Université de Montréal

Soil histories continue to structure the bacterial and oomycete communities of *Brassicaceae* host plants through time on the Canadian prairies

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Thèse présenté en vue de l'obtention du grade de *Philosophiae Doctor* (Ph. D.)

en sciences biologiques

Janvier 2023

Université de Montréal

Unité académique : Département de sciences biologiques, Faculté des arts et sciences

Cette thèse intitulée

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Résumé

Afin d'étudier l'écologie microbienne, il est nécessaire, dans un premier temps, de déterminer quels micro-organismes sont présents dans un milieu et à quel instant. Ces informations sont requises pour pouvoir ensuite développer des outils permettant de prédire l'assemblage des communautés et les fonctions que celles-ci peuvent contenir. Cependant, la multitude des processus entrant en jeu dans la structure et la composition des communautés microbiennes, rendent leur étude complexe. Parmi les nombreux processus à étudier, il est notamment question de l'échelle temporelle à prendre en compte pour comprendre l'assemblage des communautés microbiennes. En effet, les événements historiques conditionnent la composition et la biodiversité des futures communautés microbiennes. Pourtant, dans les sols, peu d'études se sont intéressées à l'impact des événements historiques dans l'assemblage des communautés microbiennes. Par conséquent, l'objectif de cette thèse était de quantifier comment les différentes histoires du sol ont influencé la structure et biodiversité des communautés bactériennes et oomycètes associées aux plantes hôtes des *Brassicaceae* à travers le temps.

Les rotations de cultures de *Brassicaceae* sont de plus en plus courantes dans le monde et ont démontré des avantages pour les cultures concernées, telles que la rétention de l'humidité du sol ou la suppression de certains agents pathogènes des plantes. En revanche, l'impact des rotations de cultures de *Brassicaceae* sur la structure et biodiversité des communautés microbiennes résidentes est peu connu. Ainsi, des terrains agricoles des prairies canadiennes ayant des expériences de rotations de cultures en cours ont été utilisés pour modéliser l'impact des histoires de sol précédemment établies sur les futures communautés microbiennes. Les communautés microbiennes des racines, de la rhizosphère, et du sol éloigné des racines des *Brassicaceae* ont été

étudiées grâce aux métabarcodes d'ARNr 16S ou ITS. La PCR quantitative et des méthodes phylogénétiques ont été utilisées pour améliorer l'analyse des communautés microbiennes.

Cette thèse illustre comment différentes histories de sol établies par les cultures de l'année précédente ont continué à structurer les communautés microbiennes de la rhizosphère tout au long de la saison de croissance, à différents stades de croissance, jusqu'à un an après leur établissement. Cependant, le phénomène de rétroactions entre plantes et micro-organismes a permis de masquer cet héritage dans la rhizosphere de différentes espèces hôtes de *Brassicacea* pour lesquelles des communautés bactériennes phylogénétiquement similaires ont été retrouvées malgré diverses histoires du sol. Nos résultats montrent également que les différentes espèces hôtes de *Brassicacea* n'avaient pas d'impact sur la structure des communautés d'oomycètes et que le stress hydrique limitait également cette structuration pour les communautés bactériennes. Dans ces deux cas, l'effet de l'histoire du sol était donc encore visible sur la structure les communautés microbiennes durant l'année subséquente.

Les découvertes selon lesquelles différentes histoires de sol persistent jusqu'à un an, même en présence de nouvelles plantes hôtes, et qu'elles peuvent continuer à façonner les communautés microbiennes ont des implications importantes pour la gestion agricole et les recherches futures sur les composants physiques de l'histoire du sol. Comprendre comment l'histoire du sol est impliquée dans la structure et la biodiversité des communautés microbiennes à travers le temps est une limitation de l'écologie microbienne et est nécessaire pour utiliser les technologies microbiennes à l'avenir pour une agriculture durable et dans toute la société.

Mots-clés : Histoire du sol, bactéries, oomycètes, *Brassicaceae*, phylogénétique, interactions plantes-microbes, écologie microbienne, biodiversité.

Abstract

A fundamental task of microbial ecology is determining which organisms are present, and when, in order to improve the predictive models of community assembly and functions. However, the heterogeneity of community assembly processes that underlie how microbial communities are formed and structured are makes assembly of taxonomic and functional profiles difficult. One reason for this challenge is the compounding effect temporal scales have on microbial communities. For example, historical events have been shown to condition future microbial community composition and biodiversity. Yet, how historical events structure microbial communities in the soil has not been well tested. Therefore, the objective of this thesis was to quantify how different soil histories influenced the structure and biodiversity of bacterial and oomycete communities associated with *Brassicaceae* host plants through time.

Brassicaceae crop rotations are increasingly common globally, and have demonstrated benefits for the crops involved, such as retaining soil moisture, or suppressing certain plant pathogens. In contrast, there is a lack of knowledge surrounding how *Brassicaceae* crop rotations impact the structure and biodiversity of resident microbial communities. As such, on-going agricultural field experiments with crop rotations on the Canadian prairies were used to model how previously established soil histories impacted future microbial communities. The *Brassicaceae* microbial communities were inferred from the roots, rhizosphere and bulk soil using 16S rRNA or ITS metabarcodes. Quantitative PCR and phylogenetic methods were used to improve the analysis of the microbial communities.

This thesis illustrates how different soil histories established by the previous year's crops continued to structure the microbial rhizosphere communities throughout the growing season, at various growth stages, and up to a year after being established. However, active plant-soil microbial

feedback allowed different *Brassicaceae* host species to mask the soil history in the rhizosphere and derive phylogenetically similar bacterial communities from these diverse soil histories. Furthermore, host plants were unable to structure the oomycete communities, and lost the ability to structure the bacterial rhizosphere communities under water stress. In both circumstances, the soil history continued to structure the microbial communities.

The findings that different soil histories persist for up to a year, even in the presence of new host plants, and can continue to shape microbial communities has important implications for agricultural management and future research on the physical components of soil history. Understanding how soil history is involved in the structure and biodiversity of microbial communities through time is a limitation in microbial ecology and is required for employing microbial technologies in the future for sustainable agriculture and throughout society.

Keywords: Soil history, bacteria, oomycetes, *Brassicaceae*, phylogenetics, plant-microbial interactions, microbial ecology, biodiversity.

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List of Acronyms & Abbreviations

ASV: amplicon sequence variant

Db-RDA: distance-based redundancy analysis

FDR: false discovery rate

ITS: internal transcribed spacer

OTU: operational taxonomic unit

PERMANOVA: permutational multivariate analysis of variance

PSF: plant-soil community feedback

rRNA: ribosomal ribonucleic acid

Acknowledgements

I would like to thank my supervisors Drs. Mohamed Hijri and Marc St-Arnaud for this opportunity. I would also like to acknowledge the support of my supervisory committee, Drs. Etienne Laliberté and Hugo Germain, my synthesis exam committee, Drs. Simon Joly and Étienne Yergeau (it was fun!), as well as my thesis examining committee, Drs. Étienne Yergeau, Geneviève Lajoie and Isabelle Laforest-Lapointe, for their time and effort in asking thoughtful questions and pushing me to improve my thesis.

I also thank post-doctoral researchers, Drs. Chih-Ying Lay, Jacynthe Masse, and Sébastien Renaut, for setting me on the right track with this project, your helpful comments, and encouragement along the way. Furthermore, this thesis would not have been possible without the support and enthusiasm of Drs. Alexis Carteron and Simon Morvan, in whom I could not have asked for a better labmate. Thank you both for your help, your expertise, and your suggestions.

I would also like to thank Drs. Simon Joly and Anne Bruneau, and their labs, Drs. Julie Faure, Boris Domenech, Carole Sinou, and Ms. Valerie Poulin, for including me and making me feel welcome at the Biodiversity Centre. Equally, thank you to other members of the faculty, Drs. Jean-François Lapierre, Daniel Kierzkowski, Anne-Lise Routier-Kierzkowska, Colin Favret, Sandra Binning, Matthew Regan, Roxane Maranger, and David Morse, for their collegiality, guidance, and mentorship.

To my other comrades-in-arms past, Drs. Stéphanie Shousha, Morgan Botrel, and Manu Chrétien, and those who remain, Adrien Frémont and Eszter Sas, thank you for all the chats, the ideas, the support, and the guidance. You each helped to lighten the load along the way.

For her endless help and support in the lab, and beyond, I would like to thank Jocelyne Ayotte. I also recognize the hard work and dedication of the many interns I have had the pleasure of mentoring, including Emmy L'Espérance for her help in establishing my lab protocols, as well as Thomas Mahbeer, Éloïse Bélanger, Marilou Tremblay, and Florance Danis, who all spent countless hours sieving soil for me in a cold basement.

Finally, to my friends, family, and the many others who deserve recognition, I thank you for your support and your patience. My sincerest gratitude; it's been a time.

I also acknowledge the generous financial support of the Schmeelk Canada Foundation, as well as contributions from l'Université de Montréal, including les Études Supérieures et Postdoctorales, le Faculté des Arts et des Sciences, et le Département des Sciences Biologiques, and from l'Institut de Recherche en Biologie Végétale, and the Québec Centre for Biodiversity Science.

Introduction

"[T]here has never been any natural animal or plant free of microorganisms"

– Zilber-Rosenberg & Rosenberg, 2008

We live in a microbial world. After ~3.7 billion years of evolution, microbes, particularly prokaryotes, are found in every environment on Earth, where they define the limits of life (Takai, 2019; Shu & Huang, 2022). The number of microbial individuals on Earth has been estimated from ~10^{26} for protists, as well as fungi, to ~10^{30} for archaea, and even higher for bacteria (Bar-On *et al*., 2018), with species estimates that range from thousands to trillions (Louca *et al*., 2019). Of the ~550 Gt of carbon that composes all biomass on Earth, prokaryotes account for ~77 Gt—second only to land plants—while fungi add ~12 Gt, and protists are another ~4 Gt (Bar-On *et al*., 2018). Where plants are not dominant, such as marine and subsurface environments, microbes account for nearly all the biomass (Bar-On *et al*., 2018). They are also fundamental drivers of global systems, including climate regulation and nutrient cycling (Guerra *et al*., 2020). As such, given their pervasive nature, studying microbial biodiversity ought to be a priority to understanding which organisms are present, when are they present, and what roles do they play.

Microbial Communities

In contrast to laboratory conditions, where microbes are typically studied in isolation, one at a time, microbes in the environment do not exist in seclusion. Rather, they live in dynamic communities, or microbiomes (Konopka *et al*., 2015; Berg *et al*., 2020), whose functions cascade

out to determine the presence or absence of other organisms, and ultimately the biodiversity and stability of ecosystems (Escalas *et al*., 2019). Moreover, microbial communities often exhibit emergent, or novel functions that arise from the diversity of organismal interactions in the community (Konopka *et al*., 2015). This underscores the importance of studying microbial community composition as a first step to determine their collective and individual functions (Konopka *et al*., 2015)

Unfortunately, culture-dependent approaches to unravel microbial community composition are tedious (Yarza *et al*., 2014). One estimate suggests that, counting the ~11 000 archaeal and bacterial species that have already been isolated, at the current rate of culturing ~600 new species per year it would take more than a thousand years to catalogue all prokaryotes (Yarza *et al*., 2014). Albeit time consuming, this kind of fundamental research is required in order to develop experimental systems that permit testing the ecological roles and interactions of different microbes (Geller & Levy, 2023). This is even more pertinent in non-model microbes and their hosts (Geisen *et al*., 2022).

Nonetheless, with the advent of high-throughput sequencing technologies, it is now possible to use culture-independent, community-scale strategies, to detail when and where specific microbes are present (Escalas *et al*., 2019; Fitzpatrick *et al*., 2020). By sequencing all, or specific pieces, of the nucleic acids extracted from an environment, meta-omics or metabarcoding approaches allow us to identify community composition, and their potential or actual functions, without having to isolate each individual microbe (Escalas *et al*., 2019; Fitzpatrick *et al*., 2020). These are useful complements to culture-based approaches, but are by no means perfect.

In the case of metabarcoding, the prokaryotic 16S rRNA gene and the eukaryotic ITS region are used as standard markers, or tags, to identify organisms present in a sample (Karst *et al*., 2018). These markers fulfil key criteria to be used as standard barcodes: first, the 16S rRNA gene and ITS region are thought to be nearly universal, for prokaryotes and eukaryotes respectively. Second, these markers have sufficiently conserved regions to allow for standard PCR primers, but also have variable regions that can allow for high resolution of taxa (Santos & Ochman, 2004). However, the utility of these markers has been hotly contested, particularly for their inability to discriminate between closely related strains (Santos & Ochman, 2004; Blakney & Patten, 2011). Nonetheless, these PCR amplified sequences, or amplicons, do provide a quick, cost-effective, first estimate of species diversity (Bulgarelli *et al*., 2012; Lundberg *et al*., 2012; Lebeis *et al*., 2015; Revillini *et al*., 2016; Lay *et al*., 2018).

As our tools and capacities continue to develop for investigating how microbial communities function, along with the importance of doing so, three broad trends have emerged in studying microbial community ecology (Konopka *et al*., 2015): 1) determining the relative strengths of each mechanism (dispersal, drift, selection, and speciation) of community ecology theory in structuring different communities (Vellend, 2010; Stegen *et al*., 2013); 2) identifying emergent properties at the community-level and how biodiversity generates these functions (Bissett *et al*., 2013; Loreau & de Mazancourt, 2013; Thibaut & Connolly, 2013; Kuang *et al*., 2022); and 3) microbial community engineering, to produce products or services (Volger *et al*., 2020; Agoussar & Yergeau, 2021; Correa-García *et al*., 2021; Zhalnina *et al*., 2021). As trends 2) and 3) fall largely beyond the scope of my work, here I am more curious in 1) the mechanisms involved in structuring microbial communities.

The interest in how these mechanisms—dispersal, drift, selection, and speciation—structure, or determine, microbial species composition (for a specific time and place; Vellend, 2010) stems from how variable microbial communities appear to be (Thompson *et al*., 2017). By studying how these mechanisms function, their relative strengths and interactions, microbial ecologists may uncover guidelines for why some organisms are found in similar environments, while others are absent (Vellend, 2010; Nemergut *et al*., 2013); i.e. how microbial communities are assembled. Furthermore, being able to accurately predict the composition of microbial communities bolsters the loftier goals of uncovering the functions of these communities and engineering them, items 2) and 3) mentioned above (Konopka *et al*., 2015; Thompson *et al*., 2017; Escalas *et al*., 2019).

Broadly, these community ecology mechanisms can be conceptualized for how they impact diversity, or the number of species present. Processes related to dispersal and speciation tend to increase local diversity, while drift and selection are thought to decrease local microbial diversity (Vellend, 2010; Nemergut *et al*., 2013). Selection, also referred to as environmental filtering, or deterministic processes, is the differential rate of survival and reproduction of organisms, as a function of biotic and abiotic interactions, and niches (Vellend, 2010; Nemergut *et al*., 2013). It has been suggested that selection is the main driver for microbial community assembly (Nemergut *et al*., 2013; Herrera Paredes & Lebeis, 2016; Goss-Souza *et al*., 2020), though this emphasis could be an artefact of the many processes that interact to determine the force of selection. Although selection pressure may typically drive a maladapted species to extinction, selection can also push the diversification, or speciation, of microbes (Vellend, 2010; Nemergut *et al*., 2013). Through a myriad of mechanisms, such as recombination, or horizontal gene transfer, microbes, especially

prokaryotes, can alter or exchange genetic elements and vary their mutation rates (Nemergut *et al*., 2013; Crits-Christoph *et al*., 2020; Chase *et al*., 2021). This allows components of microbial communities to rapidly diversify, or potentially speciate, and take advantage of changing biotic and abiotic conditions (Crits-Christoph *et al*., 2020).

Less emphasis, however, has been given to studying the impact of ecological drift, where stochastic, or random, effects impact community composition (Vellend, 2010; Nemergut *et al*., 2013). Typically, drift is thought to be more significant for small populations; for microbial populations, however, it remains unclear how small is small for drift to be important (Herrera Paredes & Lebeis, 2016). Drift may have a greater impact on founder populations, or priority effects, where the order and timing of arrival may determine community composition (Herrera Paredes & Lebeis, 2016; Debray *et al*., 2022). The impact of random ecological drift could be countered by efficient microbial dispersal (Herrera Paredes & Lebeis, 2016). For microbes, this tends to be largely passive over macro scales, as cells, or spores are moved through air, or water cycles, or by other organisms (Nemergut *et al*., 2013; Fitzpatrick *et al*., 2020; Ruuskanen *et al*., 2021; Amend *et al*., 2022). Moreover, some microbes may also be dispersed vertically across generations by being incorporated into gametes, such as seeds (Herrera Paredes & Lebeis, 2016; Shade *et al*., 2017).

Studying how drift, dispersal, selection and speciation, function may not only help describe how microbial communities are assembled, but it may also help explain and quantify some of the drivers of microbial biodiversity (Vellend, 2010; Nemergut *et al*., 2013; Thompson *et al*., 2017). Moreover, understanding how these mechanisms of community assembly function and interact also raises important questions concerning the tools and assumptions we use to identify microbial

communities. Finally, the specific context of a study will also help to shape how we analyze and understand a given microbial community.

Plant-Soil Microbial Communities

Soils are one of the largest biodiversity reservoirs on Earth (FAO *et al*., 2020), where they are home to a wide array of life, including viruses, prokaryotes, microbial eukaryotes, such as algae, oomycetes, and nematodes, fungi, and arthropods, all enmeshed in intricate interactions within a complex medium. In particular, microbes gather around the soils adjacent to root systems, or rhizosphere, to take advantage of the nutrient rich habitat the plants provide, as up to 25-40% of a plant's photosynthetically fixed-carbon is secreted out of the roots (Berendsen *et al*., 2012; Lakshmanan *et al*., 2014). This phenomenon of plants actively attracting micro-organisms from the surrounding bulk soil to the rhizosphere (Mendes *et al*., 2013; Yergeau *et al*., 2014; Vandenkoornhuyse *et a*l., 2015) is known as the rhizosphere effect (Berendsen *et al*., 2012; Yergeau *et al*., 2014; Vandenkoornhuyse *et a*l., 2015; Gkarmiri *et al*., 2017). Most prominently, the rhizosphere effect is seen by a change in microbial composition between the bulk soil and the rhizosphere, a decrease in diversity, and an increase in microbial abundance and activity (Yergeau *et al*., 2014; Lebeis *et al*., 2015). Plants invest substantial resources in rhizodeposition, not only in sugars, but also amino acids, phenols, and other secondary metabolites (Berendsen *et al*., 2012; Mendes *et al*., 2013; Lakshmanan *et al*., 2014; Yergeau *et al*., 2014), to carefully curate an assemblage of micro-organisms, that will in turn benefit them (Lakshmanan *et al*., 2014; Yergeau *et al*., 2014).

For example, Haney *et al*., (2015) and Lebeis *et al*., (2015) elegantly demonstrated how variation in *Arabidopsis thaliana* genotypes impacted the effect of colonizing bacteria, recruited from the surrounding soil. As different plant genotypes produced variations in the quantity and diversity of metabolites for rhizodeposition, these experiments illustrated how plant hosts make use of their rhizodeposition profile to order their bacterial rhizosphere and root microbiomes. Furthermore, these studies illustrate, first, that rhizosphere microbes are largely recruited from the surrounding bulk soil. Thus, the complete genetic potential of the microbiota is largely limited to what organisms are present in the nearby soil, as determined by its history (Azarbad *et al*., 2018; Hartman *et al*., 2018; Hannula *et al*., 2021) and abiotic factors (Vandenkoornhuyse *et al*., 2015). Second, the host plant's genotype is an important factor in structuring its microbial communities (Haney *et al*., 2015; Lebeis *et al*., 2015) and that the microbes are curated to perform specific functions, including increasing access to nutrients (Richardson *et al*., 2009; Weidner *et al*., 2015; Yu *et al*., 2021), temper environmental change (Lau & Lennon, 2012), or stress (Marasco *et al*., 2012; Hou *et al*., 2021), and protect against pathogens (Sikes *et al*., 2009; Mendes *et al*., 2011). Therefore, depending on which microbes are present in the nearby bulk soil can limit what functions, or genes, the host plant may have access to.

However, the evidence suggests that microbial recruitment from surrounding soils is rather more organized, as over the course of *Viridiplantae* evolution, from unicellular algae to the emergence of embryophytes on land ~450 million years ago, the lineage has been in constant contact with microbes (Remy *et al*., 1994; Redecker *et al*., 2000; Knack *et al*., 2015; Durán *et al*., 2022). Thus, over the course of time, plants and microbes have co-evolved, to the point that the presence, or absence, of microbes—or specific taxa—actually impacts the well-being of the plant;

individual plant productivity, as well as community composition, have been shown to vary based on the microbial assemblage (Bell *et al*., 2016; Klironomos, 2002; van der Heijden *et al*., 1998; van der Heijden *et al*., 2016). This has sparked discussion of plants and their associated microbes evolving as collective meta-organisms, holobionts, or hologenomes, where associated groups of genes co-occur and interact generation after generation (Zilber-Rosenberg & Rosenberg, 2008; Vandenkoornhuyse *et al*., 2015).

Two themes of experimental evidence that support the holobiont concept for plants and their associated microbes are, first, that similar bacteria are consistently found associated with plant hosts across the *Viridiplantae* linage (Yeoh *et al*., 2017; Alcaraz *et al*., 2018; Fitzpatrick *et al*., 2018; Duran *et al*., 2022), and second, that some microbes are inherited vertically via the seeds (Shade *et al*., 2017; Abdelfattah *et al*., 2021). Beginning with the 2012 descriptions of *A*. *thaliana* by Lundberg *et al*., and Bulgarelli *et al*., plant bacterial communities have been routinely found it be dominated by Proteobacteria, Bacteroidetes, and Actinobacteria. The same bacterial phyla were found again in the rhizosphere of *A*. *thaliana* by Lebeis *et al*., (2015)—Proteobacteria (~30% relative abundance), Bacteroidetes (~17%), Actinobacteria (~15%)—along with Acidobacteria (~17%) and Firmicutes (~5%). Lebeis *et al*., (2015) also found the predominant bacterial phyla in the roots to be Actinobacteria (~55%), Proteobacteria (~20%), Bacteroidetes (~10%), and Firmicutes (~10%), demonstrating a selective shift in the relative abundance of taxa between soil, rhizosphere and the interior of the root (Vandenkoornhuyse *et a*l., 2015). Furthermore, by comparing the root microbiomes of closely related *Brassicaceae A*. *thaliana* Shakdara, Landsberg, and Columbia, *A*. *lyrata*, *A*. *halleri*, and *Cardamine hirsute* Schlaeppi *et al*., (2014) found "a largely conserved and taxonomically narrow" set of microbes, again largely composed of

Proteobacteria, Bacteroidetes, and Actinobacteria. Other studies have continued to reaffirm the same bacterial phyla predominate across the *Viridiplantae* lineage, from algae to angiosperms (Yeoh *et al*., 2017; Alcaraz *et al*., 2018; Fitzpatrick *et al*., 2018; Durán *et al*., 2022).

One interpretation of the re-occurring bacterial taxa found among plants is that these taxa are perennially present in the environment, such that each generation of plant host can recruit them anew via horizontal transmission. However, evidence is accruing that plants also inherit microbes vertically via the seeds (Shade *et al*., 2017; Abdelfattah *et al*., 2021). By co-ordinating the vertical transmission of their genetic material plants and portions of their associated microbiomes ensure that the same organisms, and their genes, co-occur as a unit across generations. This has been experimentally demonstrated for bacteria, fungi and oomycetes (Saikkonen *et al*., 2002; Rodriguez *et al*., 2009; Ploch & Thines, 2011; Walters *et al*., 2018; Abdelfattah *et al*., 2021; Abdelfattah *et al*., 2022) and is strong evidence for the plant-microbial holobiont concept and understanding how these communities are formed.

Spatial, Temporal & Phylogenetic Scales in Microbial Communities

An underlying issue in plant-soil microbial communities, and microbial ecology generally, is the complexity associated with each mechanism of community assembly, as they all interact and are all compounded by spatial, temporal, and phylogenetic scales, i.e., each process will act differently according to distances, time and evolutionary position (Gonzalez *et al*., 2012; Ladau & Eloe-Fadsh, 2019). This generates a vast heterogeneity among how each process may function, as well as what the relative strength, or importance, of each community assembly processes may be. For example, abiotic selection pressures, or environmental filtering, can exist in soils between

scales of kilometers to micrometers, while biotic interactions tend to be limited to local scales (Ladau & Eloe-Fadsh, 2019). Microbial dispersal seems unlimited, yet soil communities that are millimeters apart appear different and are often described as patchy (Nemergut *et al*., 2013; Ramirez *et al*., 2017; Ruuskanen *et al*., 2021). Thus, as these examples illustrate, the fundamental task of ecology of determining community composition at a specific time and place (Vellend, 2010) is rendered more challenging by our incomplete understanding of how each mechanism involved in structuring plant-soil microbial communities is warped, or altered, depending on spatial, temporal, and phylogenetic scales (Ladau & Eloe-Fadsh, 2019).

One reason for microbial ecologists being fuzzy on how scales impact community composition is the disconnect, or bias between the scales microbes inhabit, and those of the researchers. Such a bias in perspective is compounded by the weight of research experience on plant ecology, from which microbial ecologists borrow heavily (Vellend, 2010; Revillini *et al*., 2016; Shade *et al*., 2018; Shade & Stopnisek, 2019). This bias in perspective has the potential to add a number of artefacts into analyzing plant-soil microbial communities. For example, experimental soil, or plant tissue, samples are visibly harvested—at the scale of centimeters, grams, or milliliters— even though the microbes of interest may inhabit only a fraction of those scales. Soils, for instance, are highly structured and form a lattice of potentially unique environments at different scales. This heterogeneity of soil environments has been proposed as a leading factor for the hyper-diversity observed in soil microbial communities (Upton *et al*., 2019). Unfortunately, soils are often sampled at coarse spatial scales and homogenized, or pooled (Allen *et al*., 2021; Fleishman *et al*., 2022), which may reduce the specificity, or grain, of the study, and

consequently artificially increase the diversity reported (Nemergut *et al*., 2011; Ladau & Eloe-Fadsh, 2019).

Phylogenetic uncertainty among many soil microbes can also conflate community analysis and reporting. That many microbes have poorly established species concepts is a perennial example (Cordero & Polz, 2014; Shapiro & Polz, 2014; Jain *et al*., 2018; Chase *et al*., 2021). This uncertainty has caused considerable ink to be spilled in the literature over, first, how microbes ought to be identified by blurring how community members may be counted—by binning or splitting (Callahan *et al*., 2017; Schloss, 2021). This can create difficulties or artifacts when reporting what microbes have been identified, and is further complicated by reporting disparate or uneven taxonomic levels, or conflating the vast evolutionary distances among microbes (Parks *et al*., 2018; Martiny *et al*., 2023).

Interacting with the phylogenetic and spatial scales are the diverse temporal scales microbes inhabit. For instance, many soil micro-organisms have access to various mechanisms that enable them to gain new functions within a generation through horizontal gene transfer (Tenaillon *et al*., 2012, Chase *et al*., 2021). Moreover, microbes have variable lengths to their life cycles, completing them within days to decades depending on environmental conditions (Shade *et al*., 2013a; Fuhrman *et al*., 2015). These examples can also confound reporting on microbial communities, depending on which organisms may be active, or dormant. These examples highlight some of the biases that occur by not recognizing how temporal scales can confound which organisms are actually determined to be present in a community at a specific time, and who gets counted, when and how (Lozupone & Knight, 2008; Hannula *et al*. 2019; Chase *et al*., 2021)?

Although incorporating different time scales into plant-soil microbial ecology has often been suggested (Shade *et al*., 2013b; Chaparro *et al*., 2014), methodological complexities have tended to dissuade including temporal dimensions (Shade & Stopnisek, 2019). However, with growing recognition of how important different time scales are for plant microbial communities, and with improving methods for incorporating temporal data (Shade & Stopnisek, 2019), omitting these aspects is becoming less justified. As such, new avenues of microbial temporal properties are being explored, such as microbial memory, legacy, and priority, effects (Kaisermann *et al*., 2017; Hannula *et al*. 2019; Hannula *et al*. 2021; Debray *et al*., 2022; Vermeersch *et al*., 2022). These concepts describe how previous microbial communities impact future assemblages.

Plant-Soil Feedback & Soil History

Perhaps one of the least understood contexts of how past events structure future microbial generations is in plant-soil microbial communities, as they are highly dynamic, not easily accessed or observed, and sensitive to multiple temporal scales. This is due to the reciprocal plant-soil microbial community feedback (PSF) process, where plant hosts and their associated microbial communities engage in an on-going dialogue. The existing state of the local soil environment is signalled via the extant soil microbial communities, which provide a variety of ecological roles that impact the local chemistry (Duchicela *et al*., 2013; Graf & Frei, 2013; Talbot *et al*., 2013). For their part, plant hosts continually communicate their above and below ground status to the rhizosphere through rhizodeposition, as previously discussed (Berendsen *et al*., 2012; Mendes *et al*., 2013; Lakshmanan *et al*., 2014; Yergeau *et al*., 2014; Lebeis *et al*., 2015; Korenblum *et al*., 2020; Kawasaki *et al*., 2021; Yu *et al*., 2021). Thus, through the continuing dialogue of PSF the

environmental and chemical context of the soil will be incorporated and reflected in the composition of the microbial root and rhizosphere communities (Bever *et al*., 2010; van der Putten *et al*., 2013; Revillini *et al*., 2016).

Clearly, the temporal scale of PSF will have a role in its outcome. For example, classic "home and away" studies illustrate how exchanging the established "home", or local soil, of two plant host species mid-season, or for a growing season, can negatively or positively impact the growth of the individual plants (van der Putten *et al*., 2013; Kong *et al*., 2019; Fitzpatrick *et al*., 2018: Hannula *et al*., 2021). These experiments suggest that some plant-microbial communities fair better or worse depending on the soil biotic and abiotic context, or environmental filter, such that the misalignment of the plant host and its microbial communities can be suboptimal. Furthermore, "home and away" experiments demonstrate that the rate of PSF may vary, as certain plant holobionts may re-establish their optimal soil biotic and abiotic environments more readily (van der Putten *et al*., 2013; Fitzpatrick *et al*., 2018). Nonetheless, over longer-term experiments, such as during succession or colonization events, plant hosts and their microbial communities can adapt over multiple seasons to new soil environments without penalty (Van Nuland *et al*., 2019; Ware *et al*., 2019). This further illustrates the importance of time in PSF.

More recently this interaction of temporality and PSF has come under scrutiny, where a growing body of evidence demonstrates that PSF shapes future plant-soil microbial communities (Kaisermann *et al*., 2017; Berendsen *et al*., 2018; Fitzpatrick *et al*., 2018). This suggests that the environmental filter established by PSF from a plant-soil microbial community is transmitted through time to structure future generations of the plant holobiont. This environmental filter, or context that will condition future plant-soil microbial communities is referred to as soil history, or

soil legacy (Kaisermann *et al*., 2017; Bakker *et al*., 2021; Hannula *et al*., 2021). However, studying, or predicting the impact of soil history has proven challenging, in part due to the microbial variation involved (De Long *et al*., 2019).

The soil history established by closely related plant hosts may fluctuate according to the variation among the plant host's rhizodeposition, as discussed (Van Nuland *et al*., 2019), but it may also differ based on the initial microbial load and composition (Van Nuland *et al*., 2016). Given the large diversity of soil microbes, their community structure and interactions will shape am important biotic component of PSF, and the subsequent soil history that is established (van der Putten *et al*., 2013; Van Nuland *et al*., 2016). For example, fungi are widespread soil microbes with a range of impacts on PSF (Sheng *et al*., 2022; Xi *et al*., 2022). Increased mycorrhizal fungi and saprotrophs tend to produce more positive PSF by liberating nutrients from organic matter, and then increasing the plant host's nutrient acquisition, while fungal pathogens may have positive or negative effects on PSF (van der Putten *et al*., 2013; Bennett & Klironomos, 2019; Xi *et al*., 2022). However, mycorrhiza can also have variable roles in PSF, depending on other factors involved in the feedback process, including soil chemistry, or due to yet other microbes (Hart & Reader, 2002; Maherali & Klironomos 2007; Xi *et al*., 2022).

The multi-layer interactions of soil microbial denizens, including fungi, oomycetes and bacteria, among others, may all vary the biotic component of PSF. Fungi are well known to host their own microbiota, as well as interact with other soil organisms, all of which can influence the activities of the soil microbiome, and resulting PSF (Bian *et al*., 2020; Nguyen, 2023). Fungi may also leak nutrients around their hyphae, which can generate fungal host-specific bacterial communities, analogous to how plants attract microbes to their root systems (Revillini *et al*., 2016;

Nguyen, 2023). Similarly, bacteria have also been shown to colonize the endophytic spaces of fungi, where even bacterial phytopathogens can benefit from the habitat and dispersal (Venkatesh *et al*., 2022). Conversely, fungal-bacterial antagonisms in the soil are also well documented, particularly among bacterial taxa typically associated with plants, such as *Bacillus* and *Pseudomonas* (Ansari & Ahmad, 2019; Dahlstrom & Newman, 2022: Geisen *et al*., 2022; Hansen *et al*., 2022; Hong *et al*., 2023). A high diversity of *Pseudomonas* strains has also been shown to maintain a *Pythium* disease suppressive soil through the production of anti-oomycete compounds (Oni *et al*., 2020).

