soil can harbor millions of microorganisms representing thousands of species (Torsvik et al. 1990), and since each species may show strong variations in its trait spectrum (Li et al 2021), the obtained collection still represents only a small proportion of the community diversity. The development of Sanger sequencing (Sanger 1975) and subsequent next generation sequencing methods (NGS, e.g. 454, Illumina MiSeq) overcame restrictions of structural resolution by targeting multiple organisms at the same time. Currently, 16S rRNA gene MiSeq amplicon sequencing with relatively moderate costs and moderately long read lengths is the standard procedure to analyze prokaryote community composition in various

environments (Brandt et al. 2018, Kameoka et al 2021, Paulraj et al. 2021, Schöler et al. 2017). In this thesis, we used Illumina MiSeq sequencing to investigate structural adaptations to drought in the rhizobacterial community of wheat (Chapter 1 and 2). The high resolution of this approach revealed differences in the bacterial community compositions in different samples up to species level. Unfortunately, 16S amplicon sequencing does not differentiate between the contribution of either actively growing, passive (e.g. spores) or dead organisms within the studied community (Emerson et al. 2017). Comparing the cultivation-based phosphate-solubilizing community isolated from the rhizosphere of wheat, which was dominated by species of the phyla *Pseudomonas*, *Streptomyces* and *Phyllobacterium,* with 16S rDNA gene abundances in the overall community, we found poor correlations between the high numbers on plate and the relative abundancies in the amplicon datasets (Figure 4). Although the two methods are hardly comparable for the reasons mentioned above, this discrepancy raises the question which of the cultivation-based or NGS approach is more meaningful to reveal structural and functional adaptation in rhizobacterial communities.

To bridge the gap between structural and functional diversity analyses, recent computational approaches link structural data obtained from NGS with available functional data as strategy to derive a functional profile of bacterial communities. In this line, we evaluated the tools of Tax4Fun (Aßhauer 2015) and PanFP (Jun 2015) to test the concordances between functional gene abundances and measured enzyme activity values in different agricultural systems, climate treatments and at different plant growth stages. As concluded in Chapter 2 we could find qualitative, but not necessarily quantitative concordances. This quantitative discrepancy might reflect that the passive part of the community introduced a bias to predict the extent but not the direction of expressed activity levels. The bias by dormant sub-communities can be minimized by barcoding the active part of bacterial communities based on RT-PCR of RNA and compare it to DNA extracts, as demonstrated by Zhu et al. (2020). However, at the present state, a combination of different methods may be the best option to gain a deeper and more precise understanding of plant-microbe interactions under different environmental conditions.

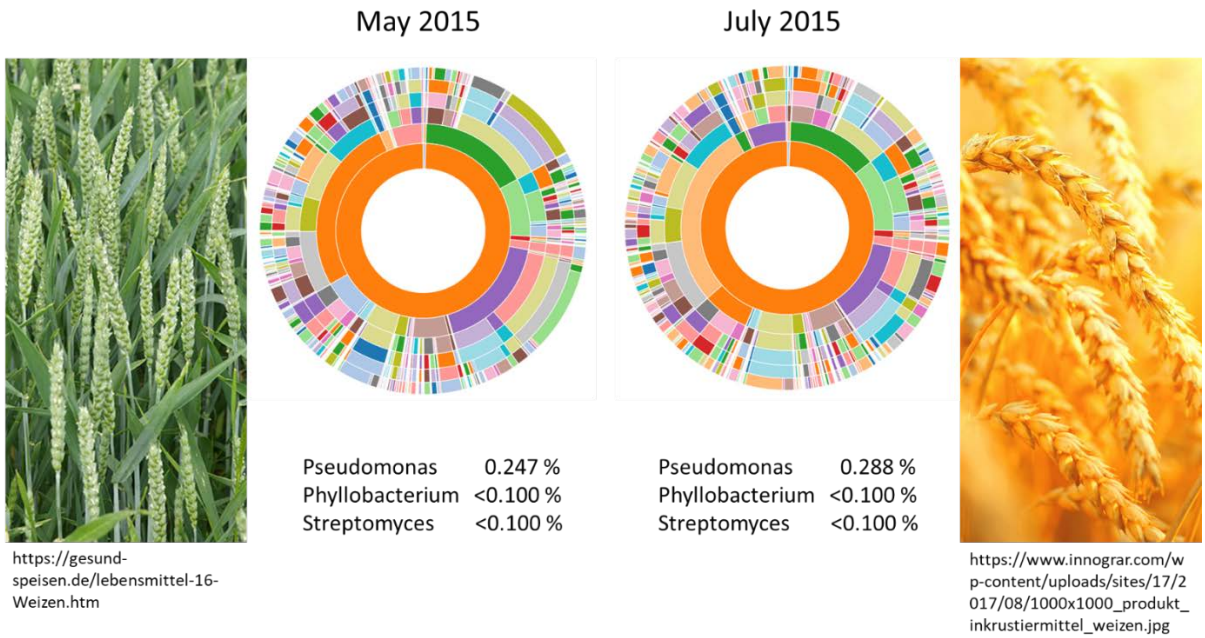


Figure 4 Rhizobacterial community composition based on 16S rDNA analysis at two growth phases in May 2015 and July 2015. From inner to outer circle the abundances and diversities of species at different taxonomy levels are presented, starting with the domain (inner circle) and ending with genus (outer circle). Percentage of total community for dominant P-solubilizing genera *Pseudomonas*, *Phyllobacterium* and *Streptomyces* are given.

Multifactorial approaches to gain a more holistic understanding of plant-microbe interactions in pot experiments

As pointed out in the introduction, different environmental parameters shape bacterial and rhizobacterial community composition, which was also confirmed in our studies (Chapter 1 to 3). In comparison to structural changes, ecosystem functions are assumed to be less affected and more resilient due to functional redundancy within soil microbial communities. Recently, Rossmann et al. (2020) described that as a consequence of domestication and breeding, highly productive cultivars form less intricate microbial co-occurrence networks compared to landraces and thus are more vulnerable to disturbances. On the other hand, de Vries et al. (2018) indicated that the recovery of such bacterial networks after drought stress is linked to soil functions creating a resilient and adapted community. Thereby, the question arises which mechanisms of microbial communities respond to changed conditions and are these common or unique for different environments. By simultaneously manipulating multiple experimental factors in field and pot experiments, we in fact revealed different, but also common adaptation

processes of bacterial communities, which can contribute to maintain productivity in agricultural systems under drought stress.