Though canonically less studied than bacteria and fungi, oomycetes are also important members of soil communities, especially given their outsized impact as phytopathogens (Kamoun *et al*., 2015), and a tendency to linger in soils (Martin & Loper, 1999; Fernández-Pavía *et al*., 2004; Kikway *et al*., 2022; Subila & Suseela, 2022). Furthermore, beginning with Ploch and Thines (2011), a number of studies have shown that oomycetes are also widespread plant endophytes, particularly among the *Brassicaceae* plant family (Sapp *et al*., 2018; Macía-Vicente *et al*., 2020). However, there is considerably less known about oomycete soil communities, and the diversity of their interactions with other microbes. Nonetheless, in briefly discussing some of these soil microbial groups and their interactions it underscores a fraction of their functional diversity. This demonstrates the complexity that is incorporated into the biotic fraction of PSF and highlights an important source of the variation in the resulting soil history.

Perhaps unsurprisingly, given this complexity, to date there have been few experiments that aim to study or predict the impact of soil microbes on establishing soil history, nor on the impact of soil history on future microbial communities (De Long *et al*., 2019). Rather, the focus

in this domain has been on examining strategies and responses of plant communities to PSF and soil history, including their growth and nutrient acquisition strategies, functional groups, or phylogenetic relatedness (Jongen *et al*., 2021; Chung, 2023; Rutten & Allan, 2023). From these different plant strategies, perhaps changes to microbial communities could be deduced, such as declines in specific pathogens, or promoting decomposers, or more copiotrophic vs oligotrophic bacteria (Jongen *et al*., 2021). However, an alternate strategy could also be "belowground-up", where exploring how soil history impacts microbial community structure will better inform us to the plant host's needs (Bennett & Klironomos, 2018; Li *et al*., 2019). Therefore, studying the plant holobiont as a whole may help tease apart how soil history established by PSF will alter the structure of the associated microbial communities. As such, a first step toward this is would be to identify which microbes may be present after different soil history scenarios.

Structure of the Thesis

In order to predict their functions, and better engineer microbial communities as technologies, there is an urgent need to better understand how bacterial communities assemble and are structured through time. In no context is this need clearer than for soil microbial communities, as they play a complex—though less studied—part in PSF and in the subsequent soil history that is established. Moreover, as soil histories impact future microbial communities (Kaisermann *et al*., 2017; Bakker *et al*., 2021; Hannula *et al*., 2021), it is relevant to investigate how we currently treat soils to better understand how microbial communities may be affected and establish baseline data on how microbial communities change through time (Chung, 2022; Geller & Levy, 2023). As such, my thesis addresses the lack of experiments, particularly field experiments (Revillini *et al*.,

2016), that aim to study and predict the impact of soil history on future microbial communities (De Long *et al*., 2019). Following the "belowground-up" strategy, I used amplicon sequencing to identify the composition of the bacterial and oomycete communities from different soil histories, with the aim to identify how changes among these communities may influence their *Brassicaceae* host plants (Bennett & Klironomos, 2018; Li *et al*., 2019).

My thesis is composed of three chapters, presented as stand-alone scientific articles, that revolve around the theme of how soil history structures future microbial communities in the soil. **Chapter 1** addresses how bacterial communities with different soil histories adapt to new plant hosts. I found that the communities converged toward similar bacterial compositions in response to new *Brassicaceae* plant hosts, regardless of their different soil histories. However, this plant-induced adaptation broke down if the new host was water-stressed, such that the previous soil histories continued to drive the bacterial community structure for up to a year afterward. **Chapter 2** identified a clear influence of soil history on the structure of oomycete plant pathogens the following year, regardless of the presence of a new *Brassicaceae* plant host. This study highlights a number of avenues for future research to explore regarding how to monitor and control this understudied group of pathogens. **Chapter 3** then considers the interaction of different soil histories and plant growth stages on the development of the soil bacterial communities. This experiment showed that the plant hosts rapidly mask the previously established soil history upon germinating and derive common rhizosphere communities from the surrounding bulk soil. I then present a general conclusion on my work, its limits, and perspectives on how soil history may inform microbial community assembly more broadly, as discussed above, and how future studies may be improved based on these experiments.

Chapter 1: Host plants can “re-write” soil history for bacterial communities

In order for the soil history from a previous plant–soil microbial community to condition future plant generations and the composition of their bacterial communities (Kaisermann *et al*., 2017; Berendsen *et al*., 2018; Fitzpatrick *et al*., 2018), information must be transmitted through time to impact subsequent plant–microbial generations. One set of mechanisms thought to accomplish this transmission is through plant-soil microbial community feedback (PSF), such that the biotic and abiotic context of the soil will be incorporated and reflected in the composition of the bacterial root and rhizosphere communities. However, it remains unclear what the effect of different soil histories may be, or how long they can continue to influence future plant-microbial communities. Therefore, **Chapter 1** tested the hypothesis that different soil histories established the previous year by PSF would continue to structure bacterial root and rhizosphere communities hosted by *Brassicaceae* crop plants the subsequent year.

To test the soil history hypothesis, I used an existing crop rotation field experiment as a model for how a previous PSF establishes soil history that may impact the biotic and abiotic soil conditions of a future plant-bacterial community (Yang *et al*., 2021; Liu et al., 2022). Thus, three different soil histories (fallow, lentil, wheat) were established throughout a growing season, and the following year each soil history was divided into five subplots and planted with a different *Brassicaceae* plant host. I examined the taxonomic composition and diversity of bacterial communities from the root and rhizosphere of the five different *Brassicaceae* hosts, as estimated

by amplicon sequence variants (ASVs), to identify any changes associated with soil history on the various bacterial communities.

Chapter 2: Soil history drives oomycete community structure

Crop rotations in agricultural fields are an example of applying PSF to establish beneficial soil histories (Hwang *et al*., 2015; Yang *et al*., 2021; Liu *et al*., 2022). Though extensive work has investigated how *Brassicaceae* oilseed rotations benefit the crop plants involved (recently reviewed by Yang *et al*., 2021), the influence of *Brassicaceae* crops on the biodiversity of soil oomycete phytopathogens remains unknown (Maciá-Vicente *et al*., 2020). Therefore, **Chapter 2** investigate this knowledge gap, where I hypothesized that the three soil histories established by the previous crops would structure different oomycete communities, regardless of their current *Brassicaceae* host, in both the roots and rhizosphere.

I used the same crop rotation field experiment, described briefly for Chapter 1, to model how previously established soil histories may structure future soil oomycete biodiversity (Yang et al., 2021; Liu et al., 2022). The three different soil histories (fallow, lentil, wheat) were established throughout a growing season, along with their agricultural inputs, while the following year each soil history was divided into five subplots and planted with different *Brassicaceae* plant hosts. I examined the biodiversity of the oomycete communities inferred as ASVs to identify any changes associated with soil history on the various *Brassicaceae* soil oomycete communities.

Chapter 3: Bacterial communities are structured by soil across plant growth stages

The previous experiment found that mature adult host plants "re-wrote", or masked different soil histories (Blakney *et al*., 2022). However, this does not illustrate how soil history influences the structure of bacterial communities across different growth stages of the host plant. Therefore, **Chapter 3** hypothesized that previously established soil histories would decrease in influence throughout the growing season of *Brassica napus*.

To test the "declining soil history hypothesis", I used another agricultural field experiment where soil histories were established by different crop rotations; monocrop canola (*B*. *napus*), or wheat-canola, or pea-barley-canola rotations (Harker *et al*., 2015). During the canola Test Phase, we repeatedly sampled the surrounding bulk soil, rhizosphere and roots of *B*. *napus* at different growth stages— the initial seeding conditions, seedling, rosette, bolting, and flower—from all three soil history plots. I compared the taxonomic composition and diversity of bacterial communities, as estimated using 16S rRNA metabarcoding, to identify any changes associated with soil history and growth stages on the different *B. napus* soil bacterial communities.

Chapter 1: *Brassicaceae* host plants mask the feedback from the previous year's soil history on bacterial communities, except when they experience drought

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Keywords: *Brassicaceae*, bacterial communities, soil legacy, plant-soil feedbacks, drought

Published in: *Environmental Microbiology*

DOI: https://doi.org/10.1111/1462-2920.16046

Abstract

Soil history operates through time to influence the structure and biodiversity of soil bacterial communities. Examining how different soil histories endure will help clarify the rules of bacterial community assembly. In this study, we established three different soil histories in field trials; the following year these plots were planted with five different *Brassicaceae* species. We hypothesized that the previously established soil histories would continue to structure the subsequent *Brassicaceae* bacterial root and rhizosphere communities. We used a MiSeq 16S rRNA metabarcoding strategy to determine the impact of the different soil histories on the structure and biodiversity of the bacterial root and rhizosphere communities from the five different *Brassicaceae* host plants. We found that the *Brassicaceae* hosts were consistently significant factors in structuring the bacterial communities. Four host plants (*Sinapis alba*, *Brassica napus*, *B. juncea*, *B. carinata*) formed similar bacterial communities, regardless of different soil histories. *Camelina sativa* host plants structured phylogenetically distinct bacterial communities compared to the other hosts, particularly in their roots. Soil history established the previous year was only a significant factor for bacterial community structure when the feedback of the *Brassicaceae* host plants was weakened, potentially due to limited soil moisture during a dry year. Understanding how soil history is involved in the structure and biodiversity of bacterial communities through time is a limitation in microbial ecology and is required for employing microbiome technologies in improving agricultural systems.

Introduction

Soil history is an understudied aspect involved in structuring soil microbial communities through time (Fitzpatrick *et al*., 2018; Hannula *et al*., 2021). Understanding how past events in soil history continue to shape the plant-soil microbial community is paramount for maintaining global biodiversity, agricultural productivity, and climate management (Albright *et al*., 2021). Without this knowledge, plant microbial communities cannot be predicted, design, or deployed for future needs (Albright *et al*., 2021, Busby *et al*., 2017, Zengler *et al*., 2019). As such, a clear barrier to successfully developing bacterial-microbiome technologies is an understanding of the role of soil history and time in structuring soil bacterial communities (Albright *et al*., 2021, Busby *et al*., 2017).

The ecological function and biological stability, of soils are regulated by microbial communities (Griffiths & Philippot, 2013). Soil microbes aggregate, structure, and stabilize soils (Duchicela *et al*., 2013; Graf & Frei, 2013), as well as cycle water and nutrients through the biosphere (Talbot *et al*., 2013). For plants, soil microbes increase access to nutrients (Richardson *et al*., 2009; Weidner *et al*., 2015; Yu *et al*., 2021), temper environmental change (Lau & Lennon, 2012), or stress (Marasco *et al*., 2012; Hou *et al*., 2021), and protect against pathogens (Mendes *et al*., 2011; Sikes *et al*., 2009). Soil bacterial communities help integrate these diverse signals and modulate the plant's responses (Castrillo *et al*., 2017; Hou *et al*., 2021).

In turn, plant hosts contribute to above-ground-below-ground communication by adjusting the environmental filter imposed on the surrounding soil chemistry. The plant host alters the soil chemistry through two concurrent processes; first, the host plant's own growth, development, and homeostasis is determined by its capacity to uptake nutrients from the soil, which will adjust the

soil chemistry (Hu *et al*., 2021). Second, through rhizodeposition the host plant can vary the quantity and array of compounds released into the rhizosphere as required, thereby changing its soil chemistry (Lebeis *et al*., 2015; Korenblum *et al*., 2020; Kawasaki *et al*., 2021; Yu *et al*., 2021). Modifying their rhizodeposition profile allows plants to tailor the structure of their bacterial rhizosphere community in response to variable conditions and the plant's needs (Lebeis *et al*., 2015; Korenblum *et al*., 2020; Kawasaki *et al*., 2021; Yu *et al*., 2021).

This plant-soil community, above-ground-below-ground communication generates a reciprocal feedback process (Hou *et al*., 2021). Through plant-soil community feedbacks (PSF) the environmental and chemical context of the soil will be incorporated and reflected in the composition of the bacterial root and rhizosphere communities. Thus, PSF contributes to the dynamic environmental filtering that determines how specific bacteria come to inhabit a given site (Bever *et al*., 2010; van der Putten *et al*., 2013; Revillini *et al*., 2016). Accordingly, given the reciprocal feedback, soil bacterial communities have been identified as well-established drivers of plant growth and development, and plant community assembly (Fig. 1A; Berendsen *et al*., 2012; Hannula *et al*., 2021; Yu *et al*., 2021).

Furthermore, there is evidence that current PSF impacts future plant generations and the composition of their soil bacterial communities (Kaisermann *et al*., 2017; Berendsen *et al*., 2018; Fitzpatrick *et al*., 2018). This implies that information from PSF in one plant-soil community is transmitted through time to impact subsequent plant-microbial generations, i.e., that the soil history, also referred to as soil legacy, of previous plant-soil communities condition future ones (Fig. 1A; Kaisermann *et al*., 2017; Bakker *et al*., 2021; Hannula *et al*., 2021). How long soil history lasts, or the variation in length, or impact between different soil histories on future plant-microbial

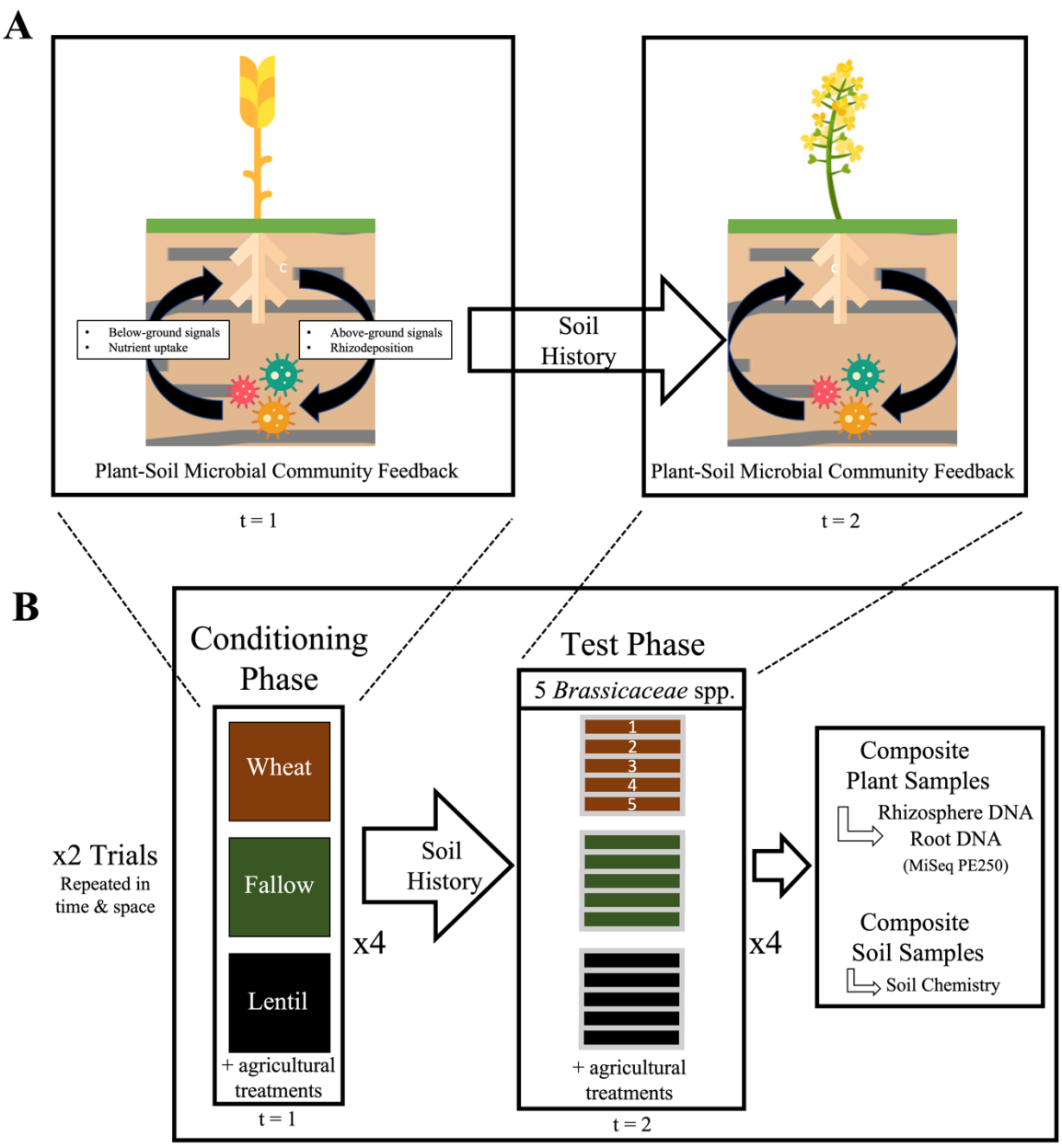

A
Below-ground signals
Nutrient uptake
Above-ground signals
Rhizodeposition
Plant-Soil Microbial Community Feedback
t = 1
Soil History
Plant-Soil Microbial Community Feedback
t = 2
B
x2 Trials
Repeated in time & space
Conditioning Phase
Wheat
Fallow
Lentil
+ agricultural treatments
t = 1
x4
Soil History
Test Phase
5 Brassicaceae spp.
1
2
3
4
5
+ agricultural treatments
t = 2
x4
Composite Plant Samples
Rhizosphere DNA
Root DNA
(MiSeq PE250)
Composite Soil Samples
Soil Chemistry

Figure 1. Conceptual design of the experiment. (A) Plant-soil microbial community, above-ground-below-ground communication generates a reciprocal feedback process, where plants use rhizosdeposition to tailor the microbial community to their needs. In turn, the microbial community services the plant host, including by increasing nutrient access. The soil history established by one plant-soil microbial community (t =1) is then projected through time to impact subsequent plant-microbial generations (t =2). (B) Two field trials were set-up on adjacent sites in a split-plot design replicated in four complete blocks. Three soil histories were established in the Conditioning Phase (t =1) of a two-phase cropping sequence, consisting of wheat, lentil, or left fallow (brown, black, green, respectively), plus their standard agricultural management practices and inputs. The following year (t =2), during the Test Phase, the conditioned plots were each subdivided and five *Brassicaceae* oilseed crop species were randomly assigned to one of these five subplots. At full flower, the plants were harvested and divided into root and rhizosphere components for 16S rRNA metabarcoding by Illumina MiSeq PE250 (paired-end 250 bp).

communities is unclear. Moreover, it has been suggested that a weakness in studying PSF and the soil history they establish, has been their lack of field experiments (Revillini *et al*., 2016).

Agricultural systems provide ready-made field opportunities to investigate how soil history established a previous year continues to impact bacterial communities in subsequent years. Crop rotations and their agricultural inputs model how a previous PSF establishes a soil history that may impact the biotic and abiotic soil conditions for a future plant-bacterial community (Yang *et al*., 2021; Liu *et al*., 2022). When lentils, or other legumes, are introduced into a rotation the subsequent crops tend to have higher yields (O'Donovon *et al*., 2014; Hamel *et al*., 2018). Leguminous plants cause a shift in the soil microbial community, which can include decreasing potential pathogens (Bazghaleh *et al*., 2016; Yang *et al*., 2021). Evidence suggests that the resulting PSF among the lentil-soil community establishes more bioavailable nitrogen and moisture in the soil, which benefits the subsequent crop (O'Donovon *et al*., 2014; Hamel *et al*., 2018; Yang *et al*., 2021). Canola rotations also establish beneficial soil histories, as they reduce the growth of cereal-specific pathogens. As such, cereals tend to have higher yields when they are planted after canola (Etesami & Alikhani, 2016; Yang *et al*., 2021). Extensive work has investigated how crop rotations benefit the plants involved (recently reviewed by Yang *et al*., 2021). However, less is known concerning how crop rotations and their agricultural inputs establish soil history and subsequently impact future soil bacterial communities.

Recent work on canola (*Brassica napus* L. or *B*. *juncea* L.) bacterial communities has begun to correct this knowledge deficit (Lay *et al*., 2018; Floch *et al*., 2020; Taye *et al*., 2020; Wang *et al*., 2020; Morales Moreira *et al*., 2021). *Brassicaceae* oilseed-based rotations are common throughout the world, as demand for vegetable oil and biofuels increase (Yang *et al*.,

2021). Expanding the diversity of *Brassicaceae* oilseed species has been on-going in order to improve production by identifying varieties resistant to pathogens, and better adapted to the heat and drought stress of the Canadian Prairies (Bailey-Serres *et al*., 2019; Hossain *et al*., 2019; Liu *et al*., 2019). Studying closely related *Brassicaceae* species will help illustrate how bacterial communities may be changed through plant breeding, as well as the fine-tuning of bacterial community structures in response to different soil histories (Bailey-Serres *et al*., 2019).

To study the impact of previously established soil history on the biodiversity and structure of soil bacterial communities, we took advantage of an existing agricultural field experiment. Three different soil histories (fallow, lentil, wheat) were established; the following year each soil history was divided into five subplots and planted with a different *Brassicaceae* oilseed species. This design allowed us to test the hypothesis that soil histories established the previous year by PSF—i.e. lentil and wheat, along with their respective microbial communities and agricultural treatments—would continue to structure the subsequent *Brassicaceae* bacterial root and rhizosphere communities. As such, we predict i) if the fallow soil history does not influence the five *Brassicaceae* soil bacterial communities, then each community should appear distinct, ii) if the lentil and wheat soil histories continue to structure the *Brassicaceae* bacterial communities, then these communities should reflect their soil histories and not the variation of the five *Brassicaceae* host plants. To test the soil history hypothesis, we examined the taxonomic composition and diversity of bacterial communities from the root and rhizosphere, as estimated by amplicon sequence variants (ASVs), to identify any changes associated with soil history on the various *Brassicaceae* soil bacterial communities.

Materials & Methods

Site and experimental design

A field experiment was conducted at the experimental farm of Agriculture and Agri-Food Canada's Research and Development Centre, in Swift Current, Saskatchewan (50°15′N, 107°43′W). The site is located in the semi-arid region of the Canadian Prairies; according to the weather station at the research farm, the 2016 and 2017 growing seasons (May, June and July) had 328.4 mm and 55.0 mm of precipitation, respectively; compared to the 30-year average [1981-2010] of 169.2 mm. The daily temperature averages for the 2016 and 2017 seasons were 15.6°C and 15.9°C, respectively, while the 30-year average was 14.93°C. The farm is on a Brown Chernozem with a silty loam texture (46% sand, 32% silt, and 22% clay), and has been well-described previously (see Liu *et al*., 2019 & 2020).

The experiment was established in a field previously growing spring wheat (*Triticum aestivum* cultivar AC Lillian). A two-phase cropping sequence—consisting of a Conditioning Phase the first year, and a Test Phase in the second year (Fig. 1)—was repeated in two field trials, Trial 1, 2015-2016, and Trial 2, 2016-2017, on adjacent sites (Fig. S1A & B). On each site, the experimental design was a split-plot replicated in four complete blocks. In the Conditioning Phase, three soil history treatments were randomly assigned to the main plots, consisting of spring wheat (*Triticum aestivum*, cv. AC Lillian), red lentil (*Lens culinaris* cv. CDC Maxim CL), or left fallow (Fig. 1 & S1). Thus, the Conditioning Phase established a soil history composed of either wheat, lentil, or fallow, plus their respective management plans as described below (Hossain *et al*., 2019; Liu *et al*., 2019).

In the Test Phase, the 12 Conditioning Phase plots were each subdivided and five *Brassicaceae* oilseed crop species were randomly assigned to one of these five subplots (Fig. 1 & S1). The *Brassicaceae* crops seeded were Ethiopian mustard (*Brassica carinata* L., cv. ACC110), canola (*B. napus* L., cv. L252LL), oriental mustard (*B. juncea* L., cv. Cutlass), yellow mustard (*Sinapis alba* L., cv. Andante), and camelia (*Camelina sativa* L., cv. Midas). The Test Phase established the *Brassicaceae* host PSF, composed of the individual *Brassicaceae* genotypes, their soil bacterial community, and their respective management plans, as described below (Hossain *et al*., 2019; Liu *et al*., 2019). In total, each field trial had 60 subplots to sample (Fig. S1 & S2). For further details of this well-described experiment, its design, and treatments, see Hossain *et al*. (2019), Liu *et al*. (2019), and Wang *et al*. (2020).

Crop management and sampling

Crops in both field trials were grown and maintained according to standard management practices, as previously described by Hossain *et al*. (2019), Liu *et al*. (2019), and Wang *et al*. (2020). A pre-seed 'burn off' herbicide treatment using glyphosate (Roundup, 900 g acid equivalent per hectare, a. e. ha^{-1}) was applied to all plots each year to ensure a clean starting field prior to seeding. Lentil seeds were treated with a commercial rhizobium-based inoculant (TagTeam at 3.7 kg ha^{-1}), which contains the phosphate-solubilizing fungi *Penicillium bilaiae*, and the lentil-nodule partner *Rhizobium leguminosarum*. Lentil and wheat were direct-seeded into wheat stubble from late April to mid-May depending on the crop and year. The herbicides glyphosate (Roundup, 900 g a. e. ha^{-1}), Assure II (36 g active ingredient per hectare, a. i. ha^{-1}), and Buctril M (560 g a.i. ha^{-1}) were applied to the fallow, lentil, and wheat plots, respectively, for

in-season weed control, while fungicides were only applied as needed. Soil tests were used to determine the rates of in-season nitrogen, phosphorus, and potassium application; no synthetic nitrogen fertilizer was applied to the lentil plots during the Conditioning Phase. Both lentil and wheat were harvested between late August and early October, depending on the crop and year.

The subsequent Test Phase *Brassicaceae* plant hosts were subjected to the same standard management practices as the Conditioning Phase, including pre-seed 'burn off', in-season herbicide and fungicide treatments as needed, and fertilized as recommended by soil tests (Table S4; Hossain *et al*. 2019, Liu *et al*. 2019, and Wang *et al*. 2020). Additionally, all *Brassicaceae* crops, except *B*. *napus*, were treated with Assure II mixed with Sure-Mix or Merge surfactant (0.5% v/v) for post-emergence grass control: Liberty (glufosinate, 593 g a.i. ha−1) was used for *B*. *napus*. We accounted for the use of the various agricultural treatments in the downstream amplicon data by considering each plant sample and their total complement of particular agricultural treatments as a unit.

Test phase *Brassicaceae* plants were sampled in mid-late July at full flowering, i.e. when 50% of the flowers on the main raceme were in bloom, as described by the Canola Council of Canada (Canola Encyclopedia: Canola Growth Stages, 2017), where flowering corresponds to higher activity in rhizosphere microbial communities (Chaparro *et al*., 2014). Four plants from two different locations within each subplot were excavated and pooled together as a composite sample (Hossain *et al*., 2019; Liu *et al*., 2019, Wang *et al*., 2020). In the field, each plant had its rhizosphere soil divided from the root material by gently scraping it off using bleach sterilized utensils into fresh collection trays. The roots were then gently washed three times with sterilized distilled water to remove any soil. Both the rhizosphere and root portions were immediately flash-

frozen and stored in liquid nitrogen vapour shipping containers until stored in the lab at -80°C (Delavaux *et al*., 2020). Based on the sampling strategy, in this study we define the rhizosphere microbiome as the microbial community in the soil in close contact with the roots (Hannula *et al*., 2021), and the root microbiome as the microbial community attached to, and within, the roots (Berendsen *et al*., 2018). Two additional soil cores were sampled from each plot, pooled, and kept on ice in coolers. These samples were homogenized in the lab, and sieved to remove rocks and roots. They were then used for soil chemistry analyses, including total carbon, nitrogen, pH, and micronutrients (see Wang *et al*., 2020 for details). Aerial portions of each harvested plant sample were retained to determine dry weight (Fig. S3).

DNA extraction from Test Phase *Brassicaceae* root and rhizosphere samples

Nucleic acids were extracted from Trial 1 Test Phase *Brassicaceae* samples, both rhizosphere and root portions. First, all the root samples were ground in liquid nitrogen via sterile mortar and pestles (Fig. S2). Total DNA and RNA were extracted from ~1.5 g of rhizosphere soil using the RNA PowerSoil Kit with the DNA elution kit (Qiagen, Germany). DNA and RNA were extracted using ~0.03 g of roots using the DNeasy Plant DNA Extraction Kit, and RNeasy Plant Mini Kit (Qiagen, Canada), respectively, following the manufacturer's instructions (see Wang *et al*. (2020) for use of the RNA samples). All remaining harvested material from Trial 1 and 2, as well as the extracted DNA from Trial 1, were kept at -80°C before being shipped to Université de Montréal's Biodiversity Centre, Montréal (QC, Canada) on dry ice for further processing (Delavaux *et al*., 2020; Lay *et al*., 2018).

Total DNA was extracted from the Trial 2 Test Phase samples; ~500 mg of rhizosphere soil was used for the NucleoSpin Soil gDNA Extraction Kit (Macherey-Nagel, Germany), and ~130 mg of roots was used for the DNeasy Plant DNA Extraction Kit (Qiagen, Germany) (Lay *et al*., 2018). A no-template extraction negative control was used with both the root and rhizosphere extractions and included with the Test Phase samples (Fig. S2), to assess the influence of the extraction kits on our sequencing results, and the efficacy of our lab preparation. All 242 extracted DNA samples (60 plots x 2 parallel field trials x 2 compartments, rhizosphere and root, +2 no-template extraction control samples) were quantified using the Qubit dsDNA High Sensitivity Kit (Invitrogen, USA), and qualitatively evaluated by mixing ~2 µL of each sample with 1 µL of GelRed (Biotium), and running it on a 0.7 % agarose gel for 30 minutes at 150 V. The no-template extraction negative controls were confirmed to not contain DNA after extraction, where the detection limit was > 0.1 ng (Qubit, Invitrogen, USA). Samples were kept at -80°C (Bell *et al*., 2016; Delavaux *et al*., 2020).

16S rRNA gene amplicon generation and sequencing to estimate the bacterial community

To estimate the composition of the bacterial communities in the rhizosphere and roots from the Test Phase *Brassicaceae* species, extracted DNA from all samples were used to prepare 16S rRNA gene amplicon libraries following Illumina's MiSeq protocols. First, all DNA samples were diluted 1:10 into 96-well plates using the Freedom EVO100 robot (Tecan, Switzerland). To assess potential bias caused by lab manipulations, sequencing and downstream bioinformatic processing, a commercially available 16S rRNA mock community, of known composition (Table S1), was included on each plate (Fig. S2) following the manufacturer's instructions (BEI Resources, USA).

The mock community contained DNA of 20 bacterial species (Table S2) in equimolar counts (10^6 copies/μL) of 16S rRNA genes.

DNA plates were stored at -20°C before ~ 5-15 ng/μL from each sample was used as template in the 16S rRNA PCR reactions (see Supplementary Methods for details), set-up in 96-well plates using the Freedom EVO100 robot (Tecan, Switzerland). A no-template PCR negative control was included on each plate, to assess the influence of the PCR reaction, and the efficacy of our lab preparation on sequencing (Fig. S2). Each sample, and all controls, were PCR amplified in two independent reactions, except four rhizosphere samples from 2017, which we were unable to amplify and were subsequently excluded hereafter (Fig. S2). Four μL of each reaction product was mixed with 1 μL of loading dye containing Gel Red (Biotium) and visualized on a 1% agarose gel after 60 minutes at 100 V. None of the no-template negative controls, from either the extractions, or the PCR reactions, contained detectable amounts of DNA after PCR amplification. All samples were then cleaned using the NucleoMag NGS Clean-Up Kit (Macherey-Nagel, Germany) following the manufacturer's instructions. The cleaned products of the duplicated 16S rRNA gene PCR reactions were then pooled together and submitted for paired-end 250 bp sequencing using Illumina's MiSeq platform (Genome Québec, Montréal) (Bell *et al*., 2016; Lay *et al*., 2018). We estimated this should provide a mean of 39 000 reads per sample, which is in line with previous studies that describe bacterial communities (Bell *et al*., 2016; Lay *et al*., 2018).

Estimating ASVs from MiSeq 16S rRNA gene amplicons

The 16S rRNA gene amplicons generated by Illumina MiSeq were used to estimate the diversity and composition of the bacterial communities present in both the rhizosphere and roots

of each Test Phase *Brassicaceae* sample. The integrity and totality of the 16S MiSeq data downloaded from Génome Québec was confirmed using their MD5 checksum protocol (Roy *et al*., 2018). Subsequently, all data was managed, and analyzed in R (4.0.3 R Core Team, 2020), and plotted using `ggplot2` (Wickham, 2016).

Instead of generating OTUs from the 16S rRNA gene amplicon data, we opted to use DADA2 for ASV inference as it generates fewer false-positives than OTUs, and reveals more low-abundant, or cryptic, microbes (Callahan *et al*., 2016a & 2017). Moreover, as ASVs are unique sequence identifiers, they are directly comparable between studies, unlike OTUs (Callahan *et al*., 2016a & 2017; Fitzpatrick *et al*., 2018). The `dada2` package (Callahan *et al*., 2016a) was first used to filter and trim all 23 313 756 raw reads, forward and reverse, from the 16S rRNA gene amplicon data generated from the control samples, the mock communities, and the Test Phase *Brassicaceae* samples, using the `filterAndTrim` function (Fig. S2). All reads were trimmed from the 3' end to 240 bp, as determined *a posteriori* to be the optimum length to maximize retention of reads (14 666 924 after filtration, 10 178 467 retained overall), and the subsequent inference of ASVs (37 445). Any residual sequencing artifacts, including primers, were trimmed from the 5', and low-quality (Q = < 20) reads removed, before ASVs were inferred (Fig. S4).

Filtered and trimmed reads were then processed through DADA2 for ASV inference (Fig. S2). Default settings were used throughout the DADA2 pipeline, except the DADA inference functions `dadaF` and `dadaR` which used the `pool ='pseudo'` argument, to increase the likelihood of identifying rare taxa. Consequently, the chimera removal function `removeBimeraDenovo` included the `method ='pooled'` argument (Callahan *et al*., 2016b).

ASVs identified from the 16S rRNA gene amplicon data were assigned taxonomy, to the species level when possible, using the Silva database (Yilmaz *et al*., 2013), and the quality of the data was assessed using the included controls (Fig. S5). Any ASVs identified as chloroplasts, or mitochondria were subsequently removed from the data, as were the 58 off-target Archaeal ASVs. Rarefaction curves confirmed that we captured the majority of the bacterial communities in both the roots and rhizosphere (Fig. S6). Test Phase *Brassicaceae* 16S rRNA sequencing data was subsequently re-analysed independently following the described protocol to avoid any biases from the six no-template negative controls and the four mock communities. These are the Test Phase *Brassicaceae* ASVs which are reported hereafter.

α-diversity of the Test Phase *Brassicaceae* rhizosphere and root communities

First, to identify any changes in abundance of the bacterial ASVs within the Test Phase *Brassicaceae* species, we estimated the absolute abundance, or size, of the bacterial communities in each Test Phase DNA sample by qPCR (Azarbad *et al*., 2018). We used the standard universal Eub338/Eub518 primers (Muyzer *et al*., 1993; Nogales *et al*., 1999; Bathe & Hausner, 2006; Davis *et al*., 2009) to detect copies of the 16S rRNA gene sequences present in each DNA sample prior to estimating their absolute abundance from a standard curve (Zhang *et al*., 2017; Azarbad *et al*., 2018; see Supplementary Methods for details, Fig. S7). Given the technical limitations of high-throughput sequencing in assessing abundance, estimating the absolute abundance by qPCR can provide data to better interpret the bacterial communities (Gloor *et al*., 2017; Props *et al*., 2017; Harrison *et al*, 2020; Jian *et al*., 2020).

Second, to visualise taxonomic diversity, ASVs were plotted as taxa cluster maps using `heat_tree` from the `metacoder` package (Foster *et al*., 2017) for the rhizosphere and roots of both sampling years, where nodes represent phyla and class: node colours represent the absolute abundance of each ASV, while node size indicates the number of unique taxa. Taxa cluster maps facilitate visualizing abundance, as well as diversity across taxonomic hierarchies (Foster *et al*., 2017). Relative and absolute abundance bar charts were plotted at the phyla level for comparison.

Finally, in order to estimate the coverage of the bacterial domain of life, we incorporated phylogenies into the phyloseq object following the method described by Callahan *et al*., 2016b (see Supplementary Methods for details). Faith's phylogenetic diversity was calculated as an α-diversity index from the Test Phase *Brassicaceae* samples using the `pd` function from the `picante` package (Kembel *et al*., 2010; sum of all branch lengths separating taxa in a community). For comparison, Simpson and Shannon's α-diversity indices were calculated (Fig. S8). Log transformed phylogenetic diversity indices were confirmed to respect normality (see Supplementary Methods for details).

We assessed differences between the mean phylogenetic diversity between soil histories, *Brassicaceae* hosts, and their interactions using a Multi-Factor ANOVA and Tukey's *post hoc* test for significant groups that respected the assumptions of normality (Azarbad *et al*., 2020, Wang *et al*., 2020; see Supplementary Methods for details). Where normality could not be respected, we used the non-parametric Kruskal-Wallis rank sum test, `kruskal.test`. Specific groups of statistical significance were identified with the *post hoc* pairwise Wilcoxon Rank Sum Tests, `pairwise.wilcox.test`, with the FDR correction on the p-values to account for multiple comparisons. As the relative and absolute abundance datasets yielded similar α-diversities, with

and without ASV rarefaction using `rarefy_even_depth` (McMurdie & Holmes, 2013), only the results incorporating the absolute abundance are reported.

Identification of differentially abundant ASVs and specific indicator species

To refine our understanding of the abundance and composition of the Test Phase *Brassicaceae* bacterial communities, we used two complementary methods to identify taxa specific to soil histories, or the *Brassicaceae* hosts (see Supplementary Methods for details). First, taxa cluster maps were used to calculate the differential abundance of ASVs between experimental groups, including rhizosphere and root compartments, *Brassicaceae* host plants, and soil histories. Second, indicator species analysis was used to detect ASVs that were preferentially abundant in pre-defined environmental groups (soil histories, or *Brassicaceae* host). A significant indicator value is obtained if an ASV has a large mean abundance within a group, compared to another group (specificity), and has a presence in most samples of that group (fidelity) (De Cáceres & Legendre; Legendre & Legendre, 2012). The fidelity component complements the differential abundance approach between taxa clusters, which only considers abundance. Moreover, given the large number of taxa in our study, it is not practical to view taxa clusters as matrices below class, whereas indicator species analysis pinpoints specific ASVs of interest.

β-diversity of the Test Phase *Brassicaceae* rhizosphere and root communities

To test for significant community differences between both field trials, compartments, soil histories and *Brassicaceae* hosts, we used the non-parametric permutational multivariate ANOVA (PERMANOVA), where any variation in the ordinated data distance matrix is divided among all

the pairs of specified experimental factors. The PERMANOVA was calculated using the `adonis` function in the `vegan` package (Oksanen *et al*., 2020), with 9999 permutations, and the experimental blocks were included as "strata". Our preliminary PERMANOVA (Table S3) used a distance matrix calculated with the Bray-Curtis formula and tested the significance of the effects of soil history, *Brassicaceae* host, compartment, and field trial (Fig. S9). This was complemented with a PERMANOVA for each trial and compartment that specifically tested treatments and hosts as experimental factors, and used a weighed UniFrac distance matrix (Lozupone & Knight, 2005; Lozupone *et al*., 2007). This distance index incorporates the phylogenetic relationship of each dataset and the absolute abundance of each ASV, as estimated by qPCR of the 16S rRNA gene (Lozupone & Knight, 2008).

We used a variance partition, as a complement to the PERMANOVA, to model the explanatory power of soil history, *Brassicaceae* host, and soil chemistry in the structure of the Test Phase *Brassicaceae* bacterial communities. We then quantified how each significant factor (ie, the explanatory variables) impacted bacterial community structure with a distance-based redundancy analysis (db-RDA) (Legendre & Legendre, 2012). First, singleton ASVs were removed before the `phyloseq` data were transformed using Hellinger's method, such that ASVs with high abundances and few zeros are treated equivalently to those with low abundances and many zeros (Legendre & de Cáceres, 2013). A weighted UniFrac index was calculated (Lozupone & Knight, 2005; Lozupone *et al*., 2007), where this distance index gives an estimate of how similar communities contain more phylogenetically related ASVs weighed by the absolute abundance of each ASV, as estimated by qPCR of the 16S rRNA gene (Lozupone & Knight, 2008). Using a weighted UniFrac index in an ordination provides one way to test if bacterial community composition follows the

evolutionary history within the *Brassicaceae* host plant family, as determining community distances based only on the number of shared taxa does not account for evolutionary distances between taxa, which are often extremely diverse among microbes (Fitzpatrick *et al*., 2018; Walters *et al*., 2018). The weighted UniFrac distance matrix was calculated using the `distance` function in `phyloseq` (McMurdie & Holmes, 2013), and gave similar results as a Bray-Curtis distance matrix.

With the `vegan` package (Oksanen *et al*., 2020), soil chemistry was standardized (Legendre & Legendre, 2012) using the `decostand` function. We modelled the explanatory power of each experimental factor in each compartment from both field trials with a variance partition of a partial RDA, using the `varpart` function, and the weighted UniFrac distance matrix (Borcard *et al*., 1992). Variation in the bacterial community data not described by the explanatory variables were quantified by the residuals. Finally, to quantify the amount of variation described by each explanatory factor, db-RDA were calculated using the `capscale` function, and plotted using `phyloseq` (McMurdie & Holmes, 2013).

Results

The bacterial rhizosphere communities had larger absolute abundances and diversity than the root communities.

In order to estimate the composition of the bacterial rhizosphere and root communities from the Test Phase *Brassicaceae* species, we used the DADA2 pipeline (Callahan *et al*., 2016 & 2017) to infer the retained16S rRNA amplicons as ASVs. We retained 10 178 467 high-quality 16S rRNA MiSeq amplicons (43 129 ±18 032 reads/sample) through the pipeline (Table 1 & Fig.

Table 1. The bacterial rhizosphere communities had more ASVs and larger absolute abundance than the root communities of five *Brassicaceae* host plants from the Test Phase of a two-year crop rotation, harvested from two field trials (Trial 1, 2016; Trial 2, 2017) from Swift Current, Saskatchewan. Raw reads were produced via Illumina's MiSeq at Génome Québec, and processed through DADA2, where 10 178 467 reads were retained (16S rRNA Reads reported here) for ASV inference. A total of 37 445 ASVs were identified across the entire dataset. Bacterial community size was estimated by qPCR as the number of copies of the 16S rRNA gene.

		16S rRNA Reads[a]	ASV Occurrence	16S rRNA Gene Copies / Sample[b]
Trial 1 Test Phase	Rhizosphere	1 139 535 (18 992 ± 4333 / sample)	14 047	25 747 358 ± 17 838 649
	Root	3 315 289 (55 254 ± 5600 / sample)	3009	2 823 077 ± 2 099 135
Trial 2 Test Phase	Rhizosphere	2 122 186 (37 896 ± 7080/ sample)	26 001	7 692 764 ± 4 988 172
	Root	3 601 457 (60 024 ± 11 835 / sample)	4307	3 509 901 ±1 818 824

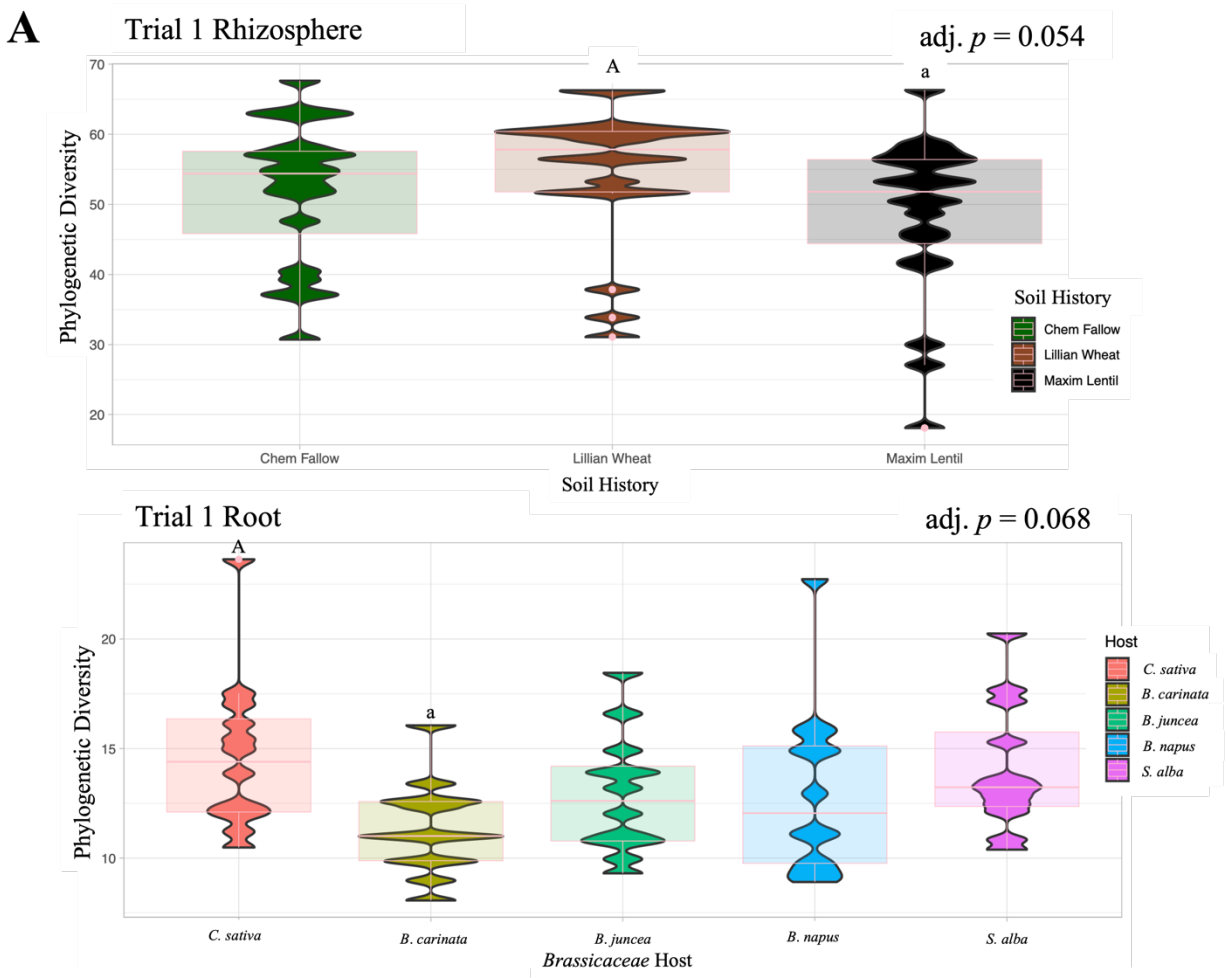
[a], Total retained reads (mean ± SD / sample)
[b], Mean number of 16S rRNA gene copies ± SD / sample

S4). More reads were retained in the Test Phase *Brassicaceae* root samples than from the rhizosphere in both field trials (Table 1). However, more bacterial ASVs were consistently identified in the rhizosphere compared to the root communities (Table 1). The absolute abundance, or size, of each Test Phase bacterial community from both trials were estimated by qPCR amplification of the 16S rRNA gene, where we observed that the bacterial rhizosphere communities were consistently larger than the root communities (Table 1). Faith's phylogenetic diversity was also consistently higher in the Test Phase bacterial rhizosphere communities, compared to the root communities (Fig. 2); Simpson and Shannon's α-diversity illustrated the same trend (Fig. S8).

ASVs were plotted as taxa clusters to the class level, where we observed that the bacterial communities from Trial 1 (Fig. 3) and 2 (Fig. S10A) were dominated by phyla *Acidobacteria* (classes *Acidobacteria* and *Blastocatellia*), *Actinobacteria* (*Actinobacteria*), *Bacteroidetes* (Bacteroidia), *Firmicutes* (*Clostridia*), *Proteobacteria* (*Gammaproteobacteria*), and *Verrucomicrobia* (*Verrucomicrobae*). These taxa were dominant in both the rhizosphere and root communities (Fig. 3 & Fig. S10). Moreover, we observed that all the taxa identified in the root communities were also present in the rhizosphere. Finally, we found no evidence for a bias among the lentil bacterial communities due to the use of the TagTeam inoculant, such as a spike of *Rhizobium leguminosarum*.

Soil history was only significant in structuring bacterial communities in the driest year

Next, we evaluated our hypothesis that the three soil histories established the previous year would continue to structure the *Brassicaceae* bacterial communities. Soil history only significantly


A
Trial 1 Rhizosphere
adj. $p = 0.054$
A
a
Phylogenetic Diversity
70
60
50
40
30
20
Chem Fallow
Lillian Wheat
Maxim Lentil
Soil History
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil
Trial 1 Root
adj. $p = 0.068$
A
a
Phylogenetic Diversity
20
15
10
C. sativa
B. carinata
B. juncea
B. napus
S. alba
Brassicaceae Host
Host
C. sativa
B. carinata
B. juncea
B. napus
S. alba

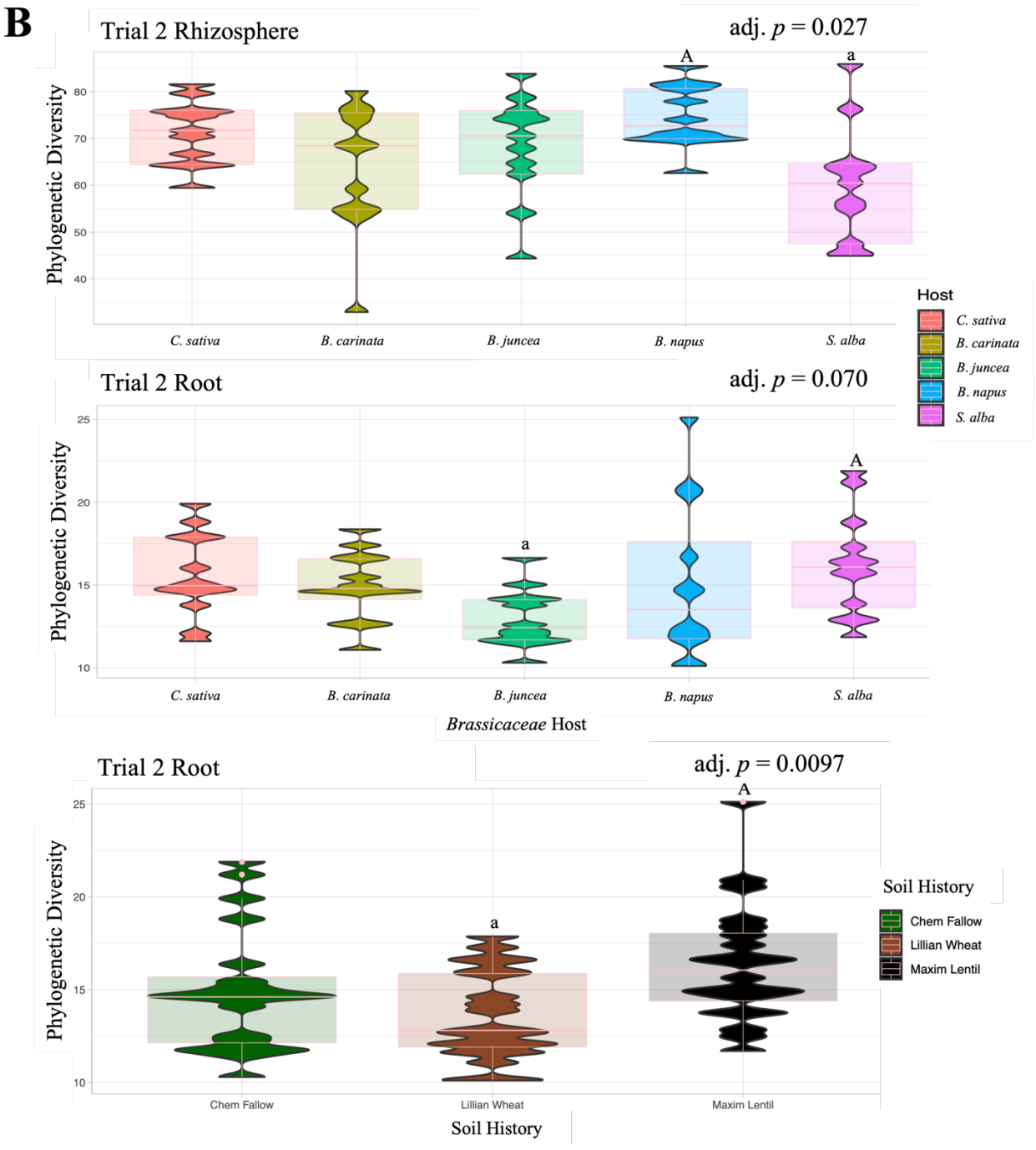
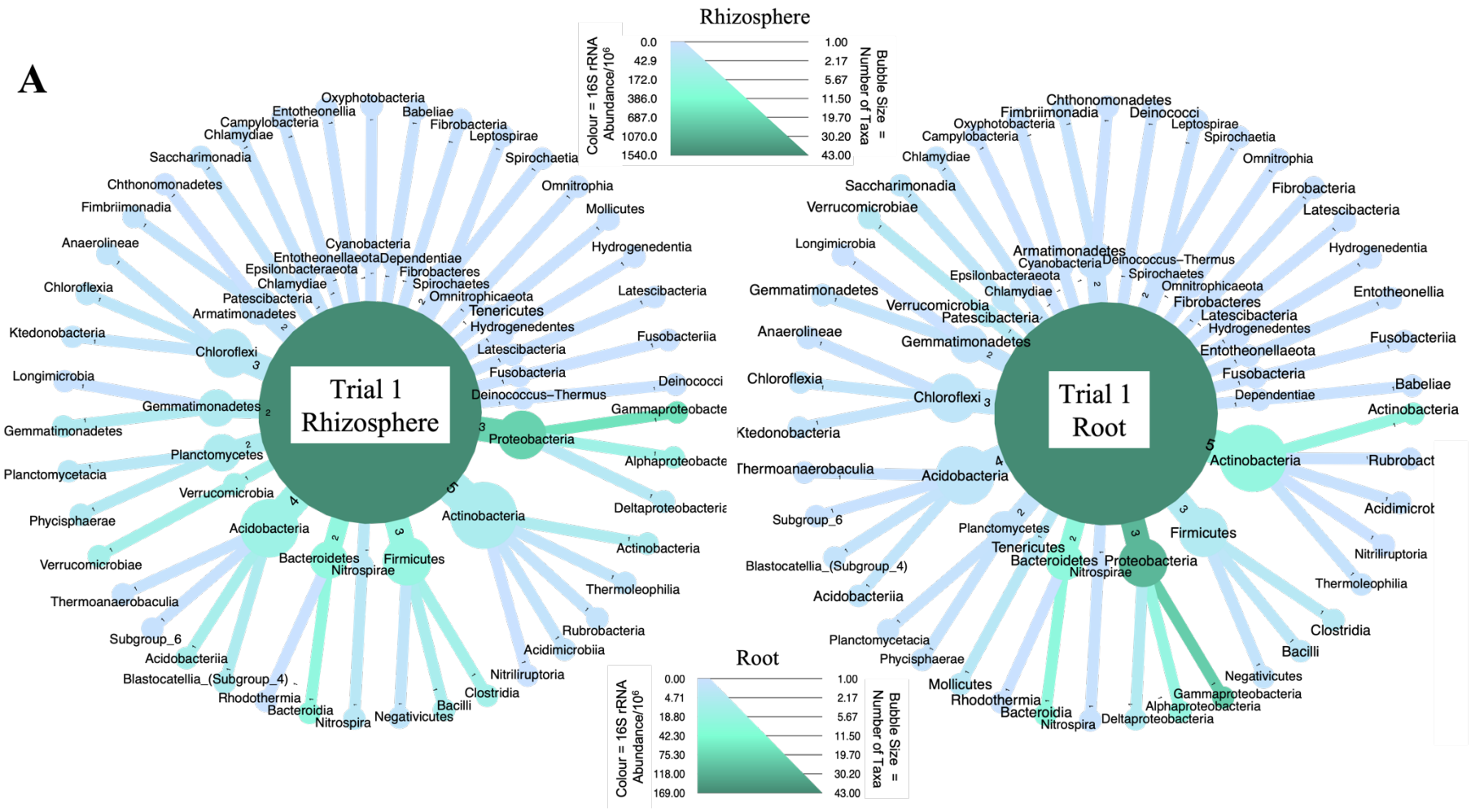


Figure 2. Phylogenetic diversity was consistently higher in the bacterial rhizosphere communities than in the root communities from five *Brassicaceae* host plants in the Test Phase of a two-year

rotation, harvested in 2016 (A, Trial 1) and 2017 (B, Trial 2) from Swift Current, Saskatchewan. Phylogenetic diversity, the sum of branch lengths of a phylogenetic tree connecting all species in the sample, accounts for the wide evolutionary distance among bacterial populations, and better represents the functions present (Hsieh & Chao, 2017). Diversity indices were log-transformed, before being tested with a nested ANOVA, which confirmed the soil histories established in the first Conditioning Phase, and the Test Phase *Brassicaceae* hosts did not interact. Statistically significant groups were identified using Tukey's *post hoc* test, and indicated with capital or lower-case letters, where capitals represent the significantly larger groups and lower-case represent the smaller groups. (A, top panel) Bacterial communities from the Test Phase rhizosphere with soil histories of growing wheat were significantly more (adj. $p = 0.054$) phylogenetically diverse than those communities with lentil soil histories. The non-parametric Kruskal test was used to test for significance among Test Phase communities grouped by soil histories. (A, bottom panel) Bacterial root communities from the Test Phase in Trial 1 with *Camelina sativa* cv. Midas hosts were more phylogenetically diverse than root communities from *Brassica carinata* (adj. $p = 0.0679$). (B, top panel) Bacterial communities from the Test Phase rhizosphere from Trial 2 with *Brassica napus* cv. canola hosts were significantly more (adj. $p = 0.027$) phylogenetically diverse than rhizosphere communities with *Sinapis alba* cv. Polish hosts. (B, middle panel) Trial 2 bacterial root communities from Test Phase *Sinapis alba* cv. Polish hosts were more phylogenetically diverse than root communities from *Brassica juncea* cv. Cutlass hosts (adj. $p = 0.070$). (B, bottom panel) Bacterial communities from the Test Phase roots from Trial 2 with soil histories of growing lentils were significantly more (adj. $p = 0.0097$) phylogenetically diverse than those communities with wheat soil histories.


A
Rhizosphere
Colour = 16S rRNA Abundance/10^6
0.0
42.9
172.0
386.0
687.0
1070.0
1540.0
Bubble Size = Number of Taxa
1.00
2.17
5.67
11.50
19.70
30.20
43.00
Trial 1 Rhizosphere
Oxyphotobacteria
Entotheonellia
Babeliae
Campylobacteria
Fibrobacteria
Chlamydiae
Leptospirae
Saccharimonadia
Spirochaetia
Chthonomonadetes
Omnitrophia
Fimbriimonadia
Mollicutes
Anaerolineae
Hydrogenedentia
Cyanobacteria
Entotheonellaeota
Dependentiae
Epsilonbacteraeota
Fibrobacteres
Chloroflexia
Chlamydiae
Spirochaetes
Latescibacteria
Patescibacteria
Omnitrophicaeota
Armatimonadetes
Tenericutes
Ktedonobacteria
Hydrogenedentes
Fusobacteriia
Chloroflexi
Latescibacteria
Longimicrobia
Fusobacteria
Deinococci
Gemmatimonadetes
Deinococcus−Thermus
Gammaproteobacte
Gemmatimonadetes
Proteobacteria
Planctomycetes
Alphaproteobacte
Planctomycetacia
Verrucomicrobia
Deltaproteobacteria
Actinobacteria
Phycisphaerae
Acidobacteria
Actinobacteria
Bacteroidetes
Firmicutes
Verrucomicrobiae
Nitrospirae
Thermoleophilia
Thermoanaerobaculia
Rubrobacteria
Subgroup_6
Acidimicrobiia
Acidobacteriia
Nitriliruptoria
Blastocatellia_(Subgroup_4)
Clostridia
Rhodothermia
Bacilli
Bacteroidia
Negativicutes
Nitrospira
Root
Colour = 16S rRNA Abundance/10^6
0.00
4.71
18.80
42.30
75.30
118.00
169.00
Bubble Size = Number of Taxa
1.00
2.17
5.67
11.50
19.70
30.20
43.00
Trial 1 Root
Chthonomonadetes
Fimbriimonadia
Deinococci
Oxyphotobacteria
Leptospirae
Campylobacteria
Spirochaetia
Chlamydiae
Omnitrophia
Saccharimonadia
Fibrobacteria
Verrucomicrobiae
Latescibacteria
Longimicrobia
Armatimonadetes
Hydrogenedentia
Cyanobacteria
Deinococcus−Thermus
Epsilonbacteraeota
Spirochaetes
Gemmatimonadetes
Chlamydiae
Omnitrophicaeota
Entotheonellia
Verrucomicrobia
Fibrobacteres
Patescibacteria
Latescibacteria
Anaerolineae
Hydrogenedentes
Gemmatimonadetes
Entotheonellaeota
Fusobacteriia
Chloroflexia
Fusobacteria
Babeliae
Chloroflexi
Dependentiae
Actinobacteria
Ktedonobacteria
Actinobacteria
Rubrobact
Thermoanaerobaculia
Acidobacteria
Acidimicrot
Subgroup_6
Planctomycetes
Firmicutes
Tenericutes
Nitriliruptoria
Bacteroidetes
Proteobacteria
Blastocatellia_(Subgroup_4)
Nitrospirae
Thermoleophilia
Acidobacteriia
Clostridia
Planctomycetacia
Bacilli
Phycisphaerae
Negativicutes
Mollicutes
Gammaproteobacteria
Rhodothermia
Alphaproteobacteria
Bacteroidia
Deltaproteobacteria
Nitrospira

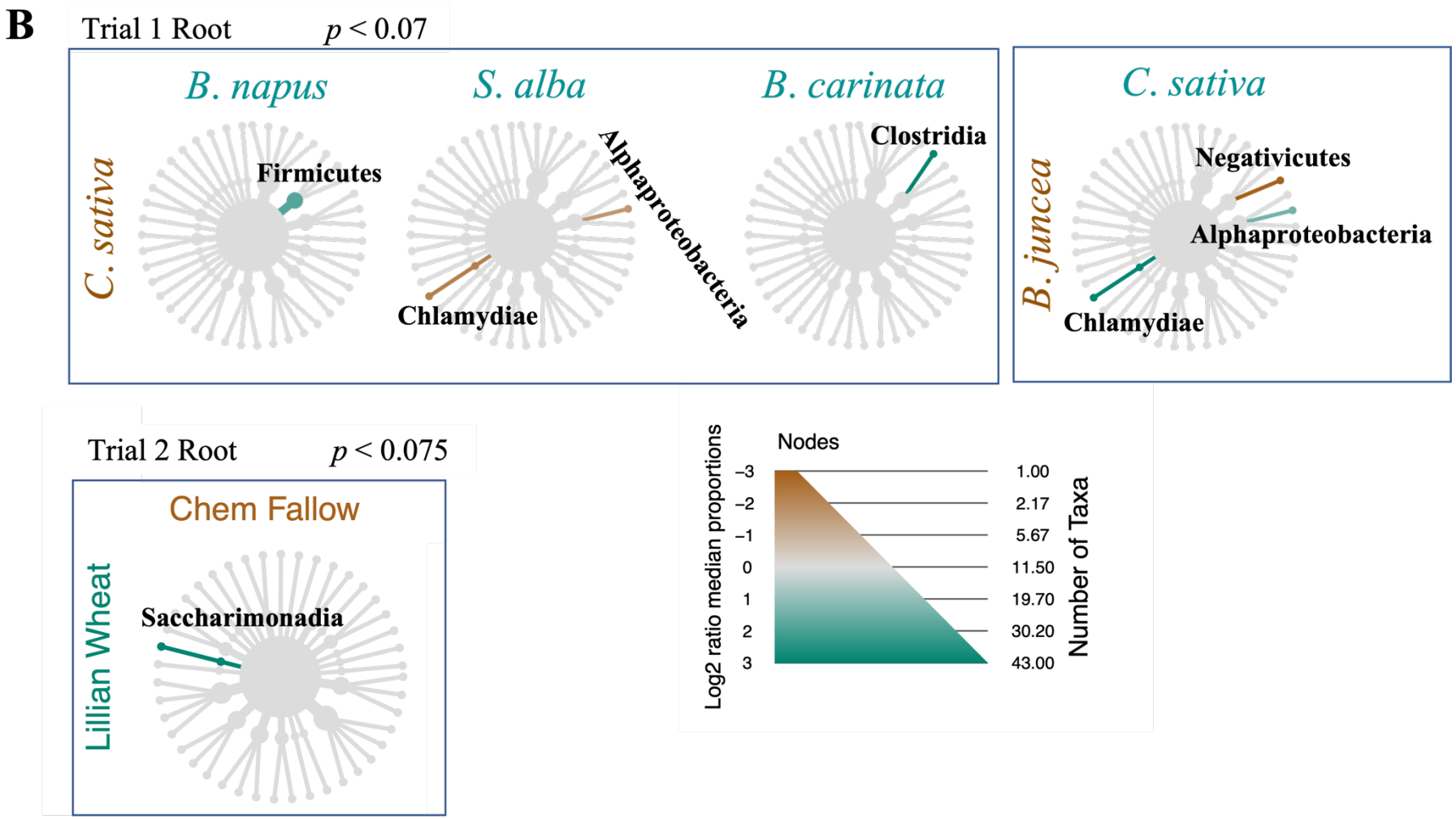

B
Trial 1 Root
p < 0.07
B. napus
S. alba
B. carinata
C. sativa
Firmicutes
Chlamydiae
Alphaproteobacteria
Clostridia
C. sativa
B. juncea
Negativicutes
Alphaproteobacteria
Chlamydiae
Trial 2 Root
p < 0.075
Chem Fallow
Lillian Wheat
Saccharimonadia
Nodes
Log2 ratio median proportions
−3
−2
−1
0
1
2
3
1.00
2.17
5.67
11.50
19.70
30.20
43.00
Number of Taxa

Figure 3. The abundance and composition of the bacterial communities varies noticeably between the rhizosphere (A, left) and root (A, right), as represented by taxa clusters of ASVs inferred from among the five *Brassicaceae* host plants in Trial 1, harvested in 2016 during the Test Phase of a two-year rotation, from Swift Current, Saskatchewan. ASV absolute abundance, represented by the colour scale, was estimated by multiplying the 16S rRNA copy number / 10^6 by the ASV abundance, while the size of each bubble represents the number of unique taxa represented to the class level. (A, left) Trial 1 Test Phase bacterial rhizosphere communities were larger (1.5 x10^9 ASVs) than the root communities (1.69 x10^8 ASVs, right). (B) Test Phase *Brassicaceae* host plants only had significant variation in terms of composition among their root bacterial communities; among the root communities in Trial 1 there were only differences between *Brassicaceae* hosts (B, top panel, *p*. adj < 0.07), while among the root communities in Trial 2 there were significant differences between soil histories (B, bottom panel, *p*. adj < 0.075). Significantly enriched taxa, labelled in bold, were tested between each pair of host plants and soil history. Taxa that were significantly more abundant are highlighted brown or green, following the labels for each compared host. These differential taxa clusters identified significantly enriched (ie, abundant), using the non-parametric Kruskal test, followed by the *post hoc* pairwise Wilcox test, with an FDR correction.

structured the Test Phase bacterial communities in Trial 2, the driest year, in both the rhizosphere (PERM R^2 = 0.0722, p = 0.0387) and root (PERM R^2 = 0.0873, p = 0.003, Table 2). Soil history was also identified as a significant factor in Trial 2 by variance partition, both in the Test Phase rhizosphere communities (0.4%, $p < 0.01$, Fig. 4A) and root communities (4.3%, $p < 0.01$, Fig. 4B). RDA further confirmed soil history significantly structured the bacterial communities in Trial 2. The Test Phase root communities followed a clear gradient between the three soil histories (adj. R^2 = 0.0989, p = 0.001, Fig. 4C). The relationship among the Test Phase rhizosphere communities and soil history remained significant, though a similar gradient between the three soil histories was less clear (adj. R^2 = 0.0399, p = 0.019, Fig. S11A).