Of all the experimental factors studied in the pot experiment, one factor predominantly determined the response of the bacterial communities under drought: the soil type. As described in the first chapter, we decided for comparing two soil types that fundamentally differed in their physical and chemical properties (Altermann et al 2005, Schweitzer 2010). After a whole vegetation period, the fertile loamy soil showed tendencies for buffering unfavorable conditions of less water and nutrient supply independently of the plant genotype, and this, in case of extracellular enzyme activities, maintained at least a status quo compared to initial conditions without drought treatment. In contrast, unfavorable conditions in the sandy soil completely altered the composition and functioning of the rhizosphere communities by decreasing diversity and depleting available resources, respectively. These effects might simply reflect the differences in water holding capacities of the two soil types, since watering levels were applied according to maximum water holding capacities instead of soil water availability. However, comparing wheat performance in both soil types under drought and well-watered conditions in the potting system, we observed maximum difference in water use for the loamy soil in early May (phase 3: stem elongation, Hack et al. 1992, data not published), while in the sandy soil the maximum was delayed until early June (phase 6: flowering, Hack et al. 1992, data not published). This suggested a much earlier effect of drought on wheat plants and rhizosphere communities at sensitive development stages in loamy than in sandy soil, and led to an overall stronger reduction in plant biomass production (Chapter 1). Thus, changes in rhizosphere communities and functions probably result from an interplay of soil type and plant presence, whereby above- and belowground responses to drought were found to be opposite in the two soil types.

A very specific interaction effect was observed in the sandy soil when the impacts of cultivar (demanding vs. non-demanding) and farming system (conventional vs. organic) related changes were considered as well. Although mechanisms could not be fully resolved, the organic manure application in organic farming treatments may have ameliorate water retention

capacities of the sandy soil (Rawls et al 2003) and possibly fostered increased enzyme activities under drought conditions in the rhizosphere of the demanding cultivar. These findings do not only emphasize the importance of carbon concentrations to increase drought tolerance. We could also conclude from Chapter 1 that the interacting effects of different factors offer a realistic and holistic picture of different adaptation processes than solely regarding single factors.

Transferability of findings gained in the pot experiment to field conditions

While pot experiments allow the simultaneous investigation of multiple factors under similar environmental conditions, the knowledge gained in a controlled environment does not necessarily apply under field conditions, where soil properties and plant growth rates are more heterogeneous, and uncontrolled changes in the environment affect the bacterial diversity and functions (Passioura et al. 2012, McKersie et al. 1999). In the Chapters 2 and 3, we used the platform of the Global Change Experimental Facility (GCEF) as the field scale experiment. The platform includes a model-based climate treatment (ambient vs. future) and realistic agricultural treatments (conventional vs. organic) with crop rotation (wheat-barley-rapeseed/clover) (Schädler et al 2019). Moreover, the fertile loamy soil (Haplic Chernozem, Altermann et al. 2005) used for the pot experiment originated from organic and conventional farming plots of the GCEF, that allowed to test the extent to which the results of the pot experiment could be transferred to field conditions.

Unlike the pot experiment, in which water supply was controlled to either 25 % or 60 % of the soil's WHC_{max}, representing drought or well-watered conditions respectively, the climate treatment in the GCEF controls for precipitation, reducing the amount of natural precipitation by 20 % in the summer to simulate future climate (Schädler et al. 2019). Gravimetric soil moistures thus ranged between 6 % to 22 %, which equals 17 % to 63 % of the soil's WHC_{max}, even on future climate plots. Modeling long-term rather than immediate changes in the GCEF, bacterial communities will adjust over years as a result of accumulative water reduction,

whereby individual groups or species may respond more rapidly due to specification on certain ecosystem functions (reviewed in Fierer et al. 2017). Accordingly, climate treatment did not affect community composition or function in our study two years after GCEF establishment (Chapter 2), but *Streptomyces* species, however, expressed higher drought tolerance when isolated from future climate plots in the same year (Chapter 3). Creating extreme conditions of prolonged drought, as in the pot experiment, will probably led to a strong community response within one growing season. Hence, the intended transferability of findings from the pot to the field would fail if only the results of one year without extremes is considered. Given the severe heat waves in 2018 and 2019 in Central Germany (Hari et al. 2020) with hardly any precipitation from spring to fall, drought conditions in the field resembled those in the pot experiment and likely had respective consequences for community composition and function. Even though it was not part of this study, it may be of future interest, whether GCEF's climate manipulations over the years have a more additive or neutral effect on such extreme events, and where the limits of adaptation lie.

Like in the pot experiment, we observed farming management-related effects on rhizobacterial community composition and function in the field (Chapter 1 and 2). Since pre-adapted soil from the GCEF farming plots, as well as fertilization according to the GCEF management were applied in the pot experiment, the implementation of more realistic scenarios in pot experiments might be the key for such, at least directional, concordances. With respect to community composition, direct comparisons between the pot and field experiments on species level may be not applicable due to the use of different pipelines for post-processing of sequencing data ("dadasnake" by Weißbecker et al. 2020 in Chapter 1 and "DeltaMP" by Lentendu (https://github.com/lentendu/DeltaMP) in Chapter 2). At the phylum level, we found a general dominance of *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Proteobacteria* and *Verrucomicrobia* for both experiments, with abundances differing between the pot and field experiments, but not necessarily between the farming treatments. The extracellular enzyme activity potentials increased in the conventional farming system, with readily available nutrients, compared to the organic farming system, irrespective of water

treatment (results for fertile loamy Haplic Chernozem in Chapters 1 and 2). Thereby, nutrient availability might have influenced bacterial extracellular enzyme production directly, or indirectly. The indirect effect is directed by different mechanisms of plant-soil-interactions, i.e. differences in root exudation patterns (reviewed in Lambers et al. 2009), and is particularly strong in the rhizosphere due to the close spatial proximity. Accordingly, in the pot experiment, community composition differed between compartments of rhizosphere and bulk soil, whereas cultivar-specific effects on composition were missing (Chapter 1).