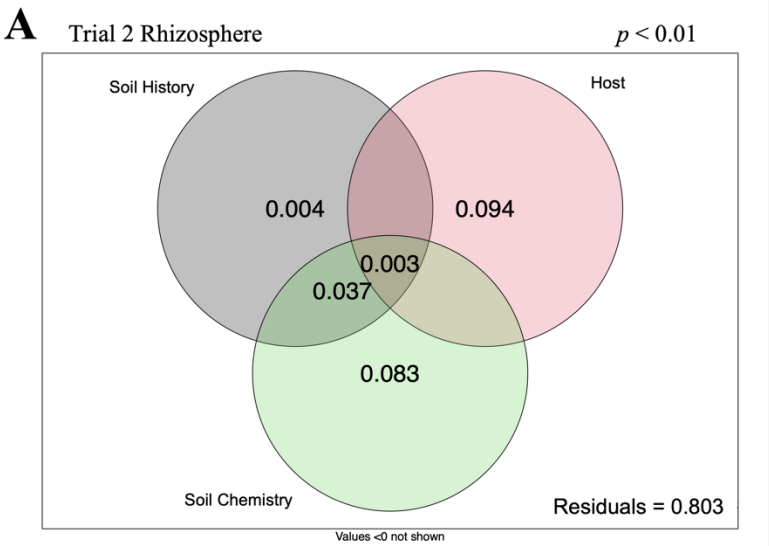
Soil history also had a significant impact on the composition of the Test Phase bacterial root communities in Trial 2. First, root communities with a lentil soil history had significantly higher phylogenetic diversity (p. adj = 0.0097, Fig. 2B, bottom panel) than root communities with wheat soil histories. Second, Test Phase root communities with wheat soil histories were enriched in *Patescibacteria* (*Saccharimonadia*) compared to Test Phase root communities with fallow soil histories (p. adj < 0.075, Fig. 3B). Finally, indicator species analysis of Trial 2 detected ASVs that were preferentially abundant in the Test Phase *Brassicaceae* roots with lentil or fallow soil histories (p. adj < 0.05, Table 3). Conversely, soil history did not significantly impact the absolute abundance, nor the composition (Fig. S10A) of the bacterial rhizosphere communities in Trial 2. Nonetheless, our data illustrated an important role for soil history in structuring the Test Phase bacterial communities in Trial 2, especially the root communities.

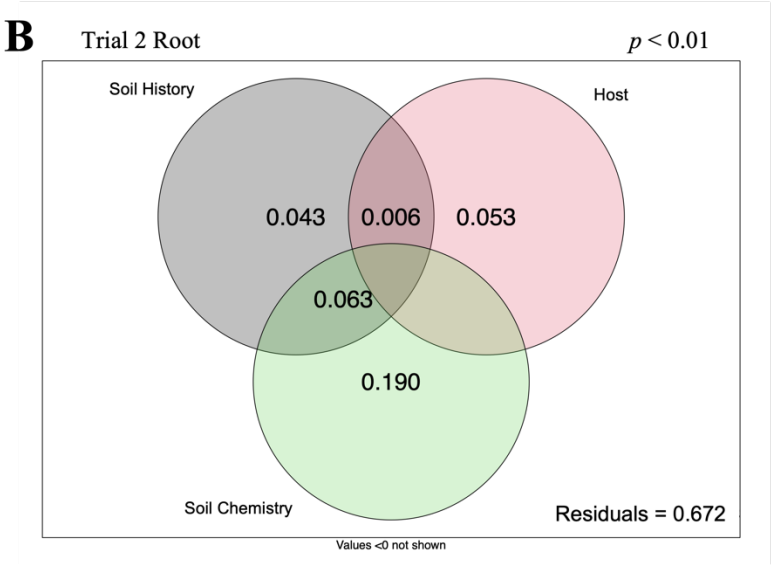
Soil history had a weak impact on the Test Phase bacterial rhizosphere communities in Trial 1 (PERM R^2 = 0.0524, p = 0.0789). Variance partition identified soil history as significant (p < 0.001; Fig. 5A) in these rhizosphere communities, though it only explained 1.6% of the variance. RDA illustrated a gradient between the Test Phase bacterial rhizosphere communities with fallow

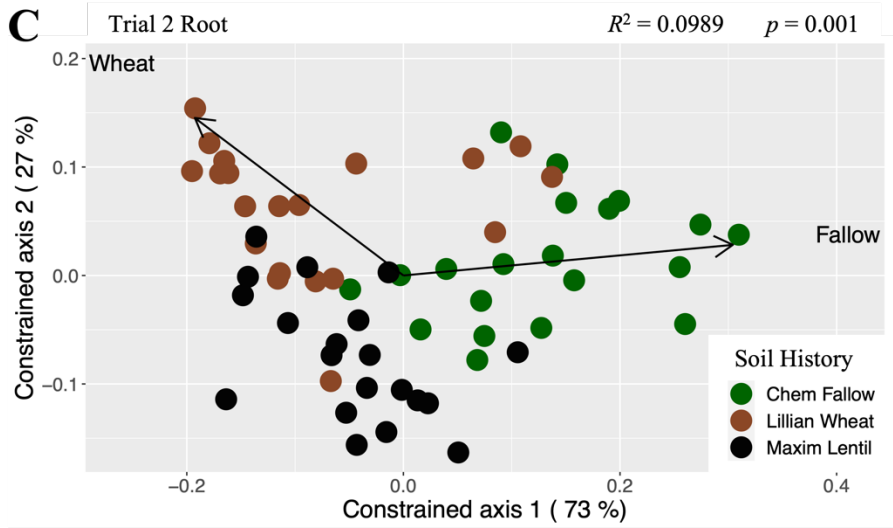
Table 2. PERMANOVA identified that the *Brassicaceae* host plants were always a significant experimental factor in structuring the bacterial rhizosphere and root communities from the Test Phase of a two-year crop rotation, harvested in 2016 (Trial 1) and 2017 (Trial 2) from Swift Current, Saskatchewan. The previous year's soil history was only significant in the Test Phase bacterial rhizosphere and root communities of Trial 2, while the *Brassicaceae* host ~ crop history interaction was never significant. The PERMANOVA was calculated using a weighted UniFrac distance matrix, with 9999 permutations.

Trial 1[a]						
Experimental Factors	Rhizosphere			Root		
	F Model	R^2	Pr (> F)	F Model	R^2	Pr (> F)
Soil History[b]	1.859	0.0524	0.0789	1.271	0.0332	0.2207
Brassicaceae Host[c]	3.912	0.2207	**0.0002**	5.267	0.2751	**0.0001**
Soil History * *Brassicaceae* Host	0.820	0.0925	0.7603	0.995	0.104	0.4577
Trial 2						
Experimental Factors	Rhizosphere			Root		
	F Model	R^2	Pr (> F)	F Model	R^2	Pr (> F)
Soil History	2.1358	0.0722	**0.0387**	2.7623	0.0873	**0.0003**
Brassicaceae Host	2.0705	0.1400	**0.0097**	1.7879	0.1130	**0.0045**
Soil History * *Brassicaceae* Host	0.6999	0.0947	0.8860	0.7014	0.0887	0.9666

[a], Values in bold indicate significant factors or interactions
[b], Fallow, wheat, or lentil
[c], *Brassica carinata*, *B. napus*, *B. juncea*, *Sinapis alba*, or *Camelina sativa*


A
Trial 2 Rhizosphere
$p < 0.01$
Soil History
Host
Soil Chemistry
0.004
0.094
0.003
0.037
0.083
Residuals = 0.803
Values <0 not shown


B
Trial 2 Root
$p < 0.01$
Soil History
Host
Soil Chemistry
0.043
0.006
0.053
0.063
0.190
Residuals = 0.672
Values <0 not shown


C
Trial 2 Root
$R^2 = 0.0989$
$p = 0.001$
Wheat
Fallow
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil
Constrained axis 2 (27 %)
Constrained axis 1 (73 %)
0.2
0.1
0.0
−0.1
−0.2
0.4

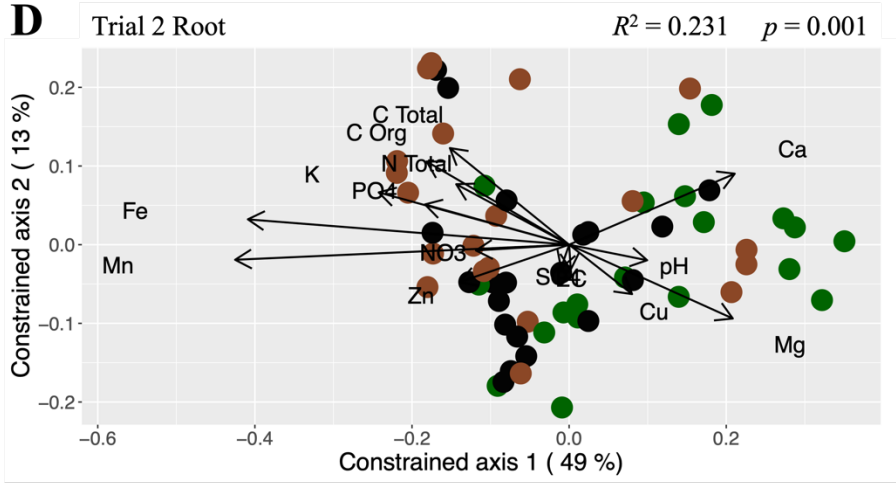
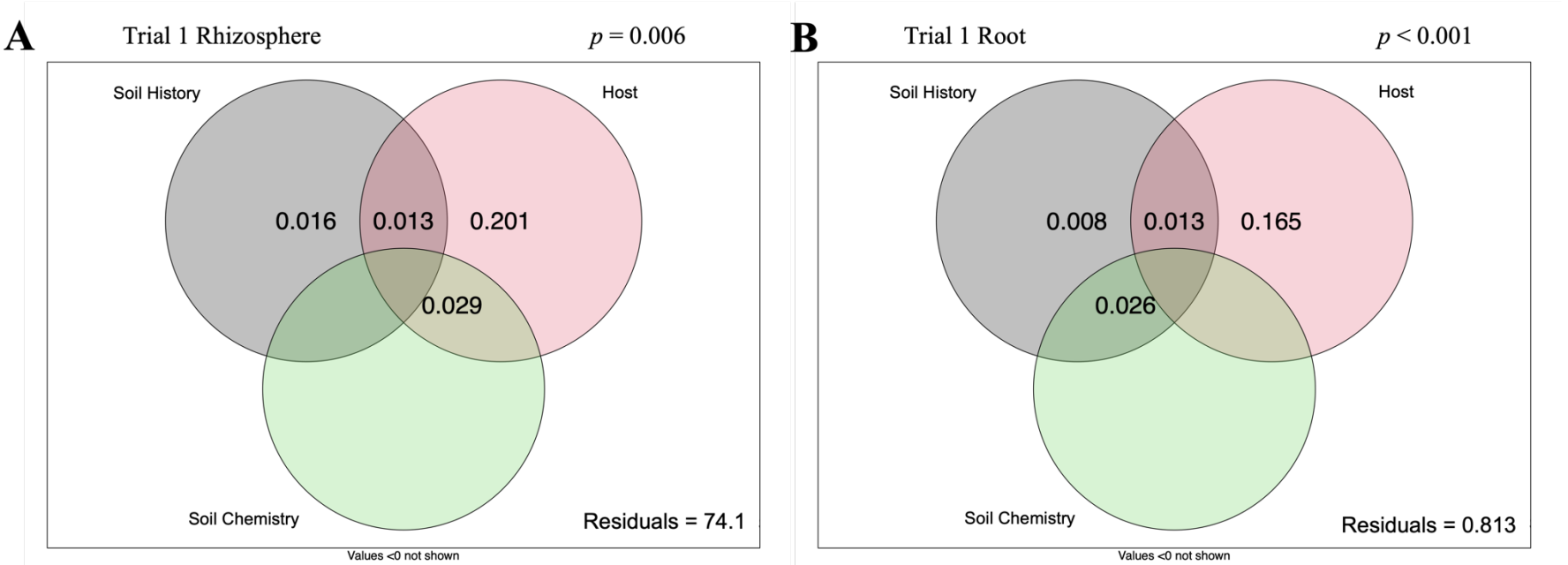

D
Trial 2 Root
$R^2 = 0.231$
$p = 0.001$
C Total
C Org
N Total
K
PO4
Fe
Mn
NO3
Zn
pH
Cu
Ca
Mg
Constrained axis 2 (13 %)
Constrained axis 1 (49 %)
0.2
0.1
0.0
−0.1
−0.2
−0.6
−0.4
0.2

Figure 4. Soil chemistry explained the most variance in the bacterial community structures in the rhizosphere and roots of five *Brassicaceae* host in Trial 2, harvested in 2017 during the Test Phase of a two-year rotation, from Swift Current, Saskatchewan. Weighted UniFrac distances were used with a variance partition (A & B), which modelled the explanatory power of each experimental factor (*Brassicaceae* host, soil history, and soil chemistry) in the Test Phase bacterial communities. Distance-based redundancy analyses (C & D) quantified how the experimental factors impacted community structure, where communities with similar phylogenetic composition appear closer together. (A) Variance partition illustrated the strong influence of *Brassicaceae* host plants (9.4%) and soil chemistry (8.3%) in explaining the Test Phase rhizosphere communities in Trial 2. (B) The influence of soil chemistry increased (19%), as did soil history (4.3%) in the Test Phase bacterial root communities in Trial 2. (C) Soil history was still significant ($R^2 = 0.0989$, $p = 0.001$) in structuring the Test Phase bacterial root communities in Trial 2, though soil chemistry was more explanatory (D, $R^2 = 0.231$, $p = 0.001$). D) pH was opposed by potassium, as well as phosphate, while calcium was contrasted by zinc, and magnesium was contrasted by total nitrogen and organic carbon. However, soil chemistry does not have a clear relationship in explaining the phylogenetic similarity between communities, as a function of soil history, nor *Brassicaceae* host plant.

Table 3. Indicator species were identified in the bacterial root communities in the Test Phase of a two-year crop rotation, harvested in 2016 (Trial 1) and 2017 (Trial 2) from Swift Current, Saskatchewan. In Trial 1, seven ASVs were identified as indicator species in the Test Phase root communities of *Camelina sativa*. In Trial 2, ten ASVs were identified from Test Phase root communities with soil history of growing lentils, while ten different ASVs were identified as indicator species from root communities with a fallow soil history. Indicator species analysis relies on abundance and site specificity to statistically test each ASV, which we report here as $p < 0.05$, with a FDR correction.

Most Closest Taxon (No. of ASVs)	Trial 1 Roots ($p < 0.05$)	Trial 2 Roots ($p < 0.05$)
Alphaproteteobacteria, *Phenylobacterium* (3x ASVs)	*C. sativa*	
Actinobacteria, *Mycobacterium*	*C. sativa*	
Gammaproteobacteria, Burkholderiales, *Aquabacteria*	*C. sativa*	
Alphaproteteobacteria, Micropepsaceae (2x ASVs)	*C. sativa*	
Bacteroidetes, *Mucilaginibacter*		Lentil
Bacteroidetes, Chitinophagales, *Chitinophaga* (2x ASVs)		Lentil
Alphaproteteobacteria, *Sphingomonas*		Lentil
Alphaproteteobacteria, Rhizobales, *Labrys*		Lentil
Alphaproteteobacteria, *Caulobacter*		Lentil
Gammaproteobacteria, Burkholderiales, *Rhizobacter* (2x ASVs)		Lentil

Actinobacteria, *Mycobacterium*	Lentil
Gammaproteobacteria, Xanthomonadales, *Luteibacter*	Lentil
Bacteroidetes, Chitinophagales, *Niastella* (4x ASVs)	Fallow
Gammaproteobacteria, *Pseudomonas* (3x ASVs)	Fallow
Gammaproteobacteria, Burkholderiales, *Massilia*	Fallow
Gammaproteobacteria, Xanthomonadales, *Lysobacter*	Fallow
Firmicutes, Bacilli, *Paenibacillus*	Fallow


A
Trial 1 Rhizosphere
p = 0.006
Soil History
Host
Soil Chemistry
0.016
0.013
0.201
0.029
Residuals = 74.1
Values <0 not shown
B
Trial 1 Root
p < 0.001
Soil History
Host
Soil Chemistry
0.008
0.013
0.165
0.026
Residuals = 0.813
Values <0 not shown

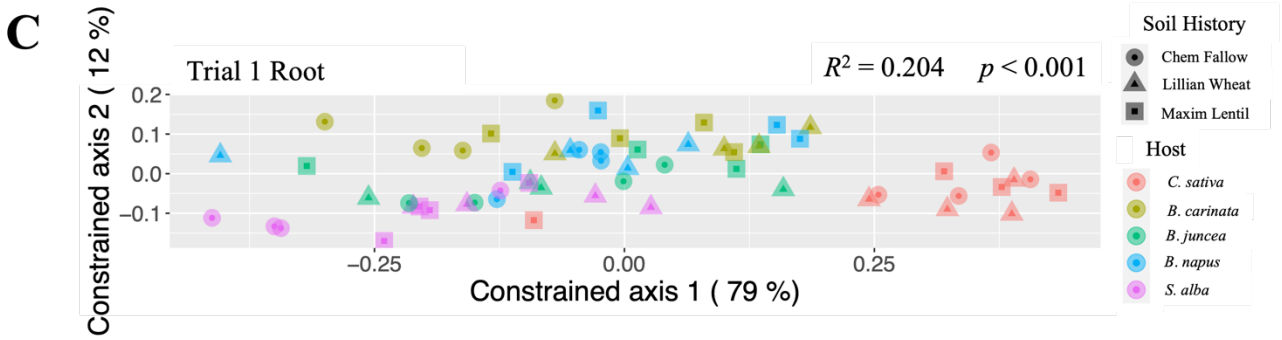

C
Trial 1 Root
$R^2 = 0.204$ $p < 0.001$
Constrained axis 2 (12 %)
Constrained axis 1 (79 %)
0.2
0.1
0.0
−0.1
−0.25
0.00
0.25
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil
Host
C. sativa
B. carinata
B. juncea
B. napus
S. alba

Figure 5. Bacterial communities were primarily structured by *Brassicaceae* host plants among rhizosphere and roots of five *Brassicaceae* host plants in Trial 1, harvested in 2016 during the Test Phase of a two-year rotation, from Swift Current, Saskatchewan. Weighted UniFrac distances were used with a variance partition (A & B), which modelled the explanatory power of each experimental factor (*Brassicaceae* host, soil history, and soil chemistry) in the Test Phase bacterial communities of Trial 1. Distance-based redundancy analyses (C) quantified how *Brassicaceae* host plants impacted community structure, where communities with similar phylogenetic composition appear closer together. Variance partition illustrated the strong influence of *Brassicaceae* host plants (20.1%) in explaining the Test Phase rhizosphere communities (A), and the root (B, 16.5%) communities in Trial 1. (C) *Brassicaceae* host plants were also significant ($R^2 = 0.204$, $p < 0.001$) in structuring the Test Phase bacterial root communities, as seen by RDA. *C. sativa* (red, at right) bacterial root communities were significantly more similar amongst themselves, than to root communities from other *Brassicaceae* host plants.

soil histories versus communities that were conditioned with lentil, or wheat (adj. R^2 = 0.027, p = 0.054, Fig. S12A). Phylogenetic diversity was also higher among Test Phase rhizosphere communities from Trial 1 with wheat soil histories (p. adj = 0.054, Fig. 2A, top panel) compared to those rhizosphere communities conditioned with lentils. The only evidence we observed where soil history significantly influenced the Test Phase bacterial root communities in Trial 1 was in the variance partition, where soil history explained 0.8% of the root communities ($p < 0.001$; Fig. 5B). Soil history was not found to be significant in the Trial 1 root communities by PERMANOVA (PERM R^2 = 0.0332, p = 0.2207, Table 2), nor by RDA.

Bacterial communities were more influenced by soil chemistry than soil history in the driest year

The variance partition used to model the explanatory power of each factor in Trial 2 revealed that the Test Phase bacterial root and rhizosphere communities were significantly explained by the current soil chemistry (Fig. 4A & B). Soil history explained 0.4% of variance in the data among the rhizosphere communities, while soil chemistry accounted for 8.3% (Fig. 4A). RDA further supported the key role of soil chemistry in the rhizosphere communities of Trial 2 (adj. R^2 = 0.27, p = 0.001, Fig. S11B). Soil chemistry also explained the most variance in the Test Phase root communities of Trial 2; 19% was attributed to soil chemistry, compared to 4.3% of the variance in the root community data being explained by soil history (Fig. 4B). RDA further supported the importance of soil chemistry in the root communities from the Test Phase of Trial 2 (adj. R^2 = 0.231, p = 0.001, Fig. 4D). These data strongly indicate that the Test Phase bacterial rhizosphere and root communities were strongly shaped by the current soil chemistry in the dry year of Trial 2, which was not detected in Trial 1.

Brassicaceae host plants were significant in structuring bacterial communities in both field trials

We further evaluated our hypothesis that soil history would continue to structure the subsequent bacterial communities by comparing the root and rhizosphere communities of the different *Brassicaceae* host plants. We found that the *Brassicaceae* host plants significantly structured the bacterial root and rhizosphere communities, in the Test Phase of both Trial 1 and 2 (Table 2). We noted there was never a significant interaction effect between soil history and *Brassicaceae* host plants, regardless of field trial, or compartment (Table 2).

In Trial 2, the *Brassicaceae* host plants were also significant in the variance partition, which explained 9.4% and 5.3% of the community variance in the Test Phase rhizosphere and roots, respectively (Fig. 4A & B). RDA illustrated that the distribution of the Test Phase root communities in Trial 2 were significantly influenced by the *Brassicaceae* host effect (adj. R^2 = 0.0307, p = 0.039, Fig. S12). However, RDA was not significant in the corresponding rhizosphere communities in Trial 2 despite being significant in the root communities.

The *Brassicaceae* host plants also modified the composition of the Test Phase bacterial communities in Trial 2. Rhizosphere communities from *B. napus* were more diverse than *S. alba* (p. adj = 0.0268, Fig. 2B). Trial 2 root communities from *S. alba* hosts were significantly more diverse than *B. juncea* (p. adj = 0.0698, Fig. 2B). Furthermore, the mean absolute abundance of the root communities from *S. alba* were significantly smaller than root communities from *B. carinata* (pairwise Wilcox test, p. adj < 0.05, data not shown).

In Trial 1, the *Brassicaceae* host plants were highly significant in structuring the Test Phase bacterial rhizosphere and root communities (Table 2). In the rhizosphere, the variance partition attributed 20.1% of the data as explained by the *Brassicaceae* hosts (Fig. 5A). Among the root communities from Trial 1, the variance partition modeled 16.5% of the data due to the *Brassicaceae* host plants (Fig. 5B). RDA illustrated how the bacterial rhizosphere communities

from Trial 1 were significantly structured by the *Brassicaceae* host (adj. R^2 = 0.147, p = 0.0001, Fig. S13B). Rhizosphere communities from *C. sativa* were particularly impacted, as they appeared the most distinctly clustered. Interestingly, *B. carinata* had particularly diffuse communities (Fig. S10B), likely related to variation in species richness across the x-axis. The *Brassicaceae* host plants structured the Test Phase root communities from Trial 1 even more drastically than their corresponding rhizosphere communities (adj. R^2 = 0.204, p = 0.001, Fig. 5C); particularly for communities from *C. sativa*, which were distinctly clustered from communities with other host plants (Fig. S14).

The *Brassicaceae* host plants created significant changes in the composition of the Test Phase root communities from Trial 1, primarily in the *C. sativa* communities. First, we found that root communities from *C. sativa* were more phylogenetically diverse than those communities from *B. carinata* (*p*. adj = 0.0679, Fig. 2 bottom panel). *C. sativa* root communities were enriched in both *Alphaproteobateria* and *Chlamydiae*, compared to root communities from *S. alba* and *B. juncea* hosts, as determined by comparing taxa clusters of the bacterial root communities between each *Brassicaceae* host (*p*. adj < 0.07, Fig. 3B). *C. sativa* root communities were also enriched in *Firmicutes* (*Negativicutes*) compared to *B. juncea* hosts (Fig. 3B). However, *C. sativa* root communities were depleted in *Firmicutes* (*Clostridia*) compared to *B. carinata* communities, and were also depleted in *Firmicutes* compared to *B. napus* communities (Fig. 3B). Finally, indicator species analysis identified seven unique ASVs that were specific to the Test Phase bacterial root communities of *C. sativa* from Trial 1 (Table 3). Taken together, our data points to a significant impact of *Brassicaceae* host plants on the structure of the Test Phase bacterial communities, especially in the roots of *C. sativa* from Trial 1, while soil chemistry and soil history were only key factors structuring the bacterial communities in the dry year of Trial 2.

Discussion

Soil history is an understudied aspect involved in structuring soil microbial communities through time (Fitzpatrick *et al*., 2018; Hannula *et al*., 2021). In this study, we took advantage of an agricultural experiment to test how soil history endures to structure subsequent soil bacterial communities. Agricultural systems bridge the gap between controlled greenhouse conditions and experiments in "natural" environments and provide the opportunity for field experiments, which are urgently needed to study PSF (Revillini *et al*. 2016). Given that crop rotations can provide advantages to the subsequent crop in agricultural systems—i.e., something is stored, or transmitted through time in the soil—we hypothesized that soil histories established the previous year through plant-soil community feedback would continue to structure the subsequent *Brassicaceae* bacterial root and rhizosphere communities. We coupled 16S rRNA metabarcoding with two field trials done over successive years that largely differed by the amount of rain received during the plant growth period. Contrary to our hypothesis, we actually found that the current *Brassicaceae* host plants were the primary factor in structuring the bacterial rhizosphere and root communities in agricultural conditions, but that this influence could be weakened.

Bacterial communities among *Brassicaceae* hosts

In opposition to our hypothesis, we found that the influence of the PSF from the current *Brassicaceae* host plants were always significant in structuring the Test Phase bacterial communities in both field trials. In Trial 2, during the dry year, the effect of the *Brassicaceae* host plant, while still significant, was noticeably weaker (Fig. 4), whereas our results from Trial 1 illustrated that the current *Brassicaceae* host plants were strong enough to obscure any influence from the previously established soil history (Fig. 5). Refuting our predictions, these results illustrated that four of the *Brassicaceae* host plants, *S. alba*, *B. carinata*, *B. juncea* and *B. napus*,

assembled essentially the same bacterial rhizosphere and root communities, over-riding any residual effects from soil history, and variation in the *Brassicaceae* management practices, and agricultural inputs. However, *C. sativa* assembled significantly different bacterial communities, most prominently in the root communities (Fig. S14). Overall, these results refute our hypothesis about the duration of soil history and highlight the capacity of plant-soil bacterial communities to re-shape their soil environment.

Traditionally, there has been robust debate surrounding the degree to which host plants play a role in shaping their microbial communities (Erlandson *et al*., 2018). More recently, however, there are a growing number of studies that demonstrate stable bacterial relationships with host plants conserved through evolutionary time. Stopnisek & Shade (2021) reported that 48 bacterial taxa were persistent in *Phaseolus vulgaris* root communities across time and space, including between soils of different continents. Zhang *et al*., (2019) reported clear differences between *Oryza sativa* L. *indica* and *japonica* cultivars, and demonstrated that the SNP variation of a rice nitrate transport gene, *NRT.1B*, was responsible for enriching the root microbiome in bacterial nitrogen-use genes. Fitzpatrick *et al*., (2018) quantified larger host impacts on the bacterial root community than in the rhizosphere for 30 angiosperms grown in a common soil. Finally, within the same bog, Wicaksono *et al*., (2021) identified similar bacterial communities, which were differentially enriched depending on if the host was a bryophyte, or a vascular plant. Thus, we see a variety of examples, through different plant taxonomic relationships, that demonstrate the important role of plant hosts in structuring their bacterial communities. Our results from five *Brassicaceae* host plants in an agricultural setting fits with this perspective of intimate PSF and interaction through evolutionary time.

To better understand how these PSF develop through time, future studies ought to be carefully designed such that they are capable of detecting the influence of host plants on structuring

their bacterial communities. First, plants grown in soils from the same source, like a "Common Garden" design, will all experience the same environmental filter, which establishes a common microbial starting point (Bouffaud *et al*., 2014). Second, detecting the impact of the host plant will be easier when the hosts are at larger evolutionary distances. Wicaksono *et al*., (2021) demonstrated clear host dominance over soil between bryophytes and vascular plants. Similarly between angiosperm families, Fitzpatrick *et al*., (2018) had sufficient evolutionary distance between host plants for clear resolution.

Detecting a clear impact of host plants within a given plant family, as we have reported here with our novel approach in the *Brassicaceae*, may create challenges; the evolutionary distance of the host plants may be insufficient to discriminate between the bacterial soil communities (Bouffaud *et al*., 2014). This may be the case for our findings for *S. alba*, *B. carinata*, *B. juncea*, and *B. napus*, which we predicted were sufficiently genetically distinct to form unique bacterial communities. Instead, we found that these host plants had phylogenetically similar bacterial communities, despite variation between their genotypes, and the agricultural management practices and inputs they were treated with.

Therefore, we encourage future studies to design experiments as though designing a phylogenetic tree, and include a diversity of host plants, at varying evolutionary distances from the hosts of interest—as if adding appropriate outgroups (Bouffaud *et al*., 2014; Fitzpatrick *et al*., 2018). In our study, *C. sativa* functioned as the outgroup to help identify any specific host plant impact on the bacterial communities from among the genus *Brassica*. We found phylogenetically distinct bacterial communities associated with *C. sativa*, particularly within the roots (Fig. 5C). Furthermore, we observed a clear trend of *C. sativa* having compositional differences compared to the other *Brassicaceae* hosts (Fig. 2, 3B & Table 3). This distinctiveness between bacterial root communities may reflect the evolutionary history of the host plants (Fig. S14). It will be important

to determine how and why this occurs, as well as if this distinct bacterial community is responsible for lower yields, and higher potential for the loss of nitrogen through denitrification, as reported by Wang *et al*., (2020).

Soil history and soil chemistry are revealed when the host plants feedback is weakened

In partial support of our hypothesis, our results illustrated that the previously established soil history was still significant a year later in structuring the Test Phase bacterial communities in Trial 2 (Fig. 4). In Trial 1, however, the strength of the current *Brassicaceae* plant-bacterial community feedback dominated the structure of the communities, and the influence of the previous soil history was minimized (Fig. 5). Moreover, despite the variation among the host plants in terms of genotypes, and agricultural management practices and inputs they were treated with, all five *Brassicaceae* plants overturned the different soil histories we tested.

Conversely, in Trial 2, both the previously established soil history, and the current *Brassicaceae* host plants were significant factors in structuring the bacterial communities (Table 2). The influence of different soil histories was most obvious by the phylogenetically distinct communities that were formed between root communities with different soil histories (Fig. 4C). This observation supports our initial hypothesis and confirms our expected prediction that the bacterial root and rhizosphere communities would reflect their soil histories over their host plants.

Nonetheless, that both soil history and the *Brassicaceae* hosts were significant in Trial 2, but not Trial 1, suggests that the dominating, homogenizing effect that the *Brassicaceae* hosts had in Trial 1 on structuring their bacterial communities was curtailed in Trial 2. As noted, all five of the *Brassicaceae* host plants dominated the structure of the bacterial communities in Trial 1, to the exclusion of any effect from the different soil histories, or any variation in soil chemistry due to the particular agricultural management practices and inputs that were employed. However, in Trial

2, we observed that the three different soil histories established the previous year structured different soil bacterial communities, with a much-reduced effect from the *Brassicaceae* host plants. This could suggest that, despite identical experimental protocols, and the use of the appropriate agricultural management practices and inputs, that another factor reduced the impact of the *Brassicaceae* host plants in Trial 2.