Former studies either support (Donn et al. 2015) or neglect (Simonin et al. 2020) the effect of wheat cultivar on surrounding conditions, but indicate a common effect of plant growth stage. At the early growth stages, the investment in plant tissue requires high amount of nutrients particularly fostering the exchange with soil communities, thereby shaping composition and function by differential production rate and spectrum of rhizodeposits. At the mature growth phases the plant mainly allocates compounds from the vegetative to generative parts, this reduces interactions on the root-soil-surface (Malhi et al. 2006, Francioli et al. 2018). Although the data were not presented in Chapter 1, sampling in the pot experiment at three different time points (March: early vegetative phase -April: intermediate vegetative phase – July: mature phase) also indicated a strong effect of the plant development stage on rhizobacterial community composition and function, which was confirmed in the field for the overall community (Chapter 2), and also at individual species level at two different growth stages (vegetative phase and mature phase) (Chapter 3). Changes in community composition in the pot and field experiments were accompanied by reduction in enzyme activities from vegetative to mature growth phases (Chapter 1 and 2). At the individual species level, community composition of dominant groups rather than activity potentials were responsible for differences between vegetative and mature growth phases (Chapter 3).

Towards a wheat core microbiome?

At the beginning of this thesis, we introduced the idea by Kavamura et al. (2021) of using multifactorial and multidisciplinary approaches to identify a possible wheat core microbiome or

key species, which may persist under different environmental conditions and promote plant growth. As such, it needs to be first clarified, how the wheat core microbiome is defined, either as the starting microbiome in the seeds or of adult plants. Second, it must be answered whether the taxa of the core microbiome play an active role or are just present in the community. Coming to the end of this thesis, some answers to these two points can be provided.

Analysis of seed microbial communities were not part of this study, but recent findings indicated the existence of a core seed microbiome. Investigating 50 different plant species, Simonin et al. (2021) found 30 bacterial and fungal taxa present in seeds of most plant species from all over the world. In our study, the rhizospheres of adult plants were investigated, and rhizobacterial communities had already experienced different biotic and abiotic impacts shaping their composition. As discussed before, abundances of phyla might differ, while especially dominant groups are present in each treatment. However, going into deeper taxonomic levels, indicator species analysis revealed that no OTUs were shared between the rhizospheres under treatments of different farming systems (conventional vs. organic) and plant growth phases (vegetative vs. mature growth phase) (Chapter 2). This does not necessarily imply, that no species at all are shared between the different rhizospheres, since the amplicon sequencing data are compositional, i.e. transformed to relative abundancies instead of absolute numbers, and rare sequences are often removed from datasets (Fernandes et al. 2014). As such, singletons were removed during the bioinformatics workflow in Chapter 2, which corresponded to 2.5 % of the total reads. It is also possible that the presence of a certain taxon might not be system-relevant, which would suggest a decoupling of high structural variability from stable functional structures (Louca et al. 2016). For instance, Bastida et al. (2021) identified the most common microbial proteins involved in different edaphic processes across biomes and concluded a higher resolution of environmental impacts using protein-based data compared to 16S rRNA gene sequencing. Most recently, Fernandes et al. (2022) identified a core functional soil mycobiome across ecosystems by metaproteomics analysis. These findings are not that surprising given the concept of functional redundancy

(Banerjee et al. 2016). However, metaproteome analysis offers new perspectives in biofertilizer creation, as such that besides living inoculants, factor-relevant bacterial metabolites may be identified and genomic information transferred to or deleted in organisms. Something comparable has already been tested for *Pseudomonas syringae*. To enhance frost tolerance of strawberries and potatoes, the ice nucleation protein has been removed from the bacterial genome (Skirvin et al. 2000). There also exist ideas for plant beneficial microbe breeding, which aim to transmit the endophytic microbiota of one plant to the next generation (Wei & Jousset 2017) and thus may be of particular interest under changing climatic conditions. In this study, functional trait approaches at individual species level revealed *Phyllobacterium* species as promising P-solubilizers under future climate conditions in the rhizosphere of wheat, which has not been described before and may be useful to create new biofertilizer (Chapter 3).

Study limitations and outlook

The aim of this study was to investigate different adaptation processes of wheat rhizobacterial communities to drought using multifactorial and multidisciplinary approaches in pot and field experiments. While the broad spectrum of factors included in this study fulfilled the intention of a multifactorial approach, the choice of analyzing methods in the three chapters was mainly driven by considerations of indirect impacts on nutrient cycling as response to drought. Certainly, direct indicators of stress in the plants, such as phytohormone regulation, oxidative enzyme production and differential gene expression levels (reviewed in Chandra et al. 2021), might provide important mechanistic insights into microbial activation and regulation of stress responses; and how they may differ under changing surrounding conditions. For instance, Gebauer et al. (submitted to Frontiers in Microbiology) studied the community composition of aminocyclopropane-1-carboxylate (ACC) deaminase carrying bacterial communities in the rhizosphere of wheat, which regulate concentrations of stress phytohormone ethylene, and found a strong farming related effect.

As already coined, for the analysis of sequencing data, we used different pipelines in the pot and field experiments, which did not allow direct comparisons of communities and, thus, the

identification of possible key players. Therefore, it would be interesting to re-analyze these datasets, also in the light of the novel bioinformatics approach determining Amplicon Sequence variants (ASVs), which offers a more precise identification of microbes and, thus of their diversity within samples, compared to conventional use of operational taxonomic units (OTUs) (Callahan et al. 2017). Despite this limitation, the use of next generation amplicon sequencing provided a high structural resolution of the rhizobacterial communities in dependency of the different factors used in this thesis. Accompanied changes in community function could not be directly linked to structural changes with the methods used in this study, but the assignment of functional capabilities of individual taxa is a prerequisite for identification of key species and a possible core microbiome (Kavamura et al. 2021). The integration of metatranscriptomics and proteomics data should overcome these restrictions, and the fast development in these techniques may introduce these methods as standard procedures in the future. As such, metatranscriptomics may be also relevant to improve existing cultivation standards in soil ecology, as it has already been described for gut microbiomes (Bomar et al. 2011).

One of the greatest potentials, which has not been explored at the time of writing this thesis, is the use of the pre-adapted communities and single species for feedback analysis in inoculation experiments under controlled conditions, but also in the field. Priming with beneficial bacteria can be performed as reinoculation experiments using pre-adapted soils (Wubs et al. 2016), as well as creation of synthetic communities (Tsolakidou et al. 2019) or single species inoculation experiments (Wehner et al. 2019). Overall, all these studies on priming indicated a significant improvement in plant performance under stress conditions, and might thus be the key for sustainable agriculture.