One hypothesis for the disparate observations between the two field trials could be that the environmental conditions were 6x drier in the Test Phase of Trial 2: 55.0 mm of precipitation versus 328.4 mm in Trial 1. The drier conditions may have restricted the growth capacity of the *Brassicaceae* host plants, which the data may suggest (Fig. S3). Water availability is a key determinant of plant performance, as it constrains nutrient uptake from the soil, and rhizodeposition. Consequently, the impact of the plant-soil bacterial feedback would be weakened, which would decrease the structure the host plants imposed on their bacterial communities, as we observed in Trial 1 (Fitzpatrick *et al*., 2018). Drought conditions have been previously observed to alter bacterial soil communities. For example, Santos-Medellín *et al*., (2021) noted that various taxa of *Actinobacteria* were enriched in rice endosphere communities, as had been observed previously (Naylor *et al*., 2017, Santos-Medellín *et al*., 2017). Fitzpatrick *et al*., (2018) went a step further and noted that across multiple plant families, the enrichment of *Actinobacteria* appears adaptive for drought conditions. Our results in Trial 2 also exhibited an enrichment in the absolute abundance of *Actinobacteria* (Fig. 3 vs Fig. S10), further suggesting that the *Brassicaceae* host plants were experiencing a drought event.

If the reciprocal plant-soil bacterial community feedback was impaired due to the availability of water, and the host plants were restricted in nutrient uptake and growth, it may account for the decreased influence of the *Brassicaceae* hosts on the bacterial communities in Trial 2. Our data illustrated that soil chemistry accounted for the largest portion of the variance among

the bacterial rhizosphere and root communities in Trial 2 (Fig. 4). In our agricultural setting, soil chemistry is largely a synthesis between previous soil history, current agricultural management practices, and PSF (Bouffaud *et al*., 2014). In the absence of plant hosts to structure the bacterial communities through rhizodeposition, or nutrient uptake, this could suggest why the current soil chemistry was so influential (Kaisermann *et al*., 2017). Finally, the weak PSF could also explain why the three soil histories remained influential a year later, as the subsequent *Brassicaceae* plant-bacterial community feedback was unable to alter the environmental filter and erase the soil history.

Conclusion

Understanding how soil history is involved in structuring bacterial communities through time is a limitation in microbial ecology, and is required for employing microbiome technologies aimed at improving agricultural systems. Here, we found that the three different soil histories established the previous year had very limited impact on the soil bacterial communities, except when the host plants suffered from drought conditions. Each soil history was consistently rewritten by the current *Brassicaceae* plants; four host plants (*Sinapis alba*, *Brassica napus*, *B. juncea*, *B. carinata*) formed nearly the same bacterial communities regardless of soil history, and variations in their management practices and agricultural inputs. *Camelina sativa* plants, however, structured phylogenetically distinct bacterial communities compared to the other hosts, particularly in their roots. These are novel findings that illustrate how the breeding and development of these crop species has had limited impact on altering their bacterial communities, and will be informative for further agricultural practices, including rotations, or intercropping. Moreover, our data demonstrates the *Brassicaceae* plants capacities to overcome the previously established soil history. This is in line with previous work by Hannula *et al*., (2021) which also demonstrated how

the impact of soil history on bacterial soil communities fades rapidly. Our data should help limit concerns that these agricultural crop rotations may negatively disrupt the soil bacterial communities, at least in the short term, and encourage planting diverse *Brassicaceae* plants to help protect against potential phytopathogens.

Our study also highlighted a potential limit of plant-soil microbial community feedback, which allowed the previously established soil history to persist and structure the bacterial root and rhizosphere communities. From our data we argued that the *Brassicaceae* host plants in our second field trial experienced drought conditions, which negatively impacted their feedback on the soil. This could explain why soil history was still able to influence the bacterial soil communities, which was not observed in our first field trial. Future studies should further investigate the limitations of soil history and host plants to structure bacterial communities; we provide some ideas for how to help design such experiments. Our results, along with others (Schlaeppi *et al*., 2014; Dombrowski *et al*., 2016; Erlandson *et al*., 2018; Vieria *et al*., 2020), demonstrate the influence and interactions between soil chemistry, host plants, and previously established soil history, in structuring soil bacterial communities. Our study illustrates how agricultural systems, PSFs and soil history, impacts soil bacterial communities and biodiversity, and offers new pathways forward for future research.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada, CRD Fund (Grant Number: CRDPJ 500507-16), Canola Council of Canada and Saskatchewan Pulse Growers, which are gratefully acknowledged. We thank Yantai Gan and Lee Poppy for setting up and managing field experiments at Swift Current. We also thank Stéphane Daigle for assistance in statistical analysis, Chih-Ying Lay, Jacynthe Masse, Chantal Hamel, and

Simon Joly and for their helpful comments and discussions. Finally, AB would like to thank Simon Morvan, Alexis Carteron, and the Quebec Centre for Biodiversity Science for teaching me to use R, and all their support in this project.

Author Contributions

AJCB prepared the libraries for sequencing, performed the qPCR experiment, analyzed the data, and wrote the manuscript with input from all co-authors. LB conducted field trials 1 and 2 and collected data; MSA, & MH designed the experiment, supervised the work, contributed reagents, analytical tools, and revised the manuscript.

Data Accessibility

Sequencing data and metadata are available at NCBI Bioproject under accession number: PRJNA786355.

Supplementary Materials

16S rRNA gene amplicon generation

The 16S PCR reactions consisted of 11.5 µL dH_2O, 5.0 µL of 10X Buffer (Qiagen, Canada), 2.5 µL of 10 µM S-D-Bact-0341-b-S-17 forward and S-D-Bact-0785-a-A-21 reverse primers, commonly referred to as 341F and 805R, respectively (Alpha DNA, Montréal, Canada; Klindworth *et al*., 2012), 1.0 µL of dNTPs (Qiagen, Canada), 0.5 µL of *T. aq* polymerase (Qiagen, Canada), and 2 µL of template DNA, for a total volume of 25 µL. Template DNA ranged in concentration from ~5-15 ng/µL. 16S rRNA gene primers, F17 and R21, indicated in Supplementary Table 2, were synthesized with the CS1 and CS2 adapters, respectively, and HPLC

purified, as per Genome Québec's (Montréal, Canada) submission requirements. PCR amplification of a 416 bp fragment from the V3-V4 region of the 16S rRNA gene (Klindworth *et al*., 2012) was run in an Eppendorf Mastercycle ProS (Germany) thermocycler, and consisted of an initial denaturation of 2 minutes at 95°C, followed by 30 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 55°C, and 1 minute elongation at 72°C, before a final elongation of 5 minutes at 72°C (Bell *et al*., 2016; Lay *et al*., 2018).

Quality control of the 16S rRNA gene amplicon MiSeq data

In order to evaluate potential biases, or flaws, in our lab preparation, sequencing, and bioinformatic analysis, we included six no-template negative controls in our pipeline, two during DNA extraction, four during 16S rRNA gene amplification, as well as four replicates of a bacterial mock community of known composition (Fig. S2). All six no-template negative controls did yield reads, to a maximum of 3750 in the rhizosphere extraction sample from Trial 2 (Fig. S5A), though they were all confirmed to contain no detectable DNA at any step during our lab manipulation. Given the drastic difference in the number of reads between the six no-template negative controls (Fig. S5A), and the replicates of the mock communities (Fig. S5B), we would suggest that the inferior numbers are sequencing artefacts. Moreover, the stringency of the filterAndTrim step eliminated nearly all the reads from these six samples (Fig. S5A).

The replicates of the bacterial mock community (Fig. S5B) also help to assess the accuracy and sensitivity of our lab preparation, sequencing, and particularly the bioinformatic analysis. The four samples were sequenced to an average of ~101 000 reads, suggesting a consistency in sequencing. Furthermore, Fig. S5B illustrates the reproducibility of the DADA2 pipeline, as each replicate looks similar through each step of the pipeline. Approximately a third of the reads were retained overall, which follows recommended standards (Callahan *et al*., 2016b). The greatest loss

of reads is consistently among the filtering step, further illustrating the stringency of this step to retain only high-quality reads for subsequent analysis.

Evaluating the inference of DADA2, the six no-template negative controls contained between 1 and 32 different ASVs identified from the few remaining reads (Fig. 5A). The majority were inferred as a single ASV identified to the phylum *Cyanobacteria* (Order Chloroplast), which we insured to have removed from the experimental Test Phase *Brassicaceae* data presented (Fig. S5A). The four mock community replicates again largely resembled each other in ASV composition. DADA2 inferred 34– 55 individual ASVs from the retained MiSeq reads, from a community composed of 20 possible ASVs (Fig. S5B). Highlighting the accuracy of the DADA2 pipeline to correctly infer ASVs, every bacterial species included in the mock community (Table S1) was detected in all four replicates.

The *Bacteroidetes*, *Deinococcus*, and *Actinobacteria*, were the most specific, as only 1, or 2, species were included in the mock community, and were accurately detected in the pipeline in each replicate (Fig. S5B). Interestingly, both mock replicates from among the endosphere samples added a third ASV identified as an *Actinobacteria*, while the other two replicates did not, despite there only being two *Actinobacteria* ASVs in the community. There was also an expansion among the ASVs identified as *Bacteriodetes* across all four replicates. Though there was only one *Bacteriodetes* included in the mock community, between five and ten were identified in the four mock replicates; the 2016 and 2017 endospheres contained six, the Trial 1 rhizosphere had 8, and the Trial 2 rhizosphere had 10. The mock community contained six *Proteobacteria*, yet eight, nine, 11, and 14, were identified in the Trial 1 rhizosphere, Trial 1 root, Trial 2 root, and Trial 2 rhizosphere, respectively. Fully half of the mock community was composed of *Firmicutes*, and from the possible ten included in the mock community, the Trial 1 rhizosphere and root both identified 15 Firmicutes ASVs, while the Trial 2 rhizosphere and root had 22, and 28, respectively

(Fig. S5B). All of these "over-identifications" may be due to the heterogenous nature of colony inoculation. Finally, while there were no bacterial specimens of *Cyanobacteria*, nor *Chloroflexi*, included in the mock community, three of the mock replicates had an ASV identified as a *Cyanobacteria*, while one of the replicates also had an ASV identified as *Chloroflexi* (Fig. S5).

Two potential confounding issues with the 16S rRNA gene sequencing data from the Test Phase Brassicaceae bacterial communities are the depth of sequencing, and batch effects from sequencing. First, to confirm that the depth of Illumina sequencing was appropriate to detect the majority of bacteria present in each sample post-processing, a rarefaction curve was plotted using the vegan package in R (Oksanen *et al*., 2020; R Core Team, 2020). Most of the samples plateau by ~15 000 reads in the rhizosphere, and ~5000 reads in the roots, which suggests that the majority of the available bacterial ASVs were estimated from the retained reads in each samples (Fig. S6).

To confirm that the Illumina MiSeq process did not introduce detectable biases into the data, an ordination was used to visual the distribution of the all the Test Phase *Brassicaceae* sequencing data (Fig. S9). Here we see that data falls into the most prominent groupings, compartment, rhizosphere, or root (Fig. S9A), and sampling year (Fig. S9B), as expected. Sequencing data produced from the same Illumina sequencing reaction that was biased in some way would be expected to group together in an unknown *a priori* fashion.

Ordination

As a preliminary exploration of the Test Phase data, the relative, and absolute abundance, were ordinated using a non-metric multidimensional scaling (NMDS) approach. First, singletons were removed from the data, which were subsequently transformed using the Hellinger transformation. The transformed data were then ordinated in phyloseq (McMurdie & Holmes, 2013), where the method was assigned as "NMDS", and the distance matrix was set as "bray".

Similar results were obtained using principal co-ordinate analysis, and a Jaccard distance matrix. Ordinations were then plotted in phyloseq, and statistically tested using PERMANOVA, as described in the Methods section, except Trials, and Compartments (rhizosphere and root), were included in the model, and we used a Bray-Curtis distance matrix.

Generating phylogenetic trees

In order to employ phylogeny-based analysis methods, such as phylogenetic diversity, or UniFrac, for analyzing diversity of the Test Phase *Brassicaceae* bacterial communities, we assembled phylogenies for each compartment, from both field trials. Following the method described by Callahan *et al*., 2016b, 16S rRNA gene sequences for each ASV inferred from the Test Phase *Brassicaceae* data were aligned using a profile-to-profile algorithm (Wang *et al*., 2004), and a dendrogram guide tree, from the `decipher` package (Wright, 2016). With the `phangorn` package (Schliep, 2011), the maximum likelihood of each site was calculated using the `dist.ml` function using a JC69 equal base frequency model, before assembling phylogenies using the neighbour-joining method. An optimized (GTR) nucleotide substitution model was fitted to the phylogeny using the `optim.pml` function. Phylogenies were subsequently added to each `phyloseq` object (McMurdie & Holmes, 2013).

Estimating absolute abundance of bacterial communities by quantitative PCR

In order to better understand and intpret the dynamics of the bacterial communities found among the rhizospheres and roots of the five *Brassicaceae* crop species, we estimated the absolute abundance of the bacterial 16S rRNA gene in each Test Phase DNA sample by qPCR (Azarbad *et al*., 2018; Props *et al*., 2017). First, a standard curve of 16S rRNA gene copy numbers was constructed. Near full-length 1.5 kb 16S rRNA gene fragments were PCR amplified using the

primers PA-27F-YM and PH-R (Bruce *et al*., 1992; Table S1) from DNA extracted from previously used soil samples (Lay *et al*., 2018). The PCR reaction and cycling conditions were as described above. The amplified 1.5 kb 16S rRNA gene fragment was visualized by a 0.7% gel electrophoresis, as described above, quantified using the QuBit dsDNA High Sensitivity Kit (Invitrogen, USA), and then serially diluted to 10^{-9}. One µL of each dilution was then used as template in a 10 µL qPCR reaction.

The 16S rRNA gene qPCR reactions consisted of 5.0 µL of Maxima SYBR Green/ROX qPCR Mix (ThermoFisher Scientific, Canada), 3.4 µL dH_2O, 0.3 µL of 10 µM Eub 338 forward and Eub 518 reverse primers (Alpha DNA, Montréal, Canada; Fierer *et al*., 2005). All qPCR reactions were set-up in triplicate in 96-well plates using the Freedom EVO100 robot (Tecan, Switzerland), with a no-template negative control included on each plate. Reactions were run in a ViiA 7 Real-Time PCR System (Life Technologies, Canada) following the same cycling conditions as described previously for the 16S rRNA PCR amplification. The Eub338/Eub518 qPCR reaction amplified a 200 bp region of the V3 region (Muyzer *et al*., 1993; Nogales *et al*., 1999; Bathe & Hausner, 2006; Davis *et al*., 2009). The number of 16S rRNA gene copies present in the serially diluted standard were calculated using the formula (Godornes *et al*., 2007):

$$\text{Number of 16S rRNA gene copies } \mu L^{-1} = \frac{\text{Avogadro's Constant x DNA (g } \mu L^{-1})}{\text{Number of base pairs x 600 Daltons}}$$

The standard curve for each diluted sample was plotted, with an R^2 value of 0.9938 and an amplification efficiency of -3.2013 (Fig. S7), falling within acceptable values (Fierer *et al*., 2005).

16S rRNA gene copy numbers were estimated for each Test Phase sample by using 1 μL of a 1:10 dilution of DNA as template in the same 16S rRNA qPCR reaction and cycling conditions as described above for the standard curve. Melt curves generated by 0.5°C increments at the end of the qPCR programme confirmed amplicon specificity, and the 16S rRNA gene copy number was determined from the standard curve. A correction to determine the absolute abundance of ASVs from the Test Phase samples was achieved by multiplying the 16S rRNA gene copy number per ng, as estimated from the qPCR reaction, by the relative abundance matrix of ASVs identified (Azarbad *et al*., 2018; Bakker, 2018).

Total community absolute abundance per sample, and their distributions within each *Brassicaceae* host and soil history, were plotted. Absolute abundance data from the rhizosphere and root of each sampling year were log transformed. We used a Multi-Factor ANOVA to test for statistical differences in the means of community size between soil histories, *Brassicaceae* hosts, and their interaction, using the anova function (see Wang *et al*., 2020 for details). All assumptions were confirmed to be respected: normality of the residuals established with a Shapiro-Wilk test, shapiro.test, while the heteroscedascity of residuals was confirmed with using a Bartlett test, bartlett.test. For significant ANOVAs, a *post hoc* Tukey's Honest Significant Difference test, TukeyHSD, was used to determine which groups were statistically different.

Identification of differentially abundant ASVs and specific indicator species

Taxa cluster maps were generated using compare_groups, in the metacoder package (Foster *et al*., 2017), the non-parametric Wilcoxon Rank Sum Tests determined if a randomly selected abundance from one group was greater on average than a randomly selected abundance from another group. As the statistical test was performed for each taxon, we used a false discovery rate (FDR) correction on the p-values to account for the multiple comparisons. When the

comparison was between more than two groups, the differential abundances were plotted onto the taxa cluster map using heat_tree_matrix (Foster *et al*., 2017).

We performed an indicator species analysis for the Test Phase ASVs identified in the rhizosphere and roots of both experiments. We obtained similar results from the relative, and absolute, abundance datasets, and so are only reporting the results using the absolute abundance. From the indicspecies package (De Cáceres & Legendre, 2009), we used the multipatt function with 9999 permutations. As the statistical test is performed for each ASV, we used the FDR correction on the p-values to account for multiple comparisons.

Cluster Analysis

We also generated cluster diagrams to further explore the relationships between bacterial communities. Using the Bray-Curtis and Unifrac (weighted and unweighted) distance matrices generated for the PERMANOVA, we produced clusters with the hclust function. Simple and complete clustering approaches, as well as different distance matrices yielded similar results. We only retained the cluster diagram from the Trial 1 bacterial root communities (Fig. S14B).

Table S1. Bacterial strains included in the mock community (BEI Resources, USA) of known composition, was included on each plate (Fig. S2). The mock community contains DNA of 20 bacterial species in equimolar counts (10^6 copies/μL) of 16S rRNA genes. Taxa have been provided to illustrate the level of comparison.

Bacteria	Taxonomy		
	Phyla	Class	Order/Family
Actinomyces odontolyticus	Actinobacteria (P)	Actinomycetales (C)	
Propionibacterium acnes	Actinobacteria (P)	Actinomycetales (C)	
Bacteroides vulgatus	Bacteroidetes (P)		
Deinococcus radiodurans	Deinococcus (P)		
Bacillus cereus	Firmicutes (P)	Bacilli (C)	Bacillales (O)/ Bacillaceae (F)
Listeria monocytogenes	Firmicutes (P)	Bacilli (C)	Bacillales (O)/ Listeriaceae (F)
Staphylococcus aureus	Firmicutes (P)	Bacilli (C)	Staphylococcaceae (F)
Staphylococcus epidermidis	Firmicutes (P)	Bacilli (C)	Staphylococcaceae (F)
Enterococcus faecalis	Firmicutes (P)	Bacilli (C)	Lactobacillales (O)/ Enterococcaceae (F)
Lactobacillus gasseri	Firmicutes (P)	Bacilli (C)	Lactobacilliaceae (F)
Streptococcus pneumoniae	Firmicutes (P)	Bacilli (C)	Streptococcaceae (F)
Streptococcus agalactiae	Firmicutes (P)	Bacilli (C)	Streptococcaceae (F)
Streptococcus mutans	Firmicutes (P)	Bacilli (C)	Streptococcaceae (F)
Clostridium beijerinckii	Firmicutes (P)	Clostridia (C)	Clostridiale (O)
Rhodobacter sphaeroides	Proteobacteria (P)	Alphaproteobacteria (C)	

Neisseria meningitides	Proteobacteria (P)	Betaproteobacteria (C)	
Helicobacter pylori	Proteobacteria (P)	Epsilonproteobacteria (C)	
Escherichia coli K12	Proteobacteria (P)	Gammaproteobacteria (C)	Enterobacteriales (O)
Acinetobacter baumannii	Proteobacteria (P)	Gammaproteobacteria (C)	Pseudomonadales (O)/ Moraxellaceae (F)
Pseudomonas aeruginosa PAO1-LAC	Proteobacteria (P)	Gammaproteobacteria (C)	Pseudomonadaceae (F)

Table S2. Primers used in this study.

Name	Sequence (5'-3')	Reference
S-D-Bact-0341-b-S-17	CCTACGGGNGGCWGCAG	Klindworth *et al*., 2012
S-D-Bact-0785-a-A-21	GACTACHVGGGTATCTAATCC	Klindworth *et al*., 2012
CS1 Adapters	ACACTGACGACATGGTTCTACA	Illumina, 2013
CS2 Adapters	TACGGTAGCAGAGACTTGGTCT	Illumina, 2013
16S PA-27F-YM	AGAGTTTGATCCTGGCTCAG	Bruce *et al*., 1992
16S PH-R	AAGGAGGTGATCCAGCCGCA	Bruce *et al*., 1992
Eub338	ACTCCTACGGGAGGCAGCAG	Fierer *et al*., 2005
Eub518	ATTACCGCGGCTGCTGG	Fierer *et al*., 2005

Table S3. PERMANOVA for all the sampled Test Phase communities identified compartment (rhizosphere, or root), year harvested (2016 for Trial 1, or 2017 for Trial 2), *Brassicaceae* host, and soil history established in the Conditioning Phase, as significant experimental factors. PERMANOVA was calculated using a Bray-Curtis distance matrix, with 9999 permutations.

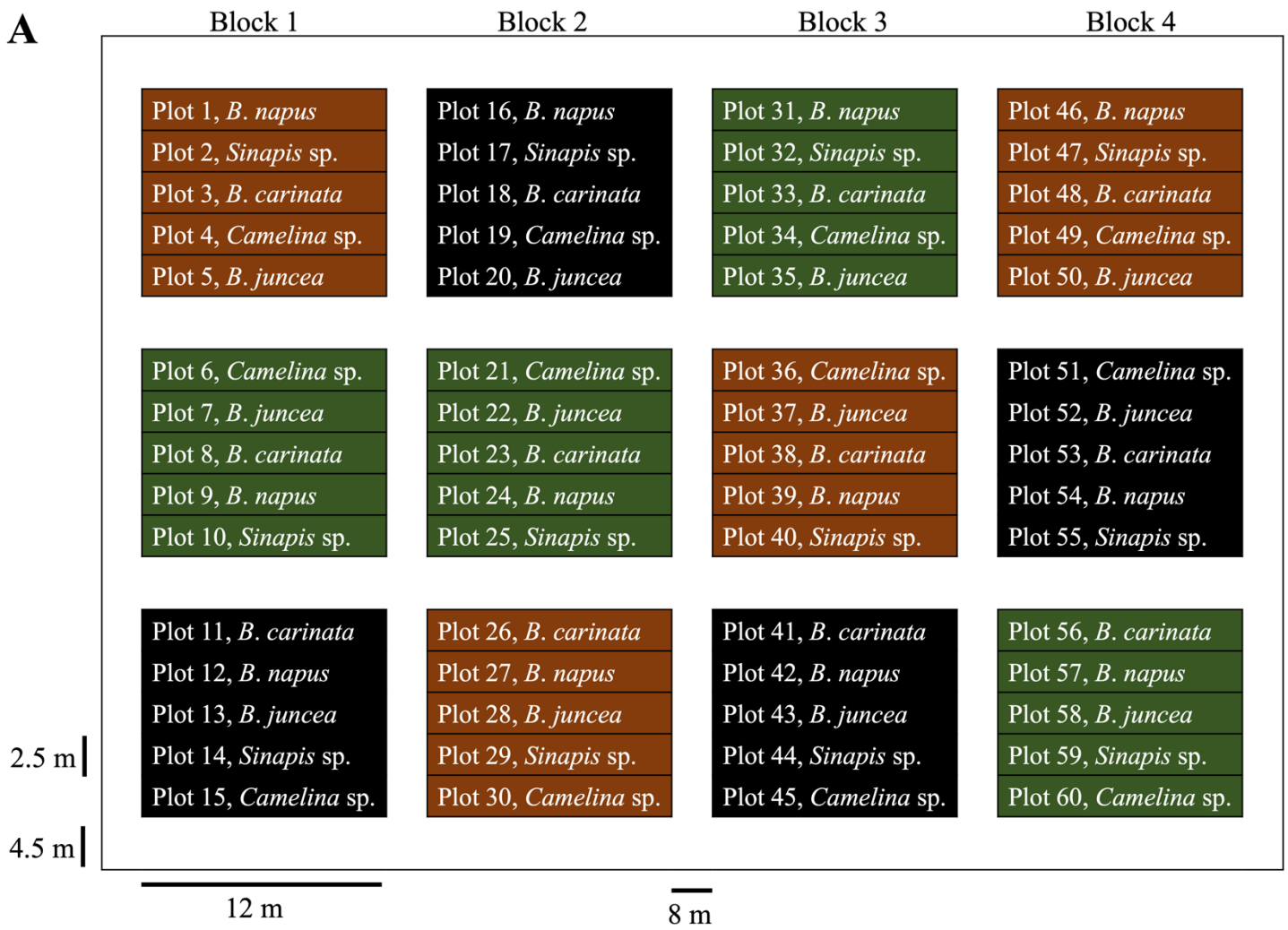
	Whole Dataset with Bray-Curtis Distances[a]		
Experimental Factors	F Model	R^2	Pr (> F)
Year[b]	25.170	0.07067	**0.001**
Compartment[c]	68.750	0.19304	**0.001**
Host[d]	1.700	0.01909	**0.003**
Crop History[e]	1.634	0.00918	**0.029**
Year ~ Compartment	14.743	0.04139	**0.001**
Year ~ Host	2.548	0.02862	**0.001**
Year ~ Crop History	2.047	0.01149	**0.005**
Compartment ~ Host	1.257	0.01412	0.071
Compartment ~ Crop History	1.257	0.00706	0.139
Host ~ Crop History	0.993	0.02232	0.458
Year ~ Compartment ~ Host	1.931	0.02169	**0.001**
Year ~ Compartment ~ Crop History	1.325	0.00744	0.087

[a], Values in bold indicate significant factors or interactions
[b], Trial 1 test phase conducted in 2016, or Trial 2 test phase conducted in 2017
[c], Rhizosphere, or root
[d], *Brassica carinata*, *B. napus*, *B. juncea*, *Sinapis alba*, or *Camelina sativa*
[e], Fallow, wheat, or lentil

Table S4. Nitrogen (N), phosphorous (P), potassium (K), and sulfur (S), available in the soil upon establishing the Test Phase, and the fertilizer that was subsequently applied during the Test Phase for the experiment at Swift Current, Saskatchewan. Adapted from Hossain *et al*., 2019.

Swift Current Test Phase Plot Fertilization (N-P_2O_5-K_2O-S kg ha $^{-1}$)			
	Soil History	Nutrients Available[a]	Fertilizer Applied
Trial 1	Chem-fallow	37-34-646-22	48-7-0-10
	Lentil	20-33-578-28	65-7-0-10
	Wheat	18-31-511-19	68-7-0-10
Trial 2	Chem-fallow	42-34-446-83	55-7-0-10
	Lentil	30-43-488-83	43-7-0-10
	Wheat	18-26-482-82	67-7-0-10

[a], Measurements taken at *Brassicaceae* planting prior to fertilizing, with available N and S measured at 0–60 cm depth, P and K at 0–15 cm depth.


A
Block 1
Block 2
Block 3
Block 4
Plot 1, B. napus
Plot 2, Sinapis sp.
Plot 3, B. carinata
Plot 4, Camelina sp.
Plot 5, B. juncea
Plot 16, B. napus
Plot 17, Sinapis sp.
Plot 18, B. carinata
Plot 19, Camelina sp.
Plot 20, B. juncea
Plot 31, B. napus
Plot 32, Sinapis sp.
Plot 33, B. carinata
Plot 34, Camelina sp.
Plot 35, B. juncea
Plot 46, B. napus
Plot 47, Sinapis sp.
Plot 48, B. carinata
Plot 49, Camelina sp.
Plot 50, B. juncea
Plot 6, Camelina sp.
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Plot 8, B. carinata
Plot 9, B. napus
Plot 10, Sinapis sp.
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Plot 39, B. napus
Plot 40, Sinapis sp.
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Plot 52, B. juncea
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Plot 11, B. carinata
Plot 12, B. napus
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Plot 26, B. carinata
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Plot 28, B. juncea
Plot 29, Sinapis sp.
Plot 30, Camelina sp.
Plot 41, B. carinata
Plot 42, B. napus
Plot 43, B. juncea
Plot 44, Sinapis sp.
Plot 45, Camelina sp.
Plot 56, B. carinata
Plot 57, B. napus
Plot 58, B. juncea
Plot 59, Sinapis sp.
Plot 60, Camelina sp.
2.5 m
4.5 m
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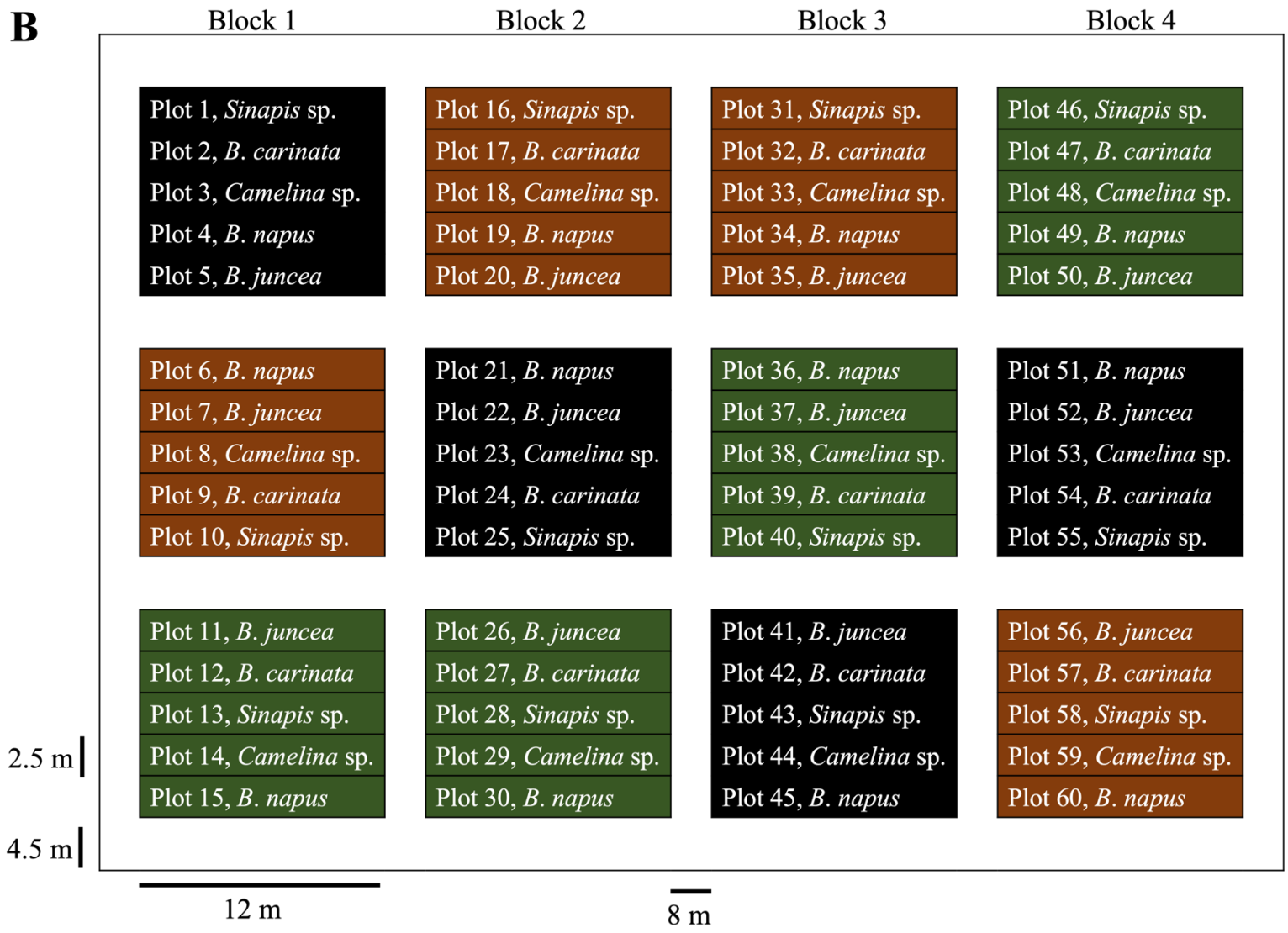
B
Block 1
Block 2
Block 3
Block 4
Plot 1, Sinapis sp.
Plot 2, B. carinata
Plot 3, Camelina sp.
Plot 4, B. napus
Plot 5, B. juncea
Plot 16, Sinapis sp.
Plot 17, B. carinata
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Plot 45, B. napus
Plot 56, B. juncea
Plot 57, B. carinata
Plot 58, Sinapis sp.
Plot 59, Camelina sp.
Plot 60, B. napus
2.5 m
4.5 m
12 m
8 m

Figure S1. Field plans for the experiment. A two-phase cropping sequence—consisting of a Conditioning Phase the first year, and a Test Phase in the second year—was repeated in two field trials, Trial 1, 2015-2016, and Trial 2, 2016-2017, on adjacent sites in a field previously growing spring wheat (*Triticum aestivum* cultivar AC Lillian). The experimental design was a split-plot replicated in four complete blocks. In the 'Conditioning Phase', three soil history treatments were randomly assigned, consisting of spring wheat (*Triticum aestivum*, cv. AC Lillian), red lentil (*Lens culinaris* cv. CDC Maxim CL), or left fallow (brown, black, green, respectively). In the 'Test Phase', the conditioned plots were each subdivided and five *Brassicaceae* oilseed crop species were randomly assigned to one of these five subplots. Thus, each experiment had 60 subplots to sample. (A) Trial 1 field plan for the *Brassicaceae* crops, which were Ethiopian mustard (*Brassica carinata* L., cv. ACC110), canola (*B*. *napus* L., cv. L252LL), oriental mustard (*B*. *juncea* L., cv. Cutlass), yellow mustard (*Sinapis alba* L., cv. Andante), and camelia (*Camelina sativa* L., cv. Midas). Boarder space between plots and blocks is in white. (B) Trial 2 field plan for the same *Brassicaceae* crops. For further details of this well-described experiment and its design, see Hossain *et al*. (2019), Liu *et al*. (2019), and Wang *et al*. (2020).

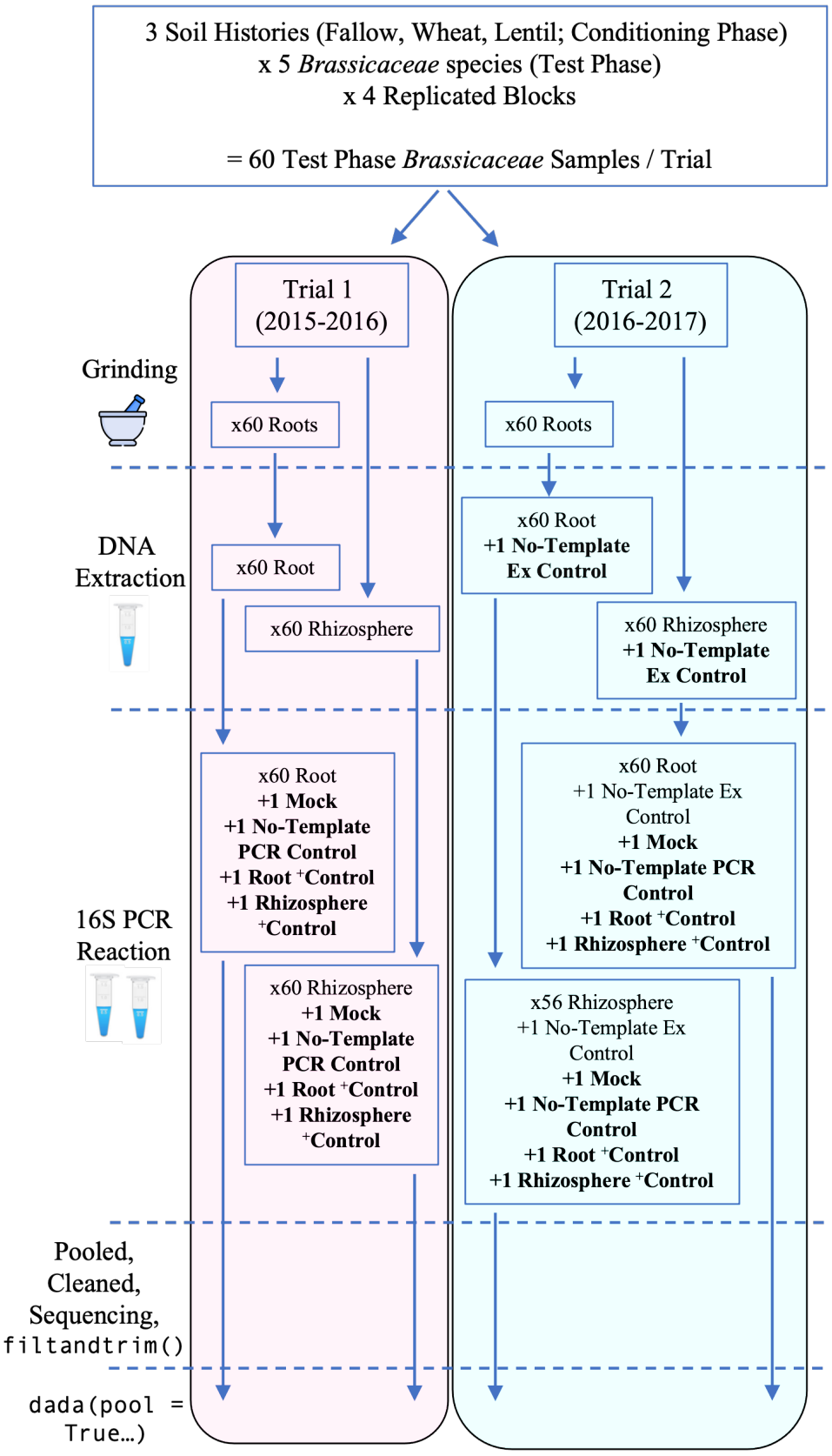

3 Soil Histories (Fallow, Wheat, Lentil; Conditioning Phase)
x 5 *Brassicaceae* species (Test Phase)
x 4 Replicated Blocks
= 60 Test Phase *Brassicaceae* Samples / Trial
Trial 1 (2015-2016)
Trial 2 (2016-2017)
Grinding
x60 Roots
x60 Roots
DNA Extraction
x60 Root
x60 Rhizosphere
x60 Root +1 No-Template Ex Control
x60 Rhizosphere +1 No-Template Ex Control
16S PCR Reaction
x60 Root +1 Mock +1 No-Template PCR Control +1 Root +Control +1 Rhizosphere +Control
x60 Rhizosphere +1 Mock +1 No-Template PCR Control +1 Root +Control +1 Rhizosphere +Control
x60 Root +1 No-Template Ex Control +1 Mock +1 No-Template PCR Control +1 Root +Control +1 Rhizosphere +Control
x56 Rhizosphere +1 No-Template Ex Control +1 Mock +1 No-Template PCR Control +1 Root +Control +1 Rhizosphere +Control
Pooled, Cleaned, Sequencing, filtandtrim()
dada(pool = True…)

Figure S2. Organization of our lab workflow for the Test Phase *Brassicaceae* samples from harvest to generating amplicon sequence variants (ASVs). The Test Phase *Brassicaceae* samples were harvested in mid-late July. Four plants from two different locations within each of the 60 subplots were excavated and pooled together as a composite sample (Hossain *et al*., 2019; Liu *et al*., 2019, Wang *et al*., 2020). In the field, each plant had its rhizosphere soil divided from the root material, both portions were immediately flash-frozen in liquid nitrogen, and kept on ice. In the lab, roots were ground in liquid nitrogen, and DNA was extracted from all the Test Phase *Brassicaceae* root and rhizosphere portions. No-template extraction controls were included to assess what contaminates, or biases, the extraction kits might impart. All DNA samples were used as templates for PCR amplification of the 16S rRNA gene as a metabarcode. All the samples were PCR amplified twice, in two independent reactions, except four rhizosphere samples from Trial 2, which we were unable to be amplify, and were subsequently excluded. We included root and rhizosphere DNA from a previous experiment as PCR positive controls, as well as a bacterial mock to assess the accuracy, bias, and sensitivity of the lab workflow and bioinformatics pipeline. We also included no-template PCR negative controls, and confirmed by gel electrophoresis that none of the no-template extraction controls, nor the no-template PCR controls, contained DNA prior to sequencing. The two independent PCR reactions were pooled together for each sample, and all controls, cleaned and submitted for paired-end 250 bp Illumina MiSeq sequencing. To help identify sequencing biases, or bath effects, a replicate of the bacterial mock community was included on each of the four plates submitted for sequencing. All reads were subsequently trimmed and processed through the DADA2 pipeline for ASV inference.

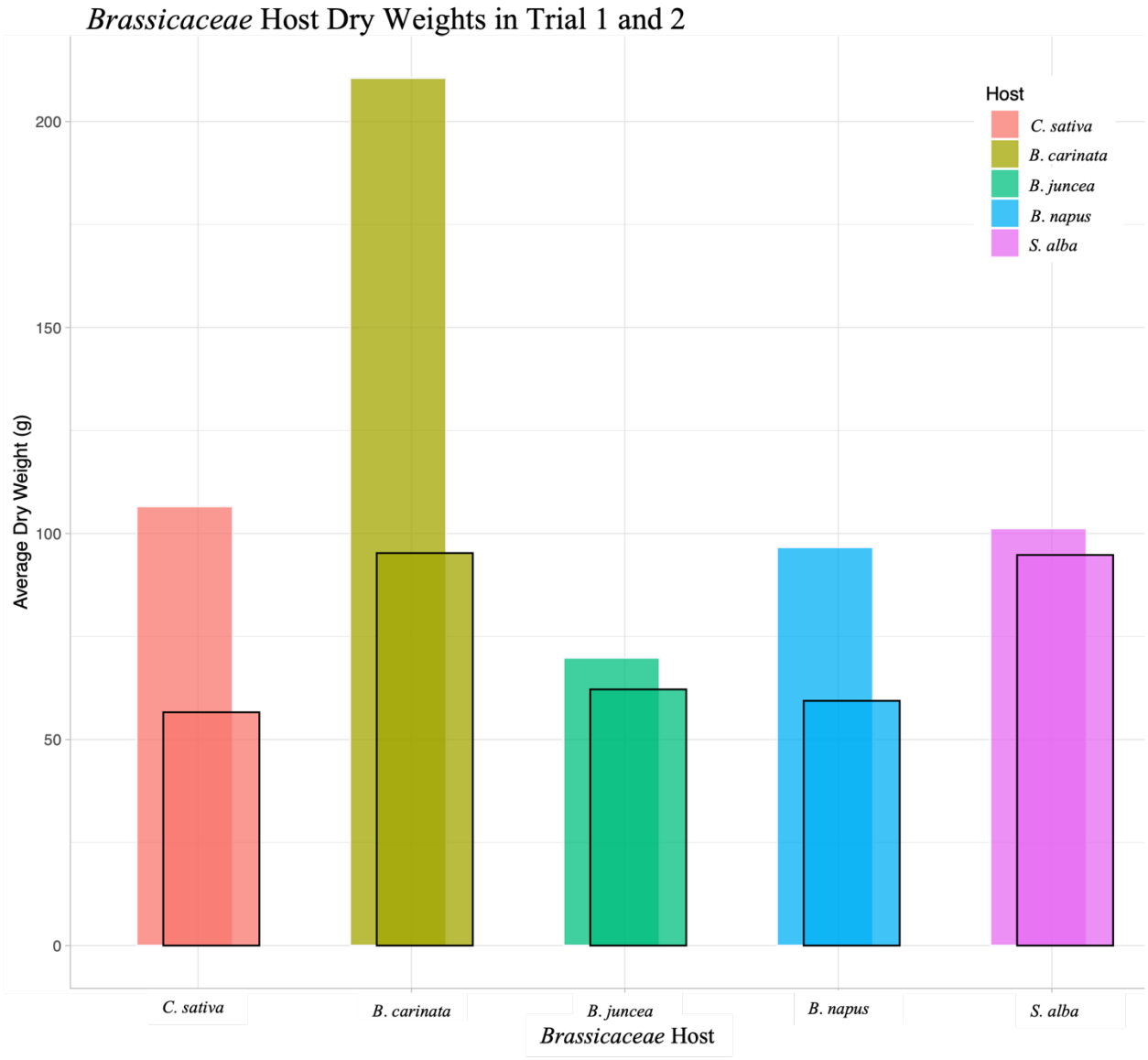


Figure S3. *Brassicaceae* host dry weights (g) decreased in Trial 2 (black outlines), compared to Trial 1 (no outlines). The Test Phase *Brassicaceae* samples were harvested in mid-late July, at Swift Current, Saskatchewan. The aerial portions were retained and dried to determine their weight.

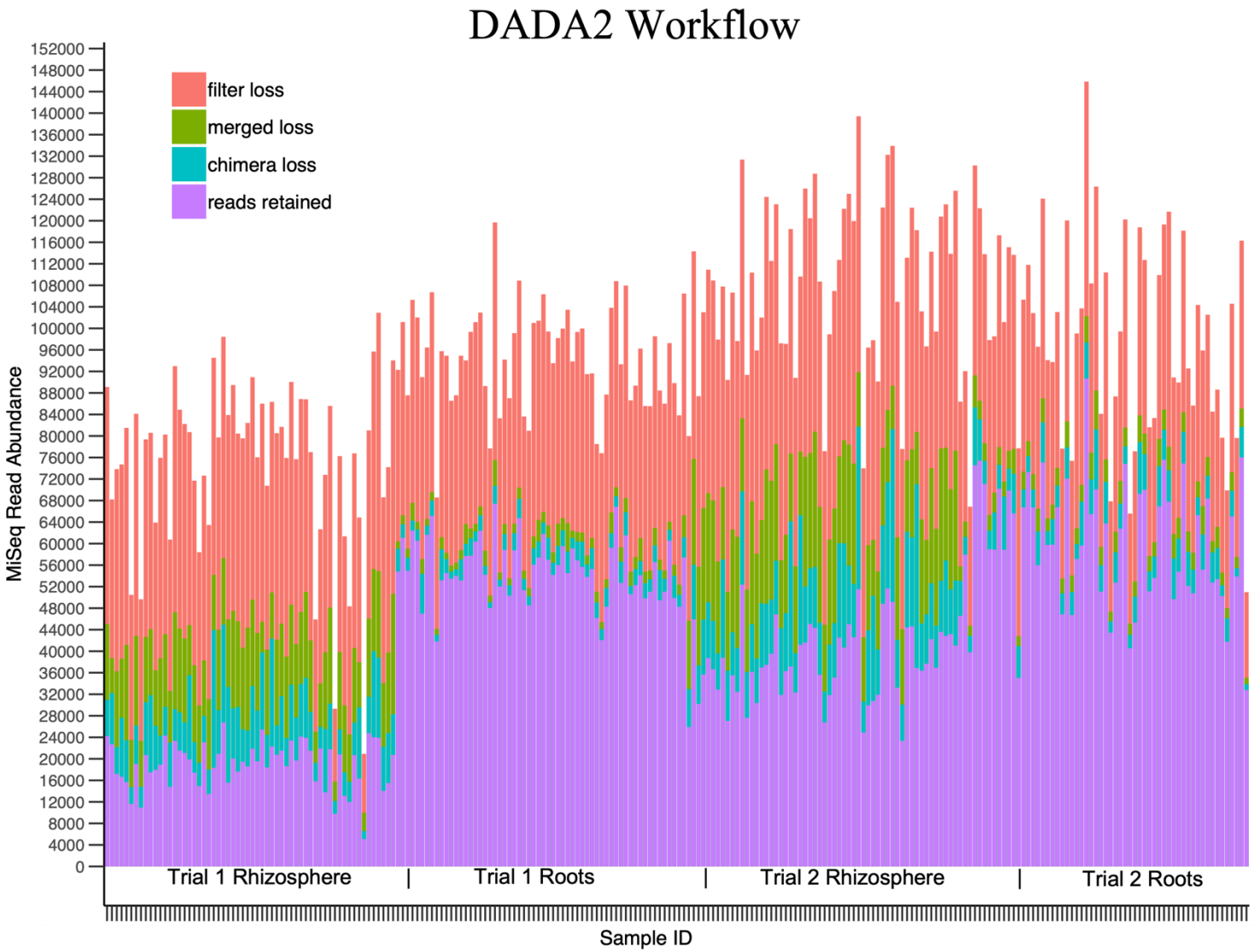
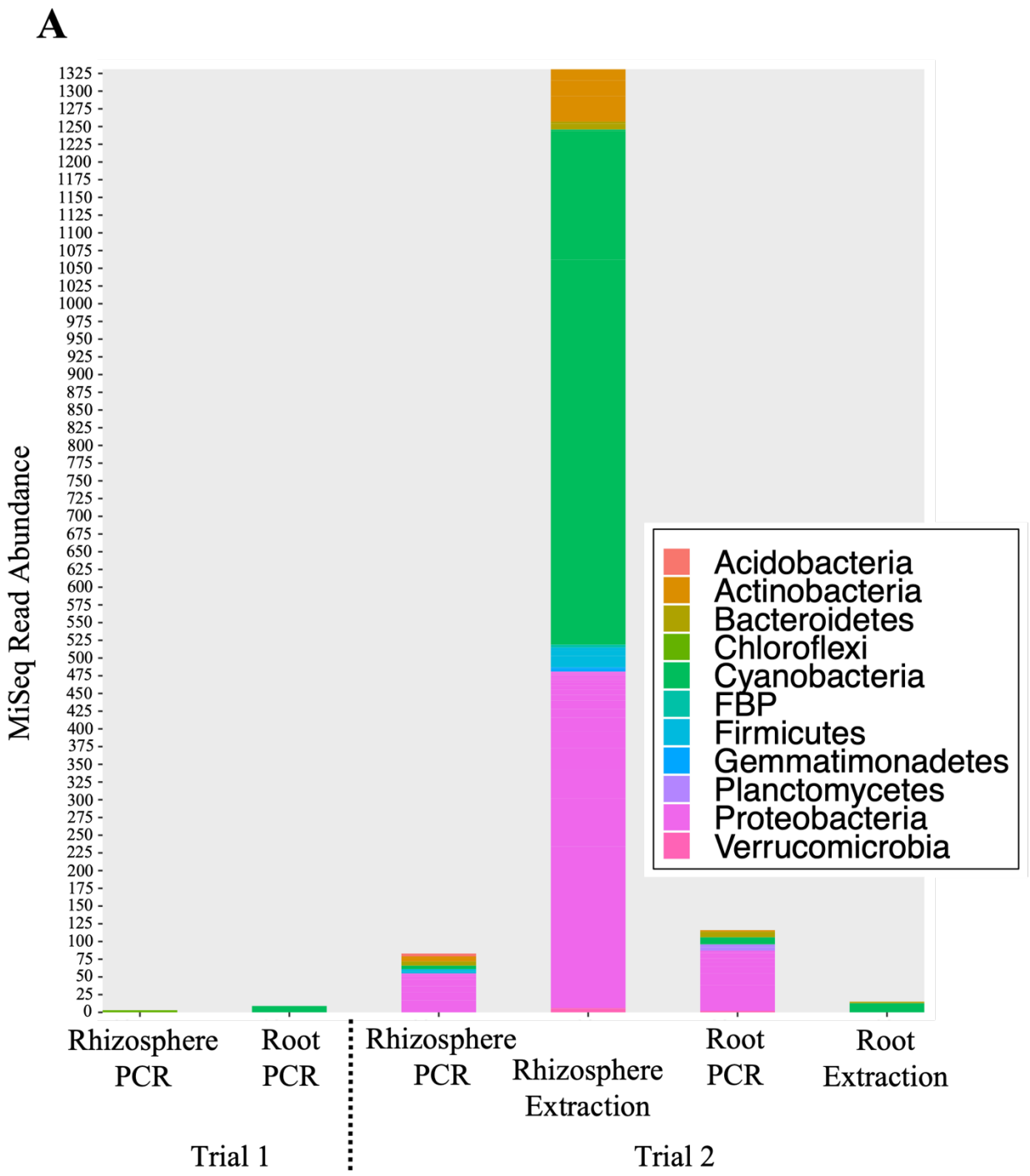

DADA2 Workflow
filter loss
merged loss
chimera loss
reads retained
MiSeq Read Abundance
152000
148000
144000
140000
136000
132000
128000
124000
120000
116000
112000
108000
104000
100000
96000
92000
88000
84000
80000
76000
72000
68000
64000
60000
56000
52000
48000
44000
40000
36000
32000
28000
24000
20000
16000
12000
8000
4000
0
Trial 1 Rhizosphere
Trial 1 Roots
Trial 2 Rhizosphere
Trial 2 Roots
Sample ID

Figure S4. The DADA2 workflow processed 23 313 756 raw reads produced from one lane of sequencing via Illumina's MiSeq at Génome Québec in order to infer amplicon sequence variants (ASVs). An average of 43 129 ±18 032 high-quality reads per Test Phase *Brassicaceae* sample, for a total of 10 178 467, were retained. Trial 2 samples tended to have more reads than the Trial 1 samples. Among the Test Phase Brassicaceae samples, the root samples retained noticeably more reads than their rhizosphere partners. Note that root samples also retained more reads during the merging and chimera steps in DADA2 than the rhizosphere samples. There was no evident difference in reads among the five *Brassicaceae* species, beyond the effects of compartment and year.


A
MiSeq Read Abundance
1325
1300
1275
1250
1225
1200
1175
1150
1125
1100
1075
1050
1025
1000
975
950
925
900
875
850
825
800
775
750
725
700
675
650
625
600
575
550
525
500
475
450
425
400
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Cyanobacteria
FBP
Firmicutes
Gemmatimonadetes
Planctomycetes
Proteobacteria
Verrucomicrobia
Rhizosphere PCR
Root PCR
Rhizosphere PCR
Rhizosphere Extraction
Root PCR
Root Extraction
Trial 1
Trial 2

B

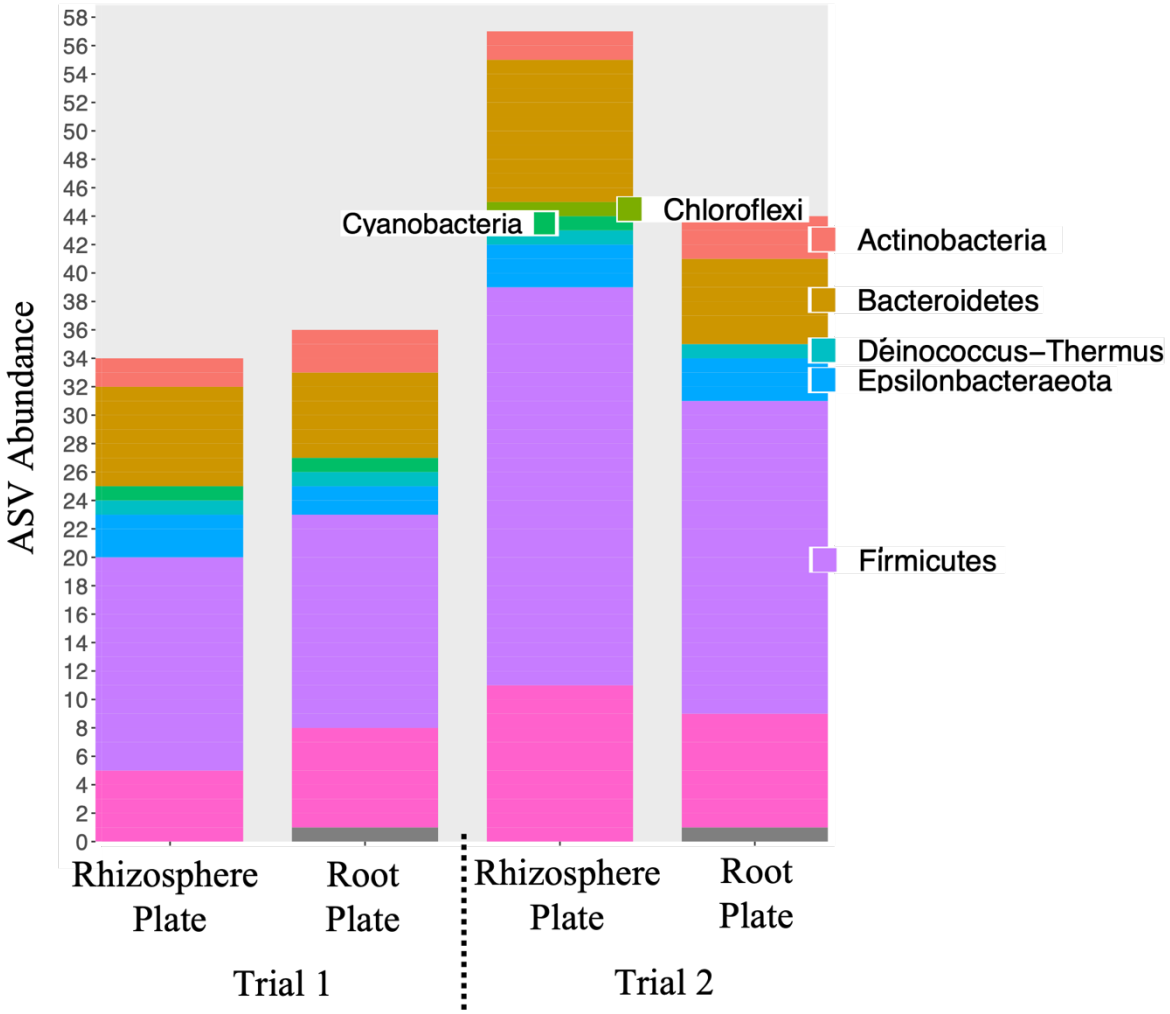

ASV Abundance
58
56
54
52
50
48
46
44
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
Cyanobacteria
Chloroflexi
Actinobacteria
Bacteroidetes
Deinococcus–Thermus
Epsilonbacteraeota
Firmicutes
Rhizosphere Plate
Root Plate
Rhizosphere Plate
Root Plate
Trial 1
Trial 2

Figure S5. High-quality MiSeq reads retained through the DADA2 pipeline from among the no-template negative controls, and mock community replicates, were inferred as inferred amplicon sequence variants (ASVs), and assigned taxonomy using the Silva database, represented here as phlya. A) ASVs inferred from among the six no-template negative controls, represented as their corresponding abundance of reads to illustrate these ASVs were derived from few reads. The negative controls had between 1 and 32 different ASVs, where most reads that were retained were inferred as ASVs identified as *Cyanobacteria* and *Proteobacteria*. B) 34 – 55 individual ASVs were inferred from among the four mock community replicates; the mock replicate prepared with the Trial 2 rhizosphere samples, followed by the mock replicate in the Trial 2 root sample, contained the most ASVs. Every bacterial group included in the mock community was detected in each replicate. The *Actinobacteria*, and *Deinococcus*, were the most specific, as 2, or 1, species were included in the mock community, respectively, and accurately detected in our bioinformatics pipeline. There was an expansion among the ASVs identified as *Bacteriodetes*, *Firmicutes*, and *Proteobacteria*. Three of the mock replicates had an ASV identified as a *Cyanobacteria*, while one of them also replicates had an ASV identified as a *Chloroflexi*, though none were included in the community's composition.

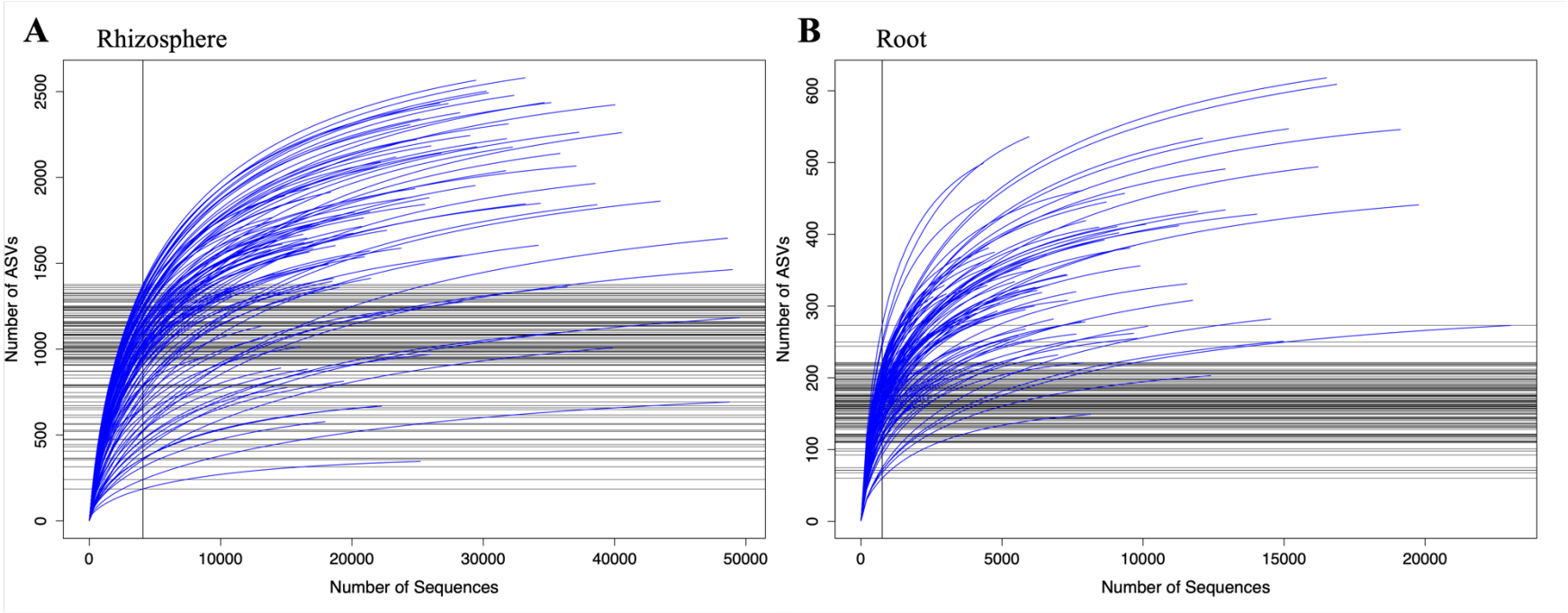


Figure S6. Rarefaction curves illustrated that the majority of the bacterial communities were identified in (A) the rhizosphere, and (B) the roots. The samples were harvested from two field trials during the Test Phase of a two-year crop rotation, in Swift Current, Saskatchewan.

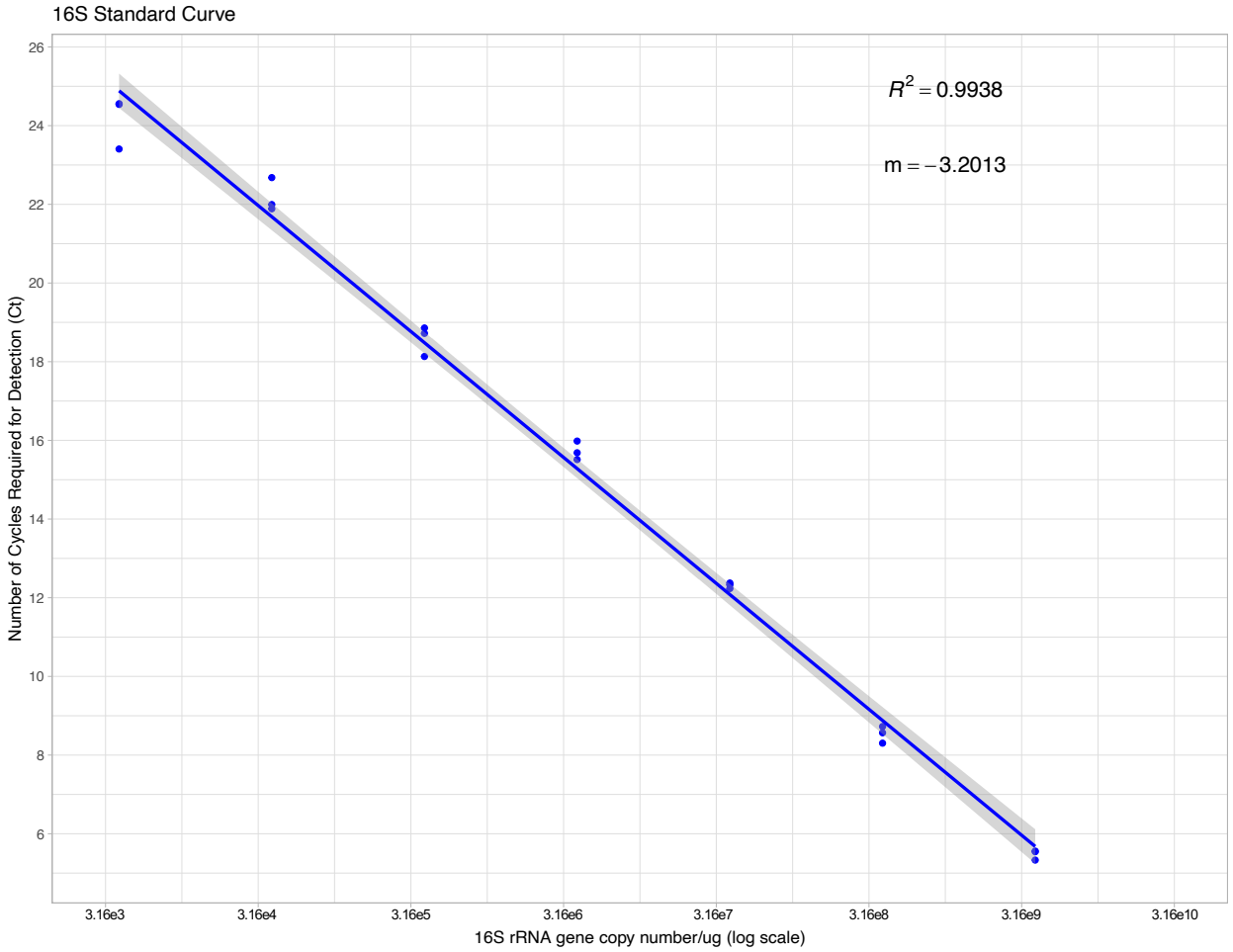
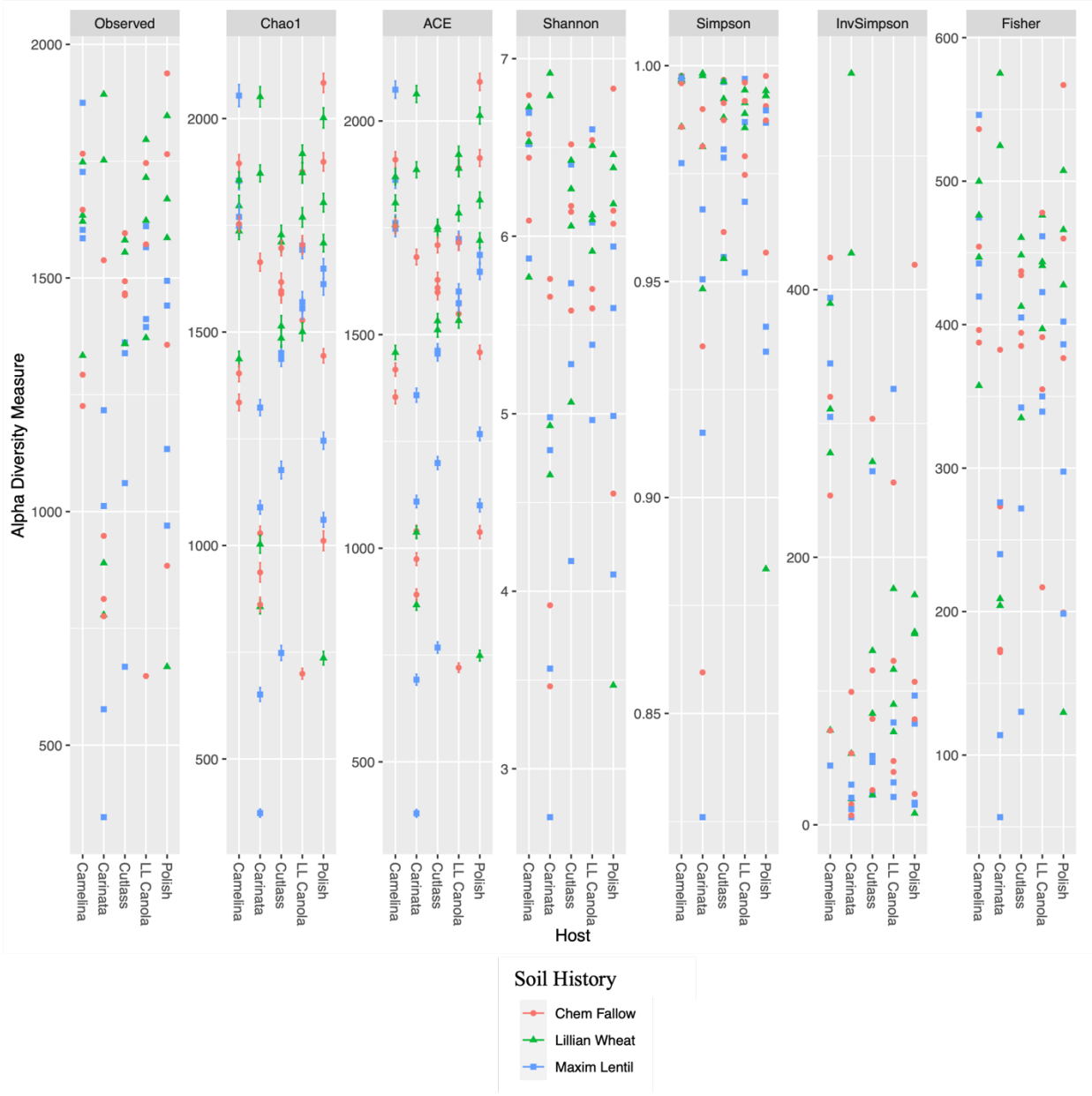


Figure S7. A standard curve of the 16S rRNA gene copy numbers (X-axis) versus the number of cycles required for detection (cycle threshold, Ct, Y-axis), as determined from the serial dilution of a quantified 16S rRNA gene.