Bibliography

Alaylar, B., Egamberdieva, D., Gulluce, M., Karadayi, M., & Arora, N. K. (2020). Integration of molecular tools in microbial phosphate solubilization research in agriculture perspective. World Journal of Microbiology and Biotechnology, 36(7), 1-12.

Altermann, M., Rinklebe, J., Merbach, I., Körschens, M., Langer, U., & Hofmann, B. (2005). Chernozem—soil of the year 2005. Journal of Plant Nutrition and Soil Science, 168(6), 725-740.

Aßhauer, K. P., Wemheuer, B., Daniel, R., & Meinicke, P. (2015). Tax4Fun: predicting functional profiles from metagenomic 16S rRNA data. Bioinformatics, 31(17), 2882-2884.

Banerjee, S., Kirkby, C.A., Schmutter, D., Bissett, A., Kirkegaard, J.A. and Richardson, A.E. (2016). Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biology and Biochemistry 97, 188-198.

Bastida, F., Jehmlich, N., Starke, R., Schallert, K., Benndorf, D., López-Mondéjar, R., ... & Delgado-Baquerizo, M. (2021). Structure and function of bacterial metaproteomes across biomes. Soil Biology and Biochemistry, 160, 108331.

Bomar, L., Maltz, M., Colston, S., & Graf, J. (2011). Directed culturing of microorganisms using metatranscriptomics. MBio, 2(2), e00012-11.

Brandt, J., & Albertsen, M. (2018). Investigation of detection limits and the influence of DNA extraction and primer choice on the observed microbial communities in drinking water samples using 16S rRNA gene amplicon sequencing. Frontiers in microbiology, 9, 2140.

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME journal, 11(12), 2639-2643.

Chandra, P., Wunnava, A., Verma, P., Chandra, A., & Sharma, R. K. (2021). Strategies to mitigate the adverse effect of drought stress on crop plants—influences of soil bacteria: A review. Pedosphere, 31(3), 496-509.

de Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., ... & Bardgett, R. D. (2018). Soil bacterial networks are less stable under drought than fungal networks. Nature communications, 9(1), 1-12.

Donn, S., Kirkegaard, J.A., Perera, G., Richardson, A.E. and Watt, M. (2015), Rhizosphere bacteria in field-grown intensive wheat crops. Environ Microbiol, 17: 610-621. https://doi.org/10.1111/1462-2920.12452

Emerson, J. B., Adams, R. I., Román, C. M. B., Brooks, B., Coil, D. A., Dahlhausen, K., ... & Rothschild, L. J. (2017). Schrödinger's microbes: tools for distinguishing the living from the dead in microbial ecosystems. Microbiome, 5(1), 1-23.

Fernandes, A. D., Reid, J. N., Macklaim, J. M., McMurrough, T. A., Edgell, D. R., & Gloor, G. B. (2014). Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome, 2(1), 1-13.

Fernandes, M. L. P., Bastida, F., Jehmlich, N., Martinović, T., Větrovský, T., Baldrian, P., ... & Starke, R. (2022). Functional soil mycobiome across ecosystems. Journal of Proteomics, 252, 104428.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology, 15(10), 579-590.

Francioli, D., Schulz, E., Buscot, F., & Reitz, T. (2018). Dynamics of soil bacterial communities over a vegetation season relate to both soil nutrient status and plant growth phenology. Microbial ecology, 75(1), 216-227.

Gebauer, L., Breitkreuz, C., Heintz-Buschart, A., Reitz, T., Buscot, F., Tarkka, M., Bouffaud, M. (submitted to Frontiers in Microbiology) Water deficit history selects plant beneficial soil bacteria differently under conventional and organic farming

Hack, H., Bleiholder, H., Buhr, L., Meier, U., Schnock-Fricke, U., Weber, E. & Witzen-Berger, A. (1997). Einheitliche Codierung der phänologischen Entwicklungsstadien mono- und dikotyler Pflanzen – Erweiterte BBCH-Skala; Allgemein-. Nachrichtenbl. Deut. Pflanzenschutzd., 44, 265-270

Hari, V., Rakovec, O., Markonis, Y., Hanel, M., & Kumar, R. (2020). Increased future occurrences of the exceptional 2018–2019 Central European drought under global warming. Scientific reports, 10(1), 1-10.

Hugerth, L. W., & Andersson, A. F. (2017). Analysing microbial community composition through amplicon sequencing: from sampling to hypothesis testing. Frontiers in microbiology, 8, 1561.

Jun, S. R., Robeson, M. S., Hauser, L. J., Schadt, C. W., & Gorin, A. A. (2015). PanFP: pangenome-based functional profiles for microbial communities. BMC research notes, 8(1), 1-7.

Kameoka, S., Motooka, D., Watanabe, S., Kubo, R., Jung, N., Midorikawa, Y., ... & Nakamura, S. (2021). Benchmark of 16S rRNA gene amplicon sequencing using Japanese gut microbiome data from the V1–V2 and V3–V4 primer sets. BMC genomics, 22(1), 1-10.

Kavamura, V. N., Mendes, R., Bargaz, A., & Mauchline, T. H. (2021). Defining the wheat microbiome: Towards microbiome-facilitated crop production. Computational and structural biotechnology journal, 19, 1200-1213.

Kloepper, J. W., & Schroth, M. N. (1981). Plant growth-promoting rhizobacteria and plant growth under gnotobiotic conditions. Phytopathology, 71(6), 642-644.

Lambers, H., Mougel, C., Jaillard, B., & Hinsinger, P. (2009). Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. Plant and soil, 321(1), 83-115.

Li, E., de Jonge, R., Liu, C., Jiang, H., Friman, V. P., Pieterse, C. M., ... & Jousset, A. (2021). Rapid evolution of bacterial mutualism in the plant rhizosphere. Nature Communications, 12(1), 1-13.

Louca, S., Jacques, S., Pires, A. P., Leal, J. S., Srivastava, D. S., Parfrey, L. W., ... & Doebeli, M. (2016). High taxonomic variability despite stable functional structure across microbial communities. Nature ecology & evolution, 1(1), 1-12.

Malhi, S. S., Johnston, A. M., Schoenau, J. J., Wang, Z. L., & Vera, C. L. (2006). Seasonal biomass accumulation and nutrient uptake of wheat, barley and oat on a Black Chernozem soil in Saskatchewan. Canadian Journal of Plant Science, 86(4), 1005-1014.