A Trial 1 Rhizosphere



B Trial 1 Root

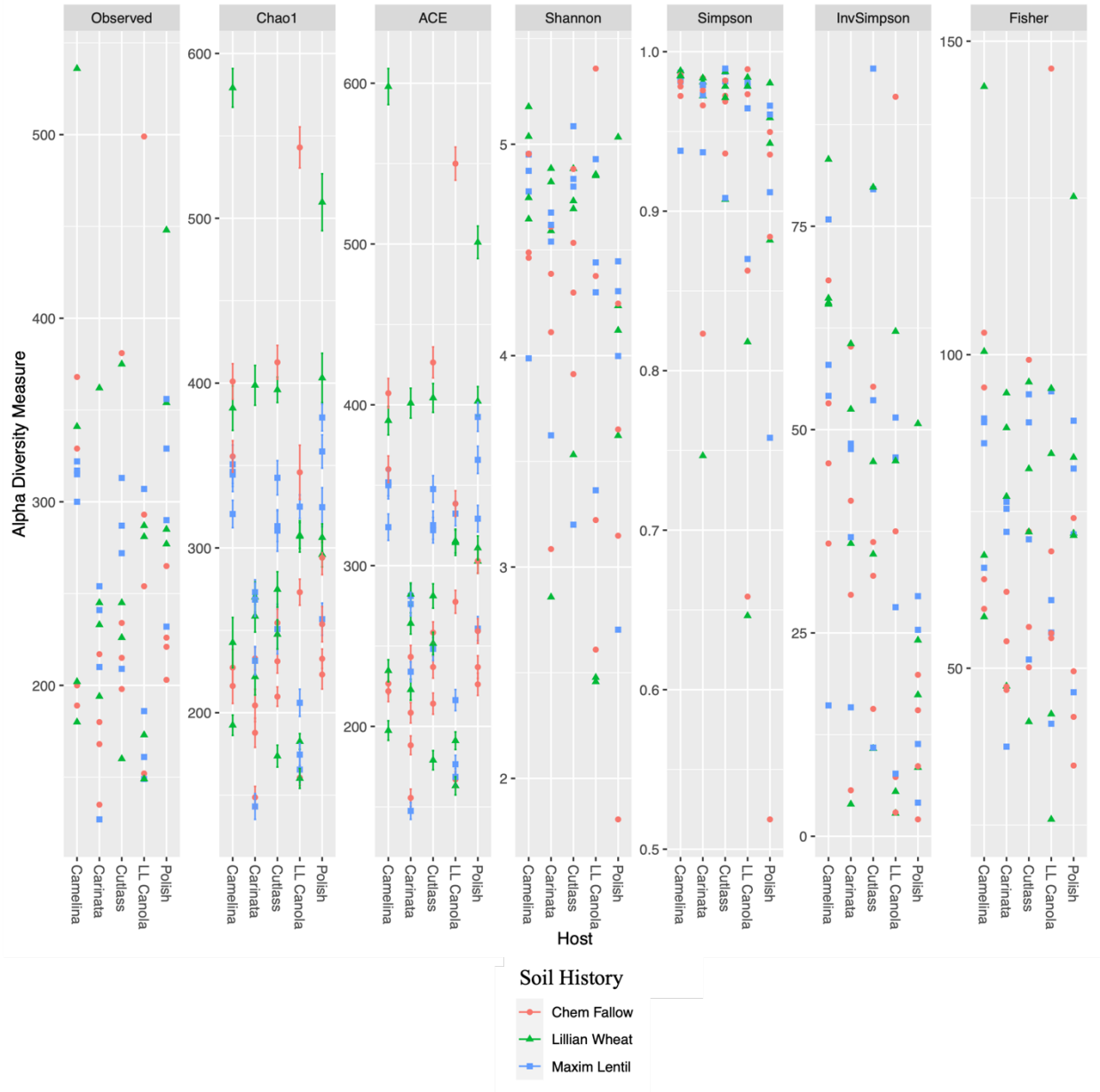


Figure S8. Taxa-based α-diversity indices (y-axis) for the rhizosphere (A) and root (B) communities from Trial 1, harvested 2016. Each α-diversity index was grouped by *Brassicaceae* host, and reflect the phylogenetic diversity observed, where communities are broadly similar across hosts, and soil histories. Similar results were also observed for the communities based on relative abundance, as well as in Trial 2 (data not shown).

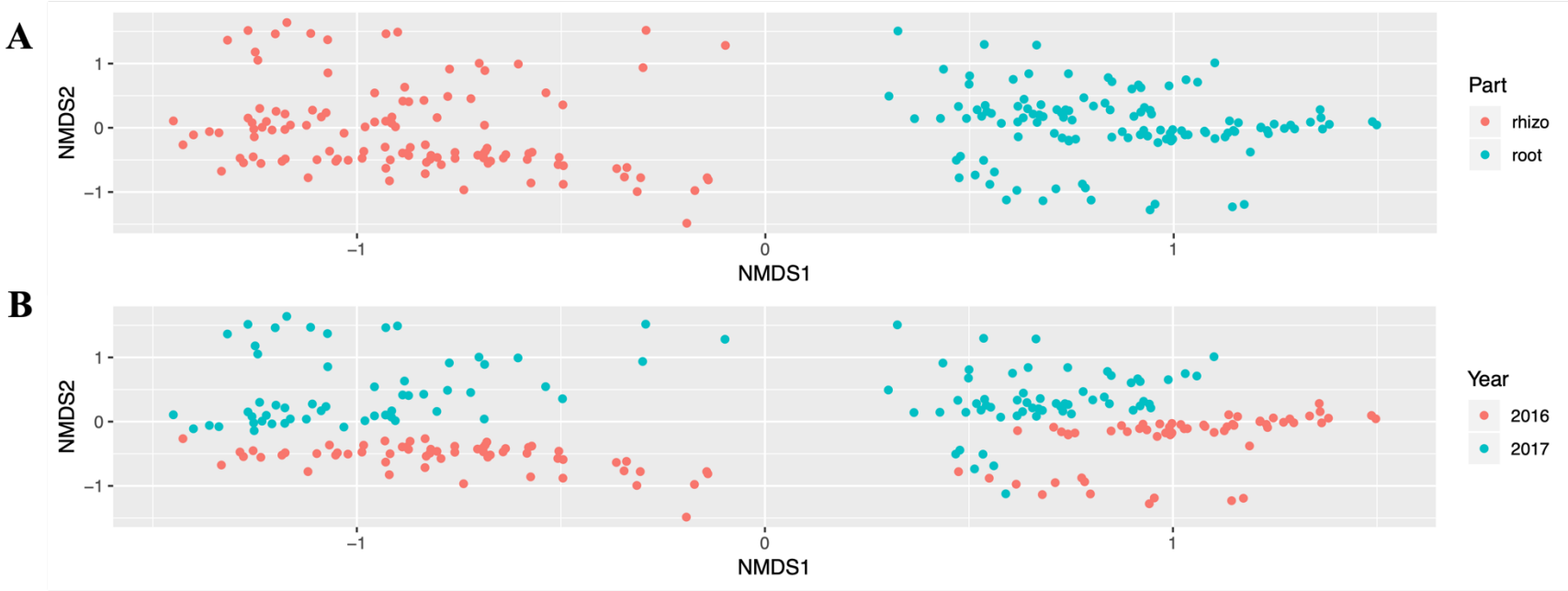
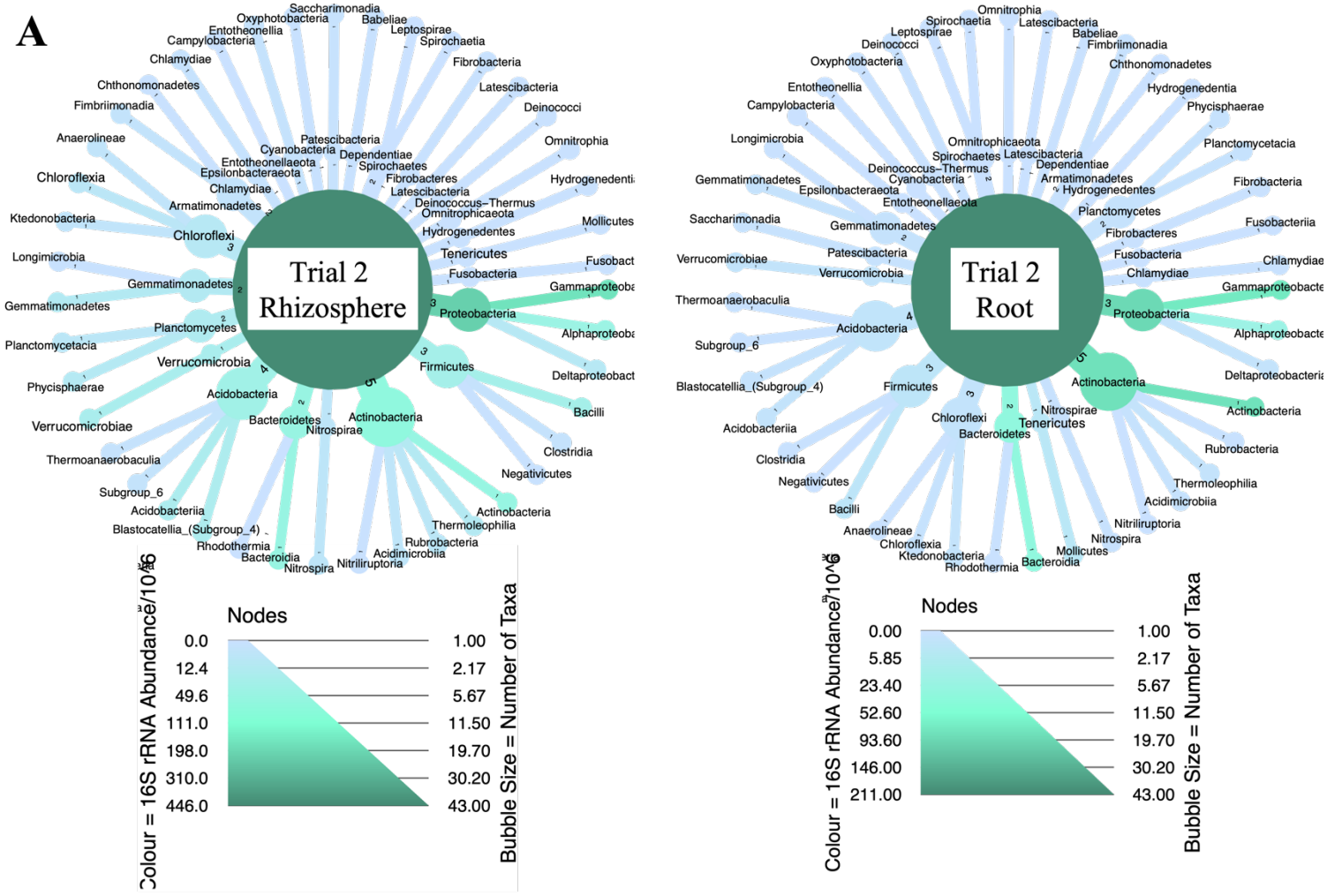


Figure S9. A non-metric multidimensional scaling (NMDS) of all the Test Phase data illustrates that the most important factors shaping the bacterial communities are: A) compartment, rhizosphere (orange), or root (turqoise) (PERMANOVA, $R^2 = 0.19304$, $p = 0.001$), and B) Trial 1 (harvested 2016, orange), or Trial 2 (harvested 2017, turqoise) (PERMANOVA, $R^2 = 0.07067$, $p = 0.001$). As all the bacterial communities appear clustered together respectively by compartment and year, regardless of other factors, we can be confident that the data is not excessively biased, and batch effects have been subsumed within these larger factors/patterns. See Table S3 for these PERMANOVA results.


A
Trial 2
Rhizosphere
Saccharimonadia
Oxyphotobacteria
Babeliae
Entotheonellia
Leptospirae
Campylobacteria
Spirochaetia
Chlamydiae
Fibrobacteria
Chthonomonadetes
Latescibacteria
Fimbriimonadia
Deinococci
Anaerolineae
Omnitrophia
Patescibacteria
Cyanobacteria
Dependentiae
Entotheonellaeota
Spirochaetes
Epsilonbacteraeota
Fibrobacteres
Hydrogenedenti
Chloroflexia
Chlamydiae
Latescibacteria
Armatimonadetes
Deinococcus−Thermus
Omnitrophicaeota
Ktedonobacteria
Chloroflexi
Hydrogenedentes
Mollicutes
Longimicrobia
Tenericutes
Fusobact
Gemmatimonadetes
Fusobacteria
Gammaproteoba
Gemmatimonadetes
Proteobacteria
Planctomycetes
Alphaproteoba
Planctomycetacia
Firmicutes
Verrucomicrobia
Deltaproteobact
Phycisphaerae
Acidobacteria
Bacilli
Bacteroidetes
Actinobacteria
Verrucomicrobiae
Nitrospirae
Clostridia
Thermoanaerobaculia
Negativicutes
Subgroup_6
Actinobacteria
Acidobacteriia
Thermoleophilia
Blastocatellia_(Subgroup_4)
Rubrobacteria
Rhodothermia
Acidimicrobiia
Bacteroidia
Nitrospira
Nitriliruptoria
Nodes
0.0
12.4
49.6
111.0
198.0
310.0
446.0
1.00
2.17
5.67
11.50
19.70
30.20
43.00
Colour = 16S rRNA Abundance/10^6
Bubble Size = Number of Taxa
Trial 2
Root
Omnitrophia
Spirochaetia
Latescibacteria
Leptospirae
Babeliae
Deinococci
Fimbriimonadia
Oxyphotobacteria
Chthonomonadetes
Entotheonellia
Hydrogenedentia
Campylobacteria
Phycisphaerae
Longimicrobia
Omnitrophicaeota
Planctomycetacia
Spirochaetes
Latescibacteria
Deinococcus−Thermus
Dependentiae
Gemmatimonadetes
Cyanobacteria
Armatimonadetes
Fibrobacteria
Epsilonbacteraeota
Hydrogenedentes
Entotheonellaeota
Planctomycetes
Saccharimonadia
Gemmatimonadetes
Fusobacteriia
Fibrobacteres
Verrucomicrobiae
Patescibacteria
Fusobacteria
Chlamydiae
Verrucomicrobia
Chlamydiae
Gammaproteobacte
Thermoanaerobaculia
Proteobacteria
Alphaproteobacte
Acidobacteria
Subgroup_6
Deltaproteobacteria
Actinobacteria
Blastocatellia_(Subgroup_4)
Firmicutes
Actinobacteria
Nitrospirae
Chloroflexi
Tenericutes
Acidobacteriia
Bacteroidetes
Rubrobacteria
Clostridia
Thermoleophilia
Negativicutes
Acidimicrobiia
Bacilli
Nitriliruptoria
Anaerolineae
Nitrospira
Chloroflexia
Mollicutes
Ktedonobacteria
Bacteroidia
Rhodothermia
Nodes
0.00
5.85
23.40
52.60
93.60
146.00
211.00
1.00
2.17
5.67
11.50
19.70
30.20
43.00
Colour = 16S rRNA Abundance/10^6
Bubble Size = Number of Taxa

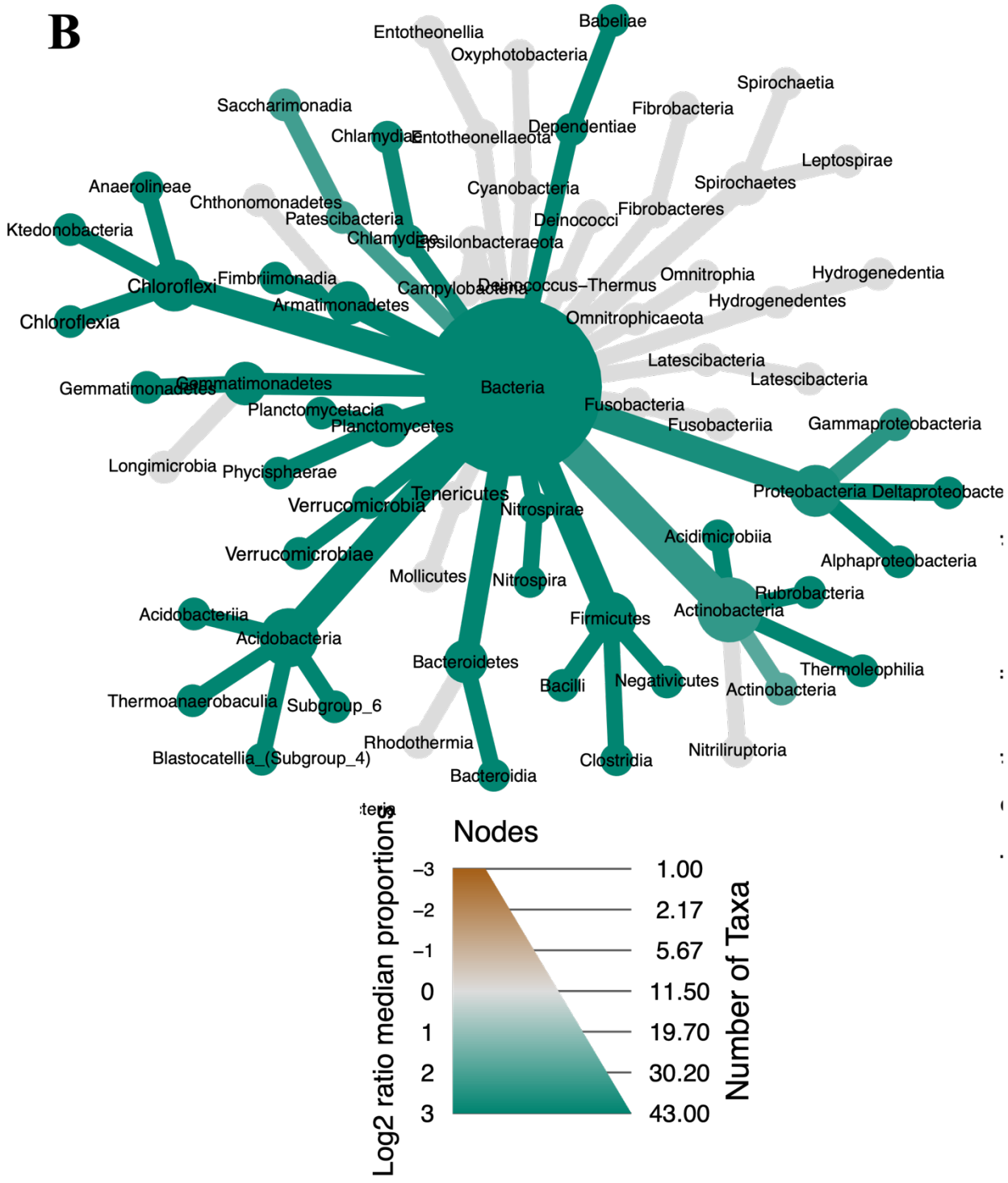

B
Entotheonellia
Babeliae
Oxyphotobacteria
Spirochaetia
Saccharimonadia
Fibrobacteria
Dependentiae
Chlamydiae
Entotheonellaeota
Leptospirae
Anaerolineae
Cyanobacteria
Spirochaetes
Chthonomonadetes
Fibrobacteres
Patescibacteria
Deinococci
Ktedonobacteria
Chlamydiae
Epsilonbacteraeota
Chloroflexi
Fimbriimonadia
Omnitrophia
Hydrogenedentia
Campylobacteria
Deinococcus−Thermus
Armatimonadetes
Hydrogenedentes
Chloroflexia
Omnitrophicaeota
Latescibacteria
Gemmatimonadetes
Bacteria
Latescibacteria
Planctomycetacia
Fusobacteria
Planctomycetes
Fusobacteriia
Gammaproteobacteria
Longimicrobia
Phycisphaerae
Proteobacteria
Deltaproteobacte
Tenericutes
Verrucomicrobia
Nitrospirae
Acidimicrobiia
Verrucomicrobiae
Alphaproteobacteria
Mollicutes
Nitrospira
Rubrobacteria
Actinobacteria
Acidobacteriia
Firmicutes
Acidobacteria
Thermoleophilia
Bacteroidetes
Bacilli
Negativicutes
Actinobacteria
Thermoanaerobaculia
Subgroup_6
Rhodothermia
Nitriliruptoria
Blastocatellia_(Subgroup_4)
Clostridia
Bacteroidia
Nodes
Log2 ratio median proportions
−3
−2
−1
0
1
2
3
Number of Taxa
1.00
2.17
5.67
11.50
19.70
30.20
43.00

Figure S10. (A) Taxa clusters of the ASVs inferred from among the rhizosphere (left) and root (right) of the Test Phase bacterial communities from Trial 2, harvested 2017, represented to the class level. The size of the taxonomic groups (bubbles) represents the number of ASVs that occurred, and the colour scale represents the absolute abundance of each ASV. (B) The differential taxa cluster between the absolute abundance of the rhizosphere and root in Trial 1, where the abundance of each taxonomic group in the cluster is compared between each compartment, using the using the non-parametric Kruskal test and the *post hoc* pairwise Wilcox test, with the FDR correction. Taxa that are significantly (p. adj < 0.05) more abundant in rhizosphere are highlighted in green.

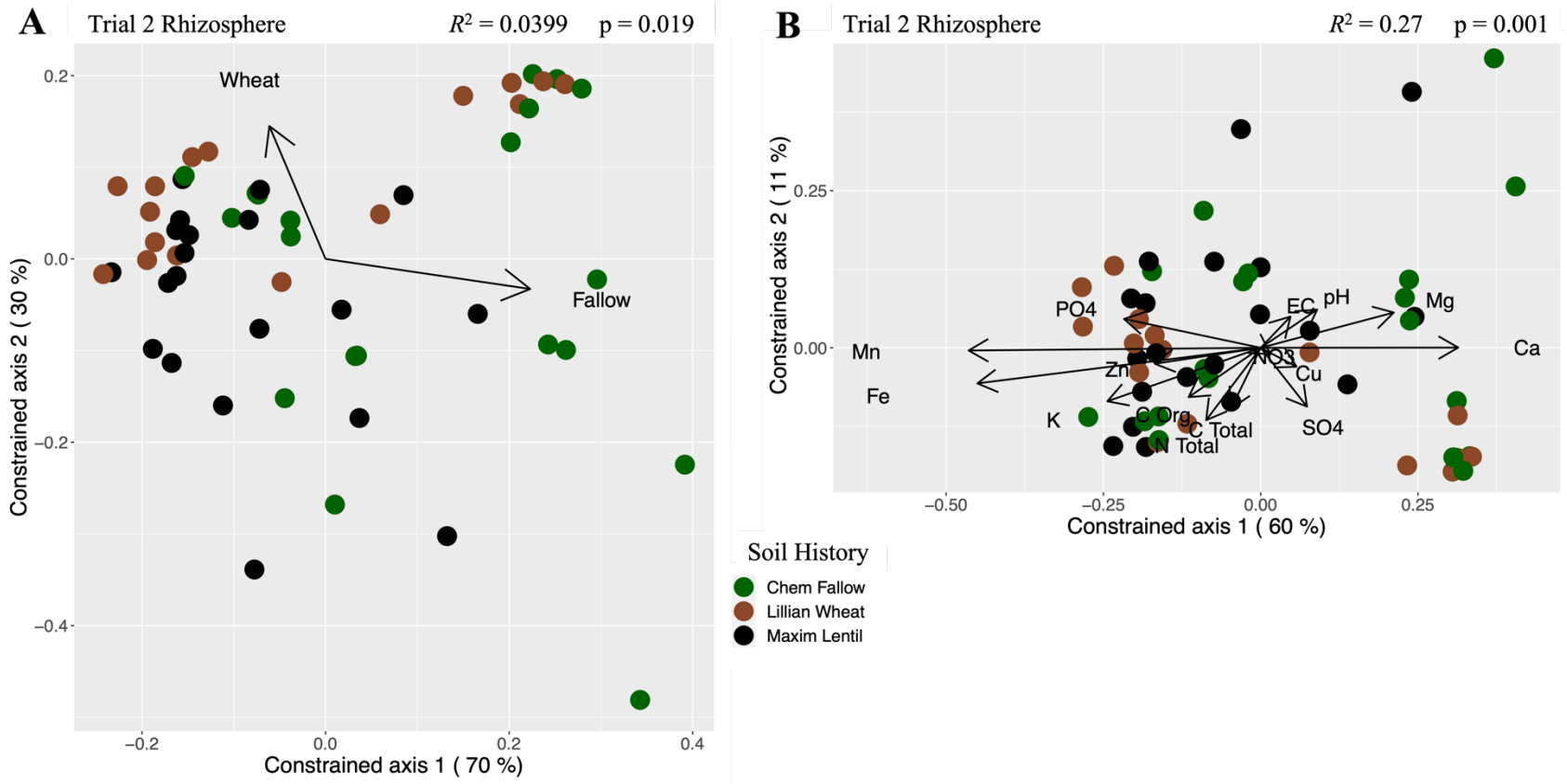

A
Trial 2 Rhizosphere
R^2 = 0.0399
p = 0.019
Wheat
Fallow
Constrained axis 2 (30 %)
Constrained axis 1 (70 %)
B
Trial 2 Rhizosphere
R^2 = 0.27
p = 0.001
PO4
EC
pH
Mg
Mn
Ca
Zn
NO3
Cu
Fe
K
C Org
C Total
SO4
N Total
Constrained axis 2 (11 %)
Constrained axis 1 (60 %)
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil

Figure S11. Bacterial rhizosphere communities exhibited significant structure according to (A) soil history, and (B) soil chemistry in Trial 2, harvested in 2017, from Test Phase of a two-year crop rotation, in Swift Current, Saskatchewan. Weighted UniFrac distances were used with a distance-based redundancy analysis. (A) Soil histories established in the Conditioning Phase were still significant a year later (R^2 = 0.0399, p = 0.019) in structuring the bacterial communities from the Test Phase rhizosphere communities in Trial 2, though it is difficult to observe a clear trend among phylogenetically similar communities being more distinctive according to their soil histories. (B) Soil chemistry was also significant in structuring the Test Phase bacterial rhizosphere communities (R^2 = 0.27, p = 0.001): manganese was contrasted by calcium, iron and zinc contrasted with magnesium, while potassium was opposed by pH, though there is not a clear trend among phylogenetically similar communities being more distinctive according to their soil chemistries. Note that the Test Phase bacterial rhizosphere communities from Trial 2 were not significantly structured by *Brassicaceae* host plants (data not shown).

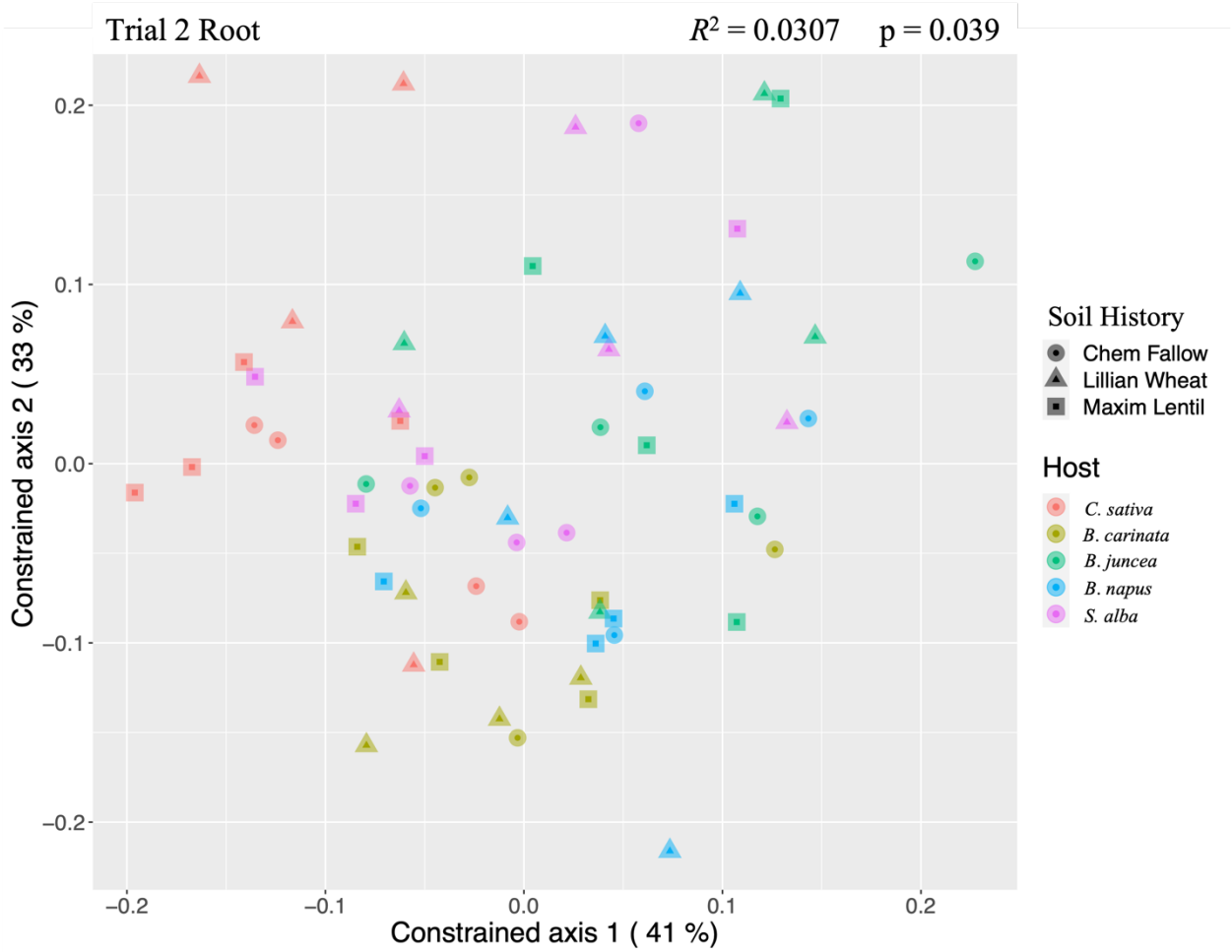


Figure S12. Bacterial root communities from Trial 2 exhibited significant structure according to their *Brassicaceae* host plants when harvested in 2017 from the Test Phase of a two-year crop rotation, in Swift Current, Saskatchewan. Weighted UniFrac distances were used with a distance-based redundancy analysis. *Brassicaceae* host plants were significant (R^2 = 0.0307, p = 0.039) in structuring the Test Phase bacterial root communities.