McKersie, B. D., Bowley, S. R., & Jones, K. S. (1999). Winter survival of transgenic alfalfa overexpressing superoxide dismutase. Plant Physiology, 119(3), 839-848.

Mustafa, S. M. T., Vanuytrecht, E., & Huysmans, M. (2017). Combined deficit irrigation and soil fertility management on different soil textures to improve wheat yield in drought-prone Bangladesh. Agricultural Water Management, 191, 124-137.

Passioura, J. B. (2012). Phenotyping for drought tolerance in grain crops: when is it useful to breeders?. Functional Plant Biology, 39(11), 851-859.

Paulraj, S., Bhat, R., Rajesh, M. K., Ramesh, S. V., Priya, U. K., Pandian, R. T. P., ... & Chowdappa, P. (2021). Data of 16S rRNA gene amplicon-based metagenomic signatures of arecanut rhizosphere soils in Yellow Leaf Disease (YLD) endemic region of India. Data in Brief, 38, 107443.

Sanger, F., & Coulson, A. R. (1975). A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. Journal of molecular biology, 94(3), 441-448.

Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya, 17, 362-370.

Rawls, W. J., Pachepsky, Y. A., Ritchie, J. C., Sobecki, T. M., & Bloodworth, H. (2003). Effect of soil organic carbon on soil water retention. Geoderma, 116(1-2), 61-76.

Rossmann, M., Perez-Jaramillo, J. E., Kavamura, V. N., Chiaramonte, J. B., Dumack, K., Fiore-Donno, A. M., ... & Mendes, R. (2020). Multitrophic interactions in the rhizosphere microbiome of wheat: from bacteria and fungi to protists. FEMS Microbiology Ecology, 96(4), fiaa032.

Schädler, M., Buscot, F., Klotz, S., Reitz, T., Durka, W., Bumberger, J., ... & Auge, H. (2019). Investigating the consequences of climate change under different land-use regimes: a novel experimental infrastructure. Ecosphere, 10(3), e02635.

Schöler, A., Jacquiod, S., Vestergaard, G., Schulz, S., & Schloter, M. (2017). Analysis of soil microbial communities based on amplicon sequencing of marker genes. Biology and Fertility of Soils, 53(5), 485-489.

Schweitzer, K. (2010). Naturnahe Böden im Berliner Stadtgebiet. Referenz für anthropogene Bodenentwicklung oder Beispiel für anthropogene Bodenveränderungen. Boden des Jahres, 13-22.

Simonin, M., Dasilva, C., Terzi, V., Ngonkeu, E. L., Diouf, D., Kane, A., ... & Moulin, L. (2020). Influence of plant genotype and soil on the wheat rhizosphere microbiome: evidences for a core microbiome across eight African and European soils. FEMS Microbiology Ecology, 96(6), fiaa067.

Simonin, M., Briand, M., Chesneau, G., Rochefort, A., Marais, C., Sarniguet, A., & Barret, M. (2021). Seed microbiota revealed by a large-scale meta-analysis including 50 plant species. bioRxiv.

Skirvin, R. M., Kohler, E., Steiner, H., Ayers, D., Laughnan, A., Norton, M. A., & Warmund, M. (2000). The use of genetically engineered bacteria to control frost on strawberries and potatoes. Whatever happened to all of that research?. Scientia horticulturae, 84(1-2), 179-189.

Torsvik, V., Goksøyr, J., & Daae, F. L. (1990). High diversity in DNA of soil bacteria. Applied and environmental microbiology, 56(3), 782-787.

Tsolakidou, M. D., Stringlis, I. A., Fanega-Sleziak, N., Papageorgiou, S., Tsalakou, A., & Pantelides, I. S. (2019). Rhizosphere-enriched microbes as a pool to design synthetic communities for reproducible beneficial outputs. FEMS Microbiology Ecology, 95(10), fiz138.

Wehner, Gwendolin; Kopahnke, Doris; Richter, Klaus; Kecke, Steffen; Schikora, Adam; Ordon, Frank (2019): Priming Is a Suitable Strategy to Enhance Resistance Towards Leaf Rust in Barley. In: Phytobiomes Journal 3 (1), S. 46–51. DOI: 10.1094/PBIOMES-09-18-0041-R.

Wei, Z., & Jousset, A. (2017). Plant breeding goes microbial. Trends in Plant Science, 22(7), 555-558.

Weißbecker, C., Schnabel, B., & Heintz-Buschart, A. (2020). Dadasnake, a Snakemake implementation of DADA2 to process amplicon sequencing data for microbial ecology. GigaScience, 9(12), giaa135.

Wubs, E. R., Van der Putten, W. H., Bosch, M., & Bezemer, T. M. (2016). Soil inoculation steers restoration of terrestrial ecosystems. Nature plants, 2(8), 1-5.

Zhu, Y., Li., Y., Zheng, N., Chapman, S. J. & Yao, H. (2020). Similar bot not identical resuscitation trajectories of the soil microbial community based on either DNA or RNA after flooding. Agronomy, 10(4), 502.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the professional and personal support of so many people in the last years. Therefore, I would like to give these special people here some space to express my gratitude.

Many thanks to Prof. François Buscot, for the opportunity and support to write this thesis in the Department of Soil Ecology, for providing your expertise and convincing arguments to get even the most arguable manuscript through a peer-reviewing process, and always the best ideas when it comes to manuscript titles.

My deepest gratitude and appreciation to my two supervisors Thomas Reitz and Mika Tarkka. Since my obligatory internship in 2014, we have come a long way together, during which you were always a great source of inspiration, open for fruitful and/or heated discussions to prepare me for academic life. Thank you so much for your professional and, even more important for me, personal commitment over the years, and I promise lifetime presents on Christmas for the both of you.

I would also like to thank my PhD-Advisory Committee (yDiv), which consisted of, besides my three supervisors, Nico Eisenhauer and Petra Högy for providing valuable input before and during the experimental phase.

All the field and lab work for this thesis would not have been completed without the help of so many people. First and foremost, I would like to thank Kerstin Hommel, which accompanied my first steps in the lab, always had a helping hand and an open ear, and more importantly became my friend, despite my severe handicap to estimate people's age. I also owe a big thanks to Beatrix Schnabel. Even though you were not officially involved in my project or part of my working group, your curiosity and critical eye often enough saved me from making mistakes and let me think twice. And, I will definitely miss the cake sessions! A great thanks also to Jacqueline (Jacki) Rose, Gabriele (Gabi) Henning, Yvonne Eckstein, Luise Kaiser, Melanie Günther and Renate Rudloff for their tremendous support in the lab, as well as to the great team at the Bad Lauchstädt research station, namely Sabine Straßenbourg, Christa Wolfram, Renate Hintz, Konrad Kirsch and Ines Merbach, for their great support in performing and harvesting the numerous field and greenhouse experiments, but also that you made me forget the hard work and every day watering by nice conversations with coffee and sweats.

Apart from the work, the personal contacts and new friendships made my life as a PhD-student to such a great experience. Because I complained this in your acknowledgement: Dear Luis, you became my absolute number one over the years. I will never forget our heated "Yes"-"No"-

“Why”-“Because” discussions, as well as our regular Friday afternoon chatting and power-napping sessions in the office, and all the other situations in and outside the UFZ, which made you to one of my best friends. I am very grateful, that you came to Germany and went with me through that time! Then somehow, the PhD-time is separated in a before-during-after Corona-time. Before Corona, I really enjoyed the regular game evenings, lunch discussions and funny projects, and also all the outbreak activities with former and actual colleagues; thank you in particular Ricardo Schöps and Kezia Goldmann (Angry BOOEKs), Anna Heintz-Buschart, Erik Schwarz, Laura Herzig, and many more for this great time. During and after Corona, especially the contact to Minh Ganther and Cynthia Albracht helped me to stay socialized and introduced some really nice new traditions, i.e. regular Discord (The Chosen Four) and Switch evenings. Thank you for your friendship and hopefully some more trips to Amsterdam (this time without marten problems…), to teach Anna how to make real pancakes.

Wie man so schön im Englischen sagt „last, but not least“, mein größter Dank gebührt meinen Eltern. Ihr seid die wichtigsten Menschen in meinen Leben. Ohne euch an meiner Seite, die mich immer angefeuert und unterstützt haben (und nur manchmal nachgefragt haben, wie lange es noch dauert…) wäre all dies nicht möglich gewesen. Nun ist es endlich geschafft, und ihr könnt die Flasche Whiskey aufmachen, die seit 2018 geduldig darauf wartet getrunken zu werden: „Slàinte!“

CURRICULUM VITAE

Personal details

Date of birth	15th of November 1989
Nationality	German
Current address	Frohburger Straße 28a, 04654 Frohburg OT Prießnitz
Electronic mail	c-breitkreuz@web.de

Education

04/2016 – present PhD in Microbial Ecology, *Helmholtz Centre for Environmental Research-UFZ GmbH, Department of Soil Ecology, Halle (Saale), Germany*
Funded by the Deutsche Bundesstiftung Umwelt (DBU) from 04/2016 to 03/2019 (granted October 2015, AZ 20015/391)

Thesis: "Impact of plant growth promoting rhizobacteria (PGPR) on stress resistance of winter wheat (*Triticum aestivum L.*)"
Advisor: Prof. Dr. François Buscot, PD Dr. Mika Tarkka, Dr. Thomas Reitz

10/2012 – 03/2015 Master studies Biology, *University of Leipzig, Leipzig, Germany*
Core subjects: Biodiversity and Evolution of plants, Microbiology

Thesis: "The impact of drought and plant communities on the functional diversity of the genus *Pseudomonas*"
performed at *Helmholtz Centre for Environmental Research-UFZ GmbH, Department of Soil Ecology, Halle (Saale), Germany*
Advisor: PD Dr. Mika Tarkka, Dr. Thomas Reitz
Reviewers: Prof. Dr. François Buscot, Dr. Alexandra Weigelt

10/2008 – 09/2012 Bachelor studies Biology, University of Leipzig, Leipzig, Germany

Thesis: "Vorstellung einer Methode zur Vermessung der Blattnervatur an herbarisierten Blättern"
University of Leipzig, Faculty of Life Sciences, Institute for Botany
Advisor/
Reviewers: Prof. Dr. Christian Wirth, Dr. Alexandra Weigelt

Work experience

04/2021 – present Researcher, Julius Kühn-Institute Federal Research Centre for Cultivated Plants, Quedlinburg, Germany
Projects: BioSam
IdeMoDeResBar - II

04/2016 – 03/2021 PhD-student in Microbial Ecology, Helmholtz Centre for Environmental Research-UFZ GmbH, Department of Soil Ecology, Halle (Saale), Germany

Research and Mentoring experience

2019 Supervision of undergraduate student Martin Sieger Pereira, obligatory internship for five months (Pontificia Universidad Javeriana, Bogotá, Columbia)

2017-2019 Mentoring and supervising students in the frame of "Soil Ecology" course offered in Master studies Biology at the University of Leipzig by Prof. Dr. François Buscot and Dr. Thomas Reitz

2017-2018 Mentoring and supervision of undergraduate student Carolin Albrecht for 14 months (Bachelor Thesis: "Dynamik mobiler und stabiler C-& N-Fraktionen im Boden über die Vegetationsperiode von Winterweizen (Triticum aestivum L.) in Abhängigkeit von Düngung, Bodentyp und Bewässerungssystem", Martin-Luther-University Halle-Wittenberg, Germany)

2016 Supervision of undergraduate student Laura Herzig, obligatory internship for three months (internship report, Martin-Luther-University Halle-Wittenberg, Germany)

Contribution to supervision of Master students (Andrea Leptin, four months in 2017; Lilith Christaller, six months in 2017; Ruben Schüchner, six months in 2016) and Bachelor students (Erik Schwarz, four months in 2016/17) regarding lab work, data analysis and thesis writing

Support in projects of Esmaeil Karimi (co-authorship, publication submitted to AMB Express), Maria Rasul (co-authorship, published in Microbiological Research 2021) and Sara Fareed Mohamed Wahdan (co-authorship, published in Environmental Microbiology 2021)

Other projects: > *Soil sampling campaigns in the Global Change Experimental Facility* to monitor every three weeks (2015-2018), seasonal and annual

dynamics in nutrient cycling (led by Dr. Thomas Reitz, data not published)

> *Tea Bag Index* for litter decomposition experiments (led by Dr. Thomas Reitz, conference talk “Land-use intensity modulates climate change impacts on annual decomposition dynamics in temperate agricultural systems”, Frontiers in experimental research on changing environments, Leipzig/Bad Lauchstädt, Germany, 2022)

> *European project “Land Use and Coverage Area frame Survey - LUCAS”* (organized by the iDiv, co-authorship in Siles et al. 2022)

Extracurricular activities

02/2019 – 02/2020	PhD representative in the Department Soil Ecology, UFZ, Halle
Graduate schools:	> *Higrade,* UFZ graduate school (finished: 30 course days, 3 UFZ presentations, presentation at international conference as first author and at Higrade Conference)
	> *yDiv,* iDiv graduate school (finished: 12 credit points)
Seminars:	> “Woche der Umwelt” („Schloss Bellevue“, Berlin, June 2016)
	> Deutsche Bundesstiftung Umwelt (DBU) scholarship holder seminars (oral presentation of results): Roggenburg (2016, one week) Marienthal (2017, one week) Volkenroda (2018, one week)
	> yDiv retreats: “Career Outside Academia” (three days, 2018) “Science collaboration and interdisciplinary projects (three days, 2019)
Public Relations work:	> “Long night of Sciences – Halle”, information booth on soil microorganisms at the UFZ (2013-2015, 2017 and 2018)
	> Interview in the documentary „The future of warming in a warming world“ (Deutsche Welle, published 22.06.2017)
	> Presentation in the frame of “168h bioinformatics” for pupils at the Martin-Luther University Halle-Wittenberg

LIST OF PUBLICATIONS AND PRESENTATIONS

Publications in peer-reviewed journals:

2022:

Gebauer, L. & **Breitkreuz, C.**; Heintz-Buschart, A.; Reitz, T.; Buscot, F., Tarkka, M.; Bouffaud, M. (2022) Legacy effects of water deficit on plant beneficial bacteria depend on the farming management. Frontiers in Microbiology

Siles, J. A., Díaz-López, M., Vera, A., Eisenhauer, N., Guerra, C. A., Smith, L. C., ...**Breitkreuz, C.**, … & Bastida, F. (2022). Priming effects in soils across Europe. Global change biology.

Karimi, E., Aliasgharzad, N., Esfandiari, E., Hassanpouraghdam, M.B., Neu, T.R., Buscot, F., Reitz, T., **Breitkreuz, C.**, Tarkka, M.T. (submitted). Biofilm forming rhizobacteria affect the physiological and biochemical responses of wheat to drought. AMB Express.

2021:

Breitkreuz, C., Heintz-Buschart, A., Buscot, F., Wahdan, S.F.M., Tarkka, M. T. & Reitz, T. (2021). "Can We Estimate Functionality of Soil Microbial Communities from Structure-Derived Predictions? A Reality Test in Agricultural Soils". Microbiology Spectrum 9(1): e00278-21.

Breitkreuz, C., Reitz, T., Schulz, E., & Tarkka, M. T. (2021). Drought and Plant Community Composition Affect the Metabolic and Genotypic Diversity of *Pseudomonas* Strains in Grassland Soils. Microorganisms, 9(8): 1677.

Breitkreuz, C., Herzig, L., Buscot, F., Reitz, T., & Tarkka, M. (2021). Interactions between soil properties, agricultural management and cultivar type drive structural and functional adaptations of the wheat rhizosphere microbiome to drought. Environmental Microbiology.

Rasul, M., Yasmin, S., Yahya, M., **Breitkreuz, C.**, Tarkka, M., & Reitz, T. (2021). The wheat growth-promoting traits of *Ochrobactrum* and *Pantoea* species, responsible for solubilization of different P sources, are ensured by genes encoding enzymes of multiple P-releasing pathways. Microbiological Research, 246, 126703.

Wahdan, S. F. M., Reitz, T., Heintz-Buschart, A., Schädler, M., Roscher, C., **Breitkreuz, C.**, ... & Buscot, F. (2021). Organic agricultural practice enhances arbuscular mycorrhizal symbiosis in correspondence to soil warming and altered precipitation patterns. Environmental Microbiology.

2020:

Breitkreuz, C., Buscot, F., Tarkka, M. and Reitz, T. (2020). Shifts Between and Among Populations of Wheat Rhizosphere *Pseudomonas*, *Streptomyces* and *Phyllobacterium* Suggest Consistent Phosphate Mobilization at Different Wheat Growth Stages Under Abiotic Stress. Frontiers in Microbiology 10:3109. doi: 10.3389/fmicb.2019.03109

Oral Presentations:

Breitkreuz, C., Heintz-Buschart, A., Buscot, F., Wahdan, S.F.M., Tarkka, M.T., Reitz, T. (2022). Multifactorial and multidisciplinary approaches to study adaptation of wheat rhizosphere communities to drought. Frontiers in experimental research on changing environments, Leipzig/Bad Lauchstädt, Germany.

Breitkreuz, C., Reitz, T., Tarkka, M., Buscot, F. (2017). Impact of plant growth promoting rhizobacteria (PGPR) on stress resistance of winter wheat. iDiv Annual conference 2017, Leipzig, Germany.

Breitkreuz, C. (2017). Impacts of climate change on PGPR: drivers of ecosystems processes in the rhizosphere of winter wheat. GCEF Meeting 2017, Halle (Saale), Germany.

Poster Presentations:

Breitkreuz, C., Buscot, F., Tarkka, M., Reitz, T., Heintz-Buschart, A. (2019). Crop plant and soil pH are strong drivers of rhizosphere microbial diversity in contrast to land use related soil enzyme activities. 8th Congress of European Microbiologists, Glasgow, Scotland.

Breitkreuz, C., Buscot, F., Tarkka, M., Heintz-Buschart, A., Reitz, T. (2018). Resilience of PGPR against future climate in two farming systems. 11th International Plant Growth-Promoting Rhizobacteria Workshop, Victoria, British Columbia, Canada.

Breitkreuz, C., Reitz, T., Heintz-Buschart, A, Tarkka, M. (2018). Functional predictions vs. measured activities of bacterial communities in agricultural systems. 17th International Symposium on Microbial Ecology, Leipzig, Germany.

Breitkreuz, C., Tarkka, M., Reitz, T., Buscot, F. (2018). Abundance and functional traits of soil bacteria are affected by land use and climatic conditions. BONARES Conference 2018: Soil as a Sustainable Resource, Berlin, Germany.

Breitkreuz, C., Buscot, F., Tarkka, M., Reitz, T. (2017) Impact of plant growth promoting rhizobacteria (PGPR) on stress resistance of winter wheat. British Ecological Society, Ecology Across Borders: Joint Annual Meeting 2017, Ghent, Belgium.

STATUTORY DECLARATION

Hereby, I, Claudia Breitkreuz, affirm that I take note and accept the doctorate regulations of the Faculty of Life Science at the University of Leipzig from September, 30th of 2019. I further affirm that the presented thesis was prepared autonomously without inadmissible help. All aids used in this thesis as well as scientific ideas which are quoted from or based on other sources were cited at the respective point.

All people who helped me to prepare the conception, to select and analyze the materials of this thesis as well as to improve the manuscripts are namely cited in the acknowledgments. With exception of the namely mentioned people no other persons were involved in the intellectual work. No PhD consultant service was employed. Third parties did not get money's worth for benefits that were conjunction with the content of this dissertation.

I declare that this dissertation has been neither presented nationally nor internationally in its entirety or in parts to any institution for the purpose of dissertation or other official or scientific examination and/or publishing.

Previously unsuccessful dissertations had no taken place.

The original document of the verification of the co-author parts are deposited in the office of the dean.

Halle (Saale), 4th of July 2022

Claudia Breitkreuz

EIDESSTATTLICHE ERKLÄRUNG

Hiermit erkläre ich, Claudia Breitkreuz, eidesstattlich, dass mir die Promotionsordnung der Fakultät für Lebenswissenschaften der Universität Leipzig vom 30.09.2019 bekannt ist und von mir anerkannt wird.

Zudem versichere ich, dass die vorliegende Dissertation von mir selbstständig und ohne unzulässige Hilfsmittel angefertigt worden ist. Sämtliche von mir verwendetet Hilfsmittel, sowie die aus fremden Quellen direkt oder indirekt übernommenen wissenschaftlichen Gedanken, sind als solche in der vorliegenden Arbeit gekennzeichnet. Alle Personen, von denen ich bei der Auswahl und Auswertung des Materials, sowie bei der Herstellung der Manuskripte Unterstützungsleistungen erhalten habe, sind an entsprechender Stelle namentlich in der/den Danksagungen genannt. Außer den genannten Personen waren keine weiteren Personen an der geistigen Herstellung der vorliegenden Arbeit beteiligt. Die Hilfe eines Promotionsberaters wurde nicht in Anspruch genommen. Dritte haben von mir für Arbeiten, die im Zusammenhang mit dem Inhalt der vorliegenden Dissertation weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zwecke der Promotion oder andere staatlicher oder wissenschaftlicher Prüfungsverfahren vorgelegt und/oder veröffentlicht wurde.

Frühere erfolglose Promotionsversuche haben nicht stattgefunden.

Der Nachweis über die Anteile der (Co-)Autorenschaften sind im Original im Dekanat der Universität Leipzig hinterlegt.

Halle (Saale), 04. Juli 2022

Claudia Breitkreuz

AUTHOR CONTRIBUTIONS

Verification of the author contributions, Claudia Breitkreuz
Impact of plant-growth promoting rhizobacteria (PGPR) on the stress resistance of wheat (Triticum aestivum L.)

Verification of the author contributions:

Title:	**Interactions between soil properties, agricultural management and cultivar type drive structural and functional adaptations of the wheat rhizosphere microbiome to drought**
Journal:	Environmental Microbiology
Authors:	Claudia Breitkreuz, Laura Herzig, François Buscot, Thomas Reitz, Mika Tarkka

Contributions of Claudia Breitkreuz (first author):

- Coordination and idea of project
- Set up of the pot experiment and maintenance
- Sample collection and laboratory work
- Data analysis and interpretation
- Manuscript conception, writing and revision

Contributions of Laura Herzig (author 2):

- Support to set up the pot experiment and maintenance
- Sample collection and laboratory work
- Data analysis and interpretation
- Manuscript revision

Contributions of François Buscot (author 3):

- Coordination and idea of project
- Manuscript revision

Contributions of Thomas Reitz (author 5):

- Coordination and idea of project
- Data interpretation
- Manuscript conception, writing and revision

Contributions of Mika Tarkka (senior author):

- Coordination and idea of project
- Data interpretation
- Manuscript conception, writing and revision

Claudia Breitkreuz Mika Tarkka

Verification of the author contributions:

Title: **Can we estimate functionality of soil microbial communities from structure-derived predictions? A reality test in agricultural soils**

Journal: Microbiology Spectrum

Authors: Claudia Breitkreuz, Anna Heintz-Buschart, François Buscot, Sara Fareed Mohamed Wahdan, Mika Tarkka, Thomas Reitz

Contributions of Claudia Breitkreuz (first author):

- Coordination and idea of project
- Sample collection and laboratory work
- Data analysis and interpretation
- Manuscript conception, writing and revision

Contributions of Anna Heintz-Buschart (author 2):

- Idea of the project
- Data analysis and interpretation
- Manuscript conception and revision

Contributions of François Buscot (author 3):

- Coordination and idea of project
- Manuscript revision

Contributions of Sara Fareed Mohamed Wahdan (author 4):

- Sample collection and laboratory work
- Manuscript revision

Contributions of Mika Tarkka (author 5):

- Coordination and idea of project
- Data interpretation
- Manuscript conception and revision

Contributions of Thomas Reitz (senior author):

- Coordination and idea of project
- Sample collection
- Data interpretation
- Manuscript conception, writing and revision

Claudia Breitkreuz

Thomas Reitz

Verification of the author contributions:

Title: **Shifts between and among populations of wheat rhizosphere *Pseudomonas*, *Streptomyces* and *Phyllobacterium* suggest consistent phosphate mobilization at different wheat growth stages under abiotic stress**

Journal: Frontiers in Microbiology

Authors: Claudia Breitkreuz, François Buscot, Mika Tarkka, Thomas Reitz

Contributions of Claudia Breitkreuz (first author):

- Coordination and idea of project
- Sample collection and laboratory work
- Data analysis and interpretation
- Manuscript conception and writing

Contributions of François Buscot (author 2):

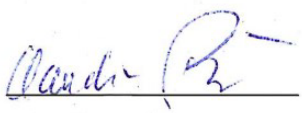
- Coordination and idea of project
- Manuscript writing and revision

Contributions of Mika Tarkka (author 3):

- Coordination and idea of project
- Data interpretation
- Manuscript conception, writing and revision

Contributions of Thomas Reitz (senior author):

- Coordination and idea of project
- Sample collection
- Data interpretation
- Manuscript conception, writing and revision



Claudia Breitkreuz

Thomas Reitz