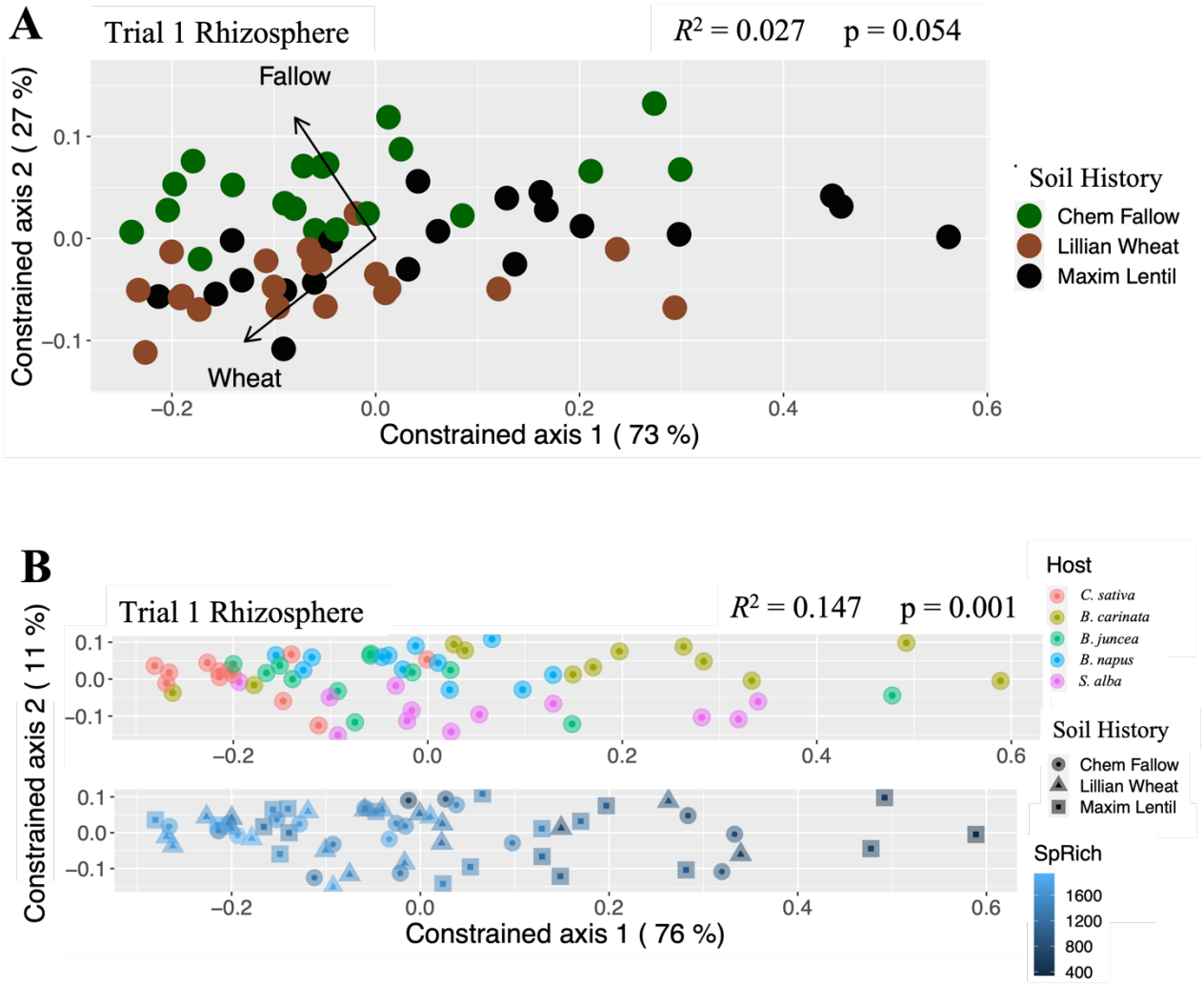

A
Trial 1 Rhizosphere
$R^2 = 0.027$ p = 0.054
Fallow
Wheat
Constrained axis 2 (27 %)
Constrained axis 1 (73 %)
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil
B
Trial 1 Rhizosphere
$R^2 = 0.147$ p = 0.001
Constrained axis 2 (11 %)
Constrained axis 1 (76 %)
Host
C. sativa
B. carinata
B. juncea
B. napus
S. alba
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil
SpRich
1600
1200
800
400

Figure S13. Bacterial rhizosphere communities exhibited significant structure according to (A) soil history, and (B) *Brassicaceae* host plants when harvested in 2016 from the Test Phase in Trial 1 of a two-year crop rotation, in Swift Current, Saskatchewan. Weighted UniFrac distances were used with a distance-based redundancy analysis. (A) Soil histories established in the Conditioning Phase were significant ($R^2 = 0.027$, $p = 0.054$) a year later in structuring the bacterial communities from the Test Phase rhizosphere communities in Trial 1, as illustrated by phylogenetically similar communities being more distinctive according to their soil histories. (B) *C. sativa* (red) bacterial rhizosphere communities were more phylogenetically similar compared to communities from other *Brassicaceae* hosts ($R^2 = 0.147$, $p = 0.001$). Communities from *Brassica carinata* appeared the least structured by host, and strongly impacted by lower species richness (SpRich). Note that the Test Phase bacterial rhizosphere communities in Trial 1 were not significantly structured by soil chemistry (data not shown).

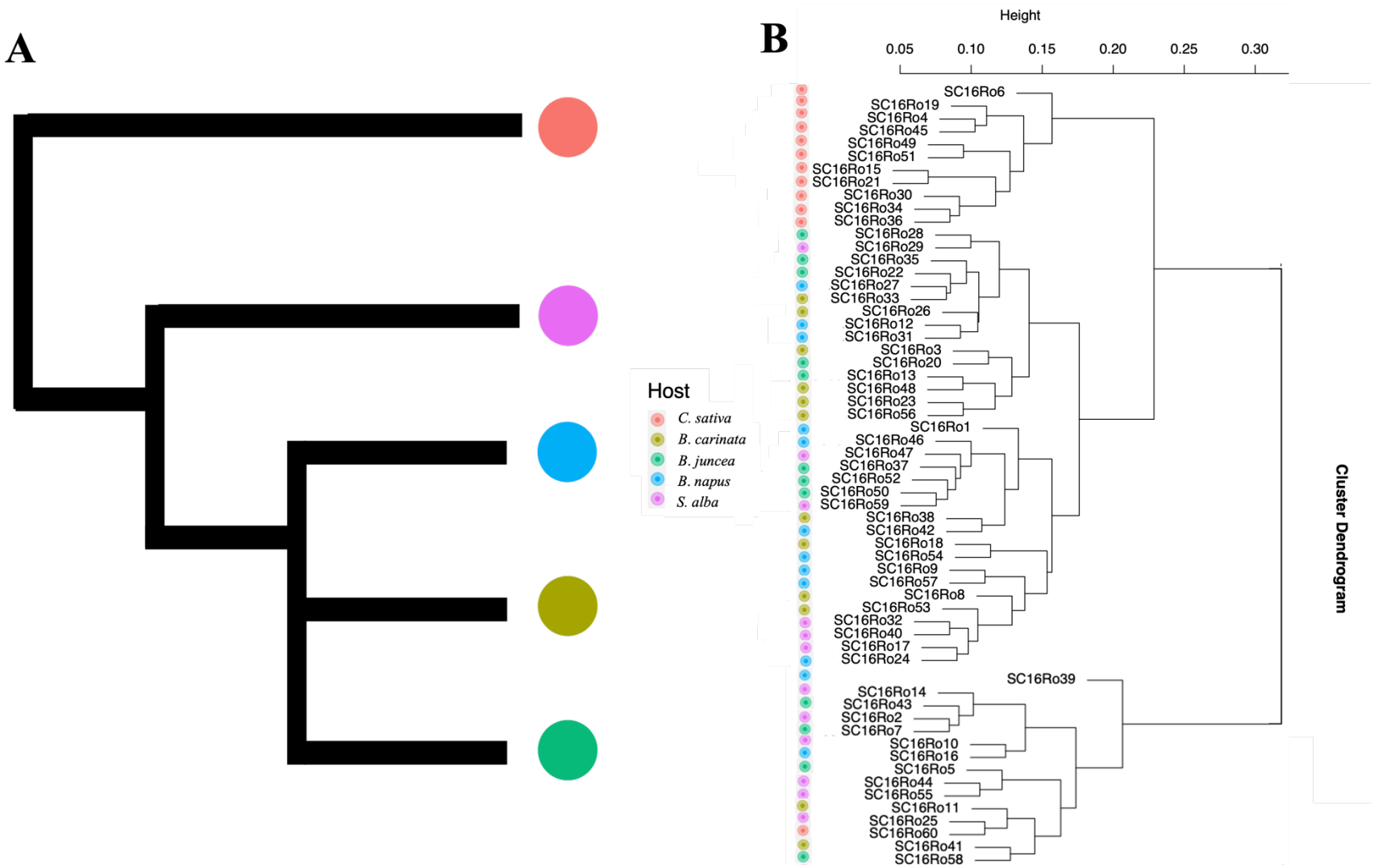

A
B
Height
0.05
0.10
0.15
0.20
0.25
0.30
Host
C. sativa
B. carinata
B. juncea
B. napus
S. alba
SC16Ro6
SC16Ro19
SC16Ro4
SC16Ro45
SC16Ro49
SC16Ro51
SC16Ro15
SC16Ro21
SC16Ro30
SC16Ro34
SC16Ro36
SC16Ro28
SC16Ro29
SC16Ro35
SC16Ro22
SC16Ro27
SC16Ro33
SC16Ro26
SC16Ro12
SC16Ro31
SC16Ro3
SC16Ro20
SC16Ro13
SC16Ro48
SC16Ro23
SC16Ro56
SC16Ro1
SC16Ro46
SC16Ro47
SC16Ro37
SC16Ro52
SC16Ro50
SC16Ro59
SC16Ro38
SC16Ro42
SC16Ro18
SC16Ro54
SC16Ro9
SC16Ro57
SC16Ro8
SC16Ro53
SC16Ro32
SC16Ro40
SC16Ro17
SC16Ro24
SC16Ro39
SC16Ro14
SC16Ro43
SC16Ro2
SC16Ro7
SC16Ro10
SC16Ro16
SC16Ro5
SC16Ro44
SC16Ro55
SC16Ro11
SC16Ro25
SC16Ro60
SC16Ro41
SC16Ro58
Cluster Dendrogram

Figure S14. Evolution of the *Brassicaceae* host plants represented in a dendrogram (A) compared to a hierarchical cluster (B) of the bacterial root communities from Trial 1, during the Test Phase of a two-year crop rotation, in Swift Current, Saskatchewan. The comparison illustrates how the unique composition of the *Camelina sativa* communities (red) may be due to the evolutionary distance between C. sativa and the other *Brassicaceae* hosts. Equally, the similarity between the bacterial communities from the other four *Brassicaceae* host plants may be due to their closer evolutionary relationships. The cluster analysis was generated using a weighted unifrac distance matrix.

Chapter 2: Soil chemistry and soil history significantly structure oomycete communities in *Brassicaceae* crop rotations.

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Keywords: *Brassicaceae*, oomycete communities, soil history, crop rotations

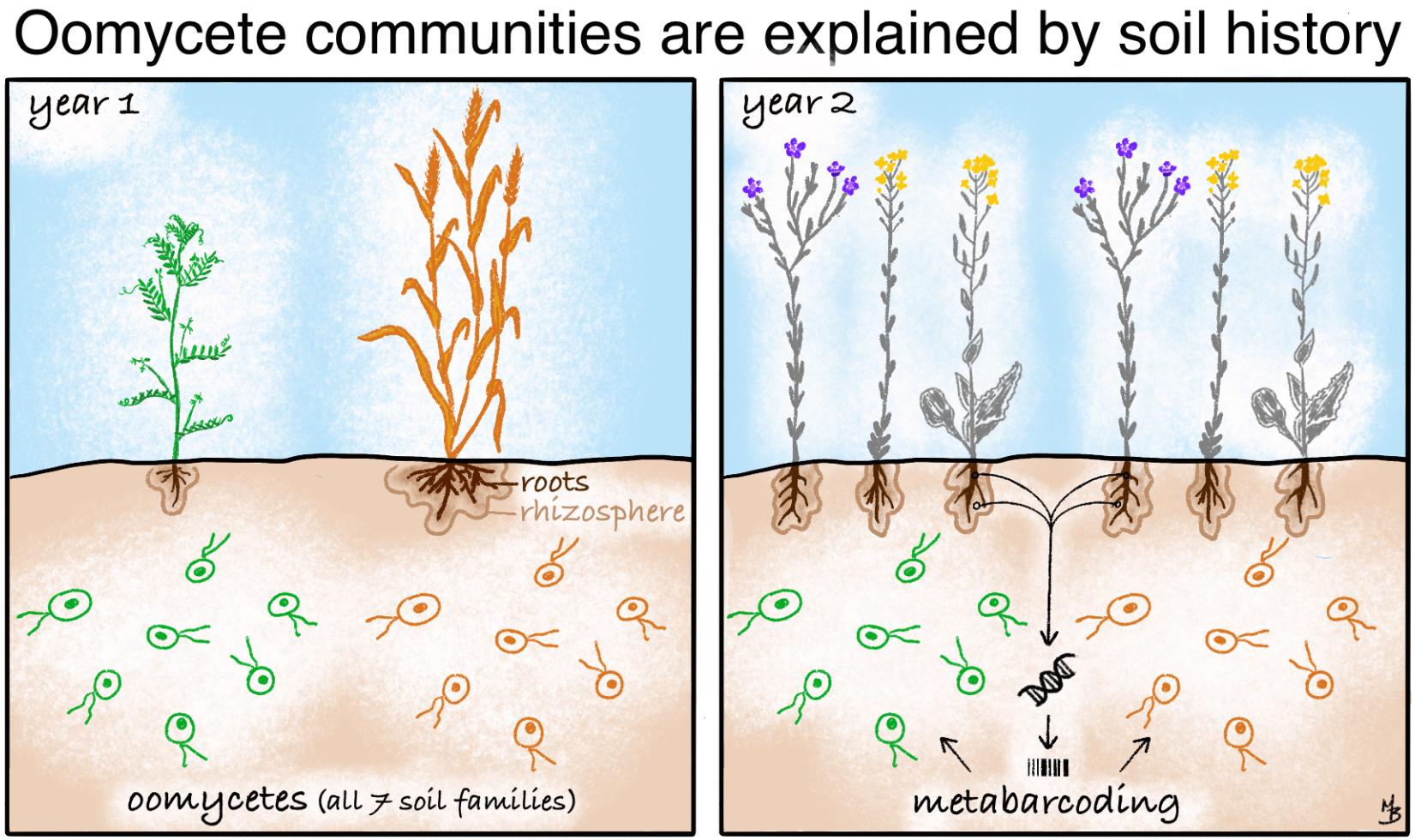
Published in: *Applied and Environmental Microbiology*

DOI: https://doi.org/10.1128/aem.01314-22

Abstract

Oomycetes are critically important soil microbial communities, especially for agriculture where they are responsible for major declines in yields. Unfortunately, oomycetes are vastly understudied compared to bacteria and fungi. As such, our understanding of how oomycete biodiversity and community structure varies through time in the soil remains poor. Soil history established by previous crops is one factor known to structure other soil microbes, but has not been investigated for its influence on oomycetes. In this study, we established three different soil histories in field trials; the following year these plots were planted with five different *Brassicaceae* crops. We hypothesized that the previously established soil histories would structure different oomycete communities, regardless of their current *Brassicaceae* crop host, in both the roots and rhizosphere. We used a nested-ITS amplicon strategy incorporated with MiSeq metabarcoding, where the sequencing data was used to infer amplicon sequence variants (ASVs) of the oomycetes present in each sample. This allowed us to determine the impact of different soil histories on the structure and biodiversity of the oomycete root and rhizosphere communities from the five different *Brassicaceae* crops. We found that each soil history structured distinct oomycete rhizosphere communities, regardless of different *Brassicaceae* crop hosts, while soil chemistry structured the oomycete communities more during a dry year. Interestingly, soil history appeared specific to oomycetes, but was less influential for bacterial communities previously identified from the same samples. These results advance our understanding of how different agricultural practices and inputs can alter edaphic factors to impact future oomycete communities. Examining how different soil histories endure and impact oomycete biodiversity will help clarify how these important communities may be assembled in agricultural soils.

Graphical Abstract



Introduction

Soil history established by previous plant-soil microbial communities condition future generations (Kaisermann *et al*., 2017; Bakker *et al*., 2021; Hannula *et al*., 2021; Blakney *et al*., 2022). These communities are subject to various biotic and abiotic factors—including the initial soil chemistry and microbes present, changes in the plant communities, and environmental extremes, such as drought—and subsequently reflect them through a plant-soil microbial feedback process (Hwang *et al*., 2015; Yang *et al*., 2021; Liu *et al*., 2022). Plant hosts alter soil chemistry, first, through their capacity to uptake nutrients from the soil (Hu *et al*., 2021), and second, through rhizodeposition. Through this mechanism host plants can vary the quantity and array of compounds released into the rhizosphere as required, thereby changing the soil chemistry (Lebeis *et al*., 2015; Korenblum *et al*., 2020; Kawasaki *et al*., 2021; Yu *et al*., 2021). Modifying their rhizodeposition profile also permits plants to actively tailor the structure of their microbial rhizosphere community in response to variable conditions and the plant's needs (Lebeis *et al*., 2015; Korenblum *et al*., 2020; Kawasaki *et al*., 2021; Yu *et al*., 2021). For example, soil microbes increase the host plant's access to nutrients (Richardson *et al*., 2009; Weidner *et al*., 2015; Yu *et al*., 2021), temper environmental change (Lau & Lennon, 2012), or stress (Marasco *et al*., 2012; Hou *et al*., 2021), and protect against pathogens (Sikes *et al*., 2009; Mendes *et al*., 2011). Soil bacterial communities in particular help integrate these diverse signals and modulate the plant's responses (Castrillo *et al*., 2017; Hou *et al*., 2021).

As such, the plant-soil microbial community generates a reciprocal feedback process that incorporates various biotic and abiotic factors (Hwang *et al*., 2015; Yang *et al*., 2021; Liu *et al*., 2022), and impacts future plant-soil microbial generations and their composition (Kaisermann *et al*., 2017; Berendsen *et al*., 2018; Fitzpatrick *et al*., 2018). Thus, information from one plant-soil

microbial community is transmitted through time to impact subsequent plant-microbial generations, i.e., that the soil history, also referred to as soil legacy, of previous plant-soil microbial communities condition future ones (Kaisermann *et al*., 2017; Bakker *et al*., 2021; Hannula *et al*., 2021). However, how different biotic and abiotic factors interact to establish soil histories are not well understood.

Drought, or water-stress, for example, is an increasingly important abiotic factor for establishing plant-soil microbial communities, as well as its growing impact on global agricultural production (Preece *et al*., 2019). On the Canadian Prairies, drought is a common experience. During the last event of the 2021 season, major crop production plunged nationally to a 15-year low due to severe drought conditions: wheat decreased by 38.5% to 21.7 M tonnes, while canola decreased by 35.4% to 12.6 M tonnes (Statistics Canada, 2021). Water availability is a key determinant of plant nutrition and performance, such that their growth becomes restricted due to drought, as they are constrained in nutrient uptake from the soil (Fitzpatrick *et al*., 2018). Soil moisture is also critical for microbial communities (reviewed by Schimel *et al*., 2007); bacteria depend on water for nutrient diffusion and mobility (Preece *et al*., 2019). Soil moisture is also a key promotor of phytopathogenic oomycete growth and dispersion (Hwang *et al*., 2015; Rojas *et al*., 2017; Karppinen *et al*., 2020). As such, there is interest in investigating how soil history and plant-microbial communities interact under dry, water-stressed conditions in an agricultural setting.

Crop rotations, complete with their agricultural inputs, model how a previous crop plant establishes a soil history by altering the biotic and abiotic soil conditions for future plant-microbial communities (Hwang *et al*., 2015; Yang *et al*., 2021; Liu *et al*., 2022). For example, when lentils, or other legumes, are introduced into a rotation they shift the soil microbial community, which

establishes more bioavailable nitrogen and moisture in the soil (O'Donovon et al., 2014; Bazghaleh *et al*., 2016; Hamel *et al*., 2018; Yang *et al*., 2021). These benefits translate to the subsequent crops, which tend to have higher yields (O'Donovon et al., 2014; Hamel *et al*., 2018).

Beneficial soil histories have also been established by canola (cultivars of *Brassica napus*, *B*. *rapa*, or *B*. *juncea*) rotations, as they are thought to reduce the growth of cereal-specific pathogens. As such, cereals tend to have higher yields when they are planted after canola (Etesami & Alikhani, 2016; Yang *et al*., 2021). As demand for vegetable oil and biofuels increases, *Brassicaceae* oilseed-based rotations are increasingly common throughout the world, such as the frequent canola-wheat rotation in Canada (Yang *et al*., 2021). Increasing *Brassicaceae* oilseed crop diversity has been on-going in order to improve production by identifying and breeding crop plants better adapted to the drought stress of the Canadian Prairies, as well as cultivars that are more resistant to pathogens (Bailey-Serres *et al*., 2019; Hossain *et al*., 2019; Liu *et al*., 2019). Though extensive work has investigated how *Brassicaceae* oilseed rotations benefit the crop plants involved (recently reviewed by Yang *et al*., 2021), less is known how these crops and their agricultural inputs impact beneficial, or pathogenic soil microbial communities.

Brassicaceae oilseed crops are known hosts to many microbial pathogens, including fungi, such as *Fusarium* sp., *Leptosphaeria* sp. (blackleg), *Rhizoctonia solani*, *Sclerotinia sclerotiorum* (stem rot), and oomycetes, like *Pythium* sp., *Phytophthora* sp., *Albugo* sp. (staghead), and *Peronospora* and *Hyaloperonospora* spp. (downy mildews) (Canola Council of Canada, 2017; Maciá-Vicente *et al*., 2020). Oomycete phytopathogens alone have had an historically outsized impact on global agriculture (Kamoun *et al*., 2015). Although particular oomycetes strains have been fairly well studied as model patho-systems with specific *Brassicaceae* species (Kamoun *et al*., 2015; Derevnina *et al*., 2016; Prince *et al*., 2017; Cevik *et al*., 2019; Mohammed *et al*., 2019),

as a whole soil oomycete biodiversity remains relatively under-studied, notably among *Brassicaceae* crops (Maciá-Vicente *et al*., 2020).

A few high-throughput sequencing studies have yielded some clues concerning soil oomycete communities: Maciá-Vicente *et al* (2020) and Sapp *et al* (2018) both illustrated oomycete communities present in agricultural and natural *Brassicaceae* soil samples, respectively. They identified root and rhizosphere communities dominated by *Pythiales* (*Pythium* sp., or *Globisporangium* sp.), with minorities of *Peronosporales* (*Phytophthora* sp., *Peronospora* sp., and *Hyaloperonospora* sp.), and *Saprolegniales* (*Aphanomyces* sp.), though they indicated substantial difficulties in assigning taxa (Sapp *et al*., 2018; Maciá-Vicente *et al*., 2020). Taheri *et al* (2017a) yielded further insights by also identifying oomycete root and rhizosphere communities dominated by *Pythiales* from various agricultural pea fields across the Canadian Prairies. Beyond these examples, there is a lack of baseline knowledge concerning how oomycete communities are structured around diverse *Brassicaceae* oilseed crops.

Crop rotations and the soil histories they establish, highlight important factors to investigate in order to better understand oomycete community dynamics. First, different crops, along with their agricultural treatments, alter edaphic factors, which may have important consequences for oomycete communities. For example, legumes tend to retain soil moisture, which is a key factor for oomycete growth as discussed (Hwang *et al*., 2015; Rojas *et al*., 2017; Karppinen *et al*., 2020). Second, crop rotations are known to shift bacterial communities, which may interact to suppress or promote, oomycete communities (Löbmann *et al*., 2016). Both of these examples highlight what impact crop rotations might have on the subsequent crop plant-oomycete community.

We took advantage of an existing agriculture experiment to investigate the impact of soil history established by the previous year's crop, and the current *Brassicaceae* oilseed host crops, on

the soil oomycete communities. Three soil histories were established by growing wheat, lentils, or being left fallow (Fig. S1A). The following year, each soil history plot was divided into five subplots and planted with a different *Brassicaceae* oilseed crop. At full flower, the root systems of these *Brassicaceae* host crops were harvested, and divided into root and rhizosphere compartments, from which environmental DNA was extracted. We hypothesized that the three soil histories established by the previous crops would structure different oomycete communities, regardless of their current *Brassicaceae* host, in both the roots and rhizosphere. We used a MiSeq metabarcoding approach to specifically target the oomycete communities for each root and rhizosphere sample, where the sequencing data was used to infer amplicon sequence variants (ASVs) to identify the oomycete biodiversity, and how this understudied group varies in agricultural soils.

Materials & Methods

Site and experimental design

A field experiment was conducted at the experimental farm of Agriculture and Agri-Food Canada's Research and Development Centre, in Swift Current, Saskatchewan (50°15′N, 107°43′W). The site is located in the semi-arid region of the Canadian Prairies; according to the weather station of the research farm, the 2016 and 2017 growing seasons (May, June and July) had 328.4 mm and 55.0 mm of precipitation, respectively; compared to the 30-year average [1981-2010] of 169.2 mm. The daily temperature averages for the 2016 and 2017 seasons were 15.6°C and 15.9°C, respectively, while the 30-year average was 14.93°C. The farm is on a Brown Chernozem with a silty loam texture (46% sand, 32% silt, and 22% clay), and has been well-described previously by Liu *et al* (2019) and Liu *et al* (2020).

The experiment was established in a field previously growing spring wheat (*Triticum aestivum* cultivar AC Lillian). A two-phase cropping sequence—consisting of a Conditioning Phase the first year, and a Test Phase in the second year (Fig. S1)—was repeated in two field trials, Trial 1, 2015-2016, and Trial 2, 2016-2017, on adjacent sites (Fig. S1B & C). On each site, the experimental design was a split-plot replicated in four complete blocks. In the Conditioning Phase, three soil history treatments were randomly assigned to the main plots, consisting of spring wheat (*Triticum aestivum*, cv. AC Lillian), red lentil (*Lens culinaris* cv. CDC Maxim CL), or left fallow (Fig. S1). Thus, the Conditioning Phase established a soil history composed of either wheat, lentil, or fallow, plus their respective management plans as described below (Hossain *et al*., 2019; Liu *et al*., 2019; Blakney *et al*., 2022).

In the Test Phase, the 12 Conditioning Phase plots were each subdivided and five *Brassicaceae* oilseed crop species were randomly assigned to one of these five subplots (Fig. S1). The *Brassicaceae* crops seeded were Ethiopian mustard (*Brassica carinata* L., cv. ACC110), canola (*B. napus* L., cv. L252LL), oriental mustard (*B. juncea* L., cv. Cutlass), yellow mustard (*Sinapis alba* L., cv. Andante), and camelia (*Camelina sativa* L., cv. Midas). Therefore, the Test Phase established the *Brassicaceae* host plant-soil microbial community feedback, composed of the individual *Brassicaceae* genotypes, their soil microbial community, and their respective management plans, as described below (Hossain *et al*., 2019; Liu *et al*., 2019; Blakney *et al*., 2022). In total, each field trial had 60 subplots to sample (Fig. S1 & S2). For further details of this well-described experiment, its design, and treatments, see Hossain *et al* (2019), Liu *et al* (2019), and Blakney *et al* (2022).

Crop rotation management and sampling

Crops in both field trials were grown and maintained according to standard management practices, as previously described by Hossain *et al* (2019), Liu *et al* (2019), and Blakney *et al* (2022). A pre-seed 'burn off' herbicide treatment using glyphosate (Roundup, 900 g acid equivalent per hectare, a. e. ha^{-1}) was applied to all plots each year to ensure a clean starting field prior to seeding. Lentil seeds were treated with a commercial rhizobium-based inoculant (TagTeam at 3.7 kg ha^{-1}). Lentil and wheat were direct-seeded into wheat stubble from late April to mid-May, depending on the crop and year. The herbicides, glyphosate (Roundup, 900 g a. e. ha^{-1}), Assure II (36 g active ingredient per hectare, a. i. ha^{-1}), and Buctril M (560 g a.i. ha^{-1}) were applied to the fallow, lentil, and wheat plots, respectively, for in-season weed control, while fungicides were only applied as needed. Soil tests were used to determine the rates of in-season nitrogen, phosphorus, and potassium application; no synthetic nitrogen fertilizer was applied to the lentil plots during the Conditioning Phase. Both lentil and wheat were harvested between late August and early October, depending on the crop and year.

The subsequent Test Phase *Brassicaceae* plant hosts were subjected to the same standard management practices as the Conditioning Phase, including pre-seed 'burn off', in-season herbicide and fungicide treatments as needed, and fertilized as recommended by soil tests (Table S1; Hossain *et al* (2019); Liu *et al* (2019); Wang *et al*., 2020). Additionally, all *Brassicaceae* crops, except *B*. *napus*, were treated with Assure II mixed with Sure-Mix or Merge surfactant (0.5% v/v) for post-emergence grass control: Liberty (glufosinate, 593 g a.i. ha−1) was used for *B*. *napus*.

Test phase *Brassicaceae* plants were sampled in mid-late July at full flowering, i.e. when 50% of the flowers on the main raceme were in bloom, as described by the Canola Council of Canada (Canola Council, 2017), where flowering corresponds to higher activity in rhizosphere

microbial communities (Chaparro *et al*., 2014). Four plants from two different locations within each subplot were excavated and pooled together as a composite sample (Hossain *et al*., 2019; Liu *et al*., 2019; Wang *et al*., 2020; Blakney *et al*., 2022). In the field, each plant had its rhizosphere soil divided from the root material by gently scraping it off using bleach sterilized utensils into fresh collection trays. The roots were then gently washed three times with sterilized distilled water to remove any soil. Both the rhizosphere and root portions were immediately flash-frozen and stored in liquid nitrogen vapour shipping containers until stored in the lab at -80°C (Delavaux *et al*., 2020). Based on the sampling strategy, in this study we define the rhizosphere microbiome as the microbial community in the soil in close contact with the roots (Hannula *et al*., 2021), and the root microbiome as the microbial community attached to, and within, the roots (Berendsen *et al*., 2018). Two additional soil cores were sampled from each plot, pooled, and kept on ice in coolers. These samples were homogenized in the lab and sieved to remove rocks and roots. They were then used for soil chemistry analyses, including total carbon, nitrogen, pH, and micronutrients (see Wang *et al*., 2020 for details). Aerial portions of each harvested plant sample were retained to determine dry weight (Fig. S3).

DNA extraction from Test Phase *Brassicaceae* root and rhizosphere samples

Nucleic acids were extracted from Trial 1 Test Phase *Brassicaceae* samples, for both rhizosphere and root portions. First, all the root samples were ground in liquid nitrogen via sterile mortar and pestles (Fig. S2). Total DNA and RNA were extracted from ~1.5 g of rhizosphere soil using the RNA PowerSoil Kit with the DNA elution kit (Qiagen, Germany). DNA and RNA were extracted using ~0.03 g of roots using the DNeasy Plant DNA Extraction Kit, and RNeasy Plant Mini Kit (Qiagen, Canada), respectively, following the manufacturer's instructions (see Wang *et*

al., 2020 for use of the RNA samples). All remaining harvested material from Trial 1 and 2, as well as the extracted DNA from Trial 1, were kept at -80°C before being shipped to Université de Montréal's Biodiversity Centre, Montréal (QC, Canada) on dry ice for further processing (Lay *et al*., 2018; Delavaux *et al*., 2020).

Total DNA was extracted from the Trial 2 Test Phase samples; ~500 mg of rhizosphere soil was used for the NucleoSpin Soil gDNA Extraction Kit (Macherey-Nagel, Germany), and ~130 mg of roots was used for the DNeasy Plant DNA Extraction Kit (Qiagen, Germany) (Lay *et al*., 2020). A no-template extraction negative control was used with both the root and rhizosphere extractions and included with the Test Phase samples (Fig. S2), to assess the influence of the extraction kits on our sequencing results, and the efficacy of our lab preparation. All 242 extracted DNA samples (60 plots x 2 parallel field trials x 2 compartments, rhizosphere and root, +2 no-template extraction control samples) were quantified using the Qubit dsDNA High Sensitivity Kit (Invitrogen, USA), and qualitatively evaluated by mixing ~2 µL of each sample with 1 µL of GelRed (Biotium), and running it on a 0.7 % agarose gel for 30 minutes at 150 V. The no-template extraction negative controls were confirmed to not contain DNA after extraction, where the detection limit was > 0.1 ng (Qubit, Invitrogen, USA). Samples were kept at -80°C (Lay *et al*., 2018; Delavaux *et al*., 2020).

Assembly of oomycete mock community

To assess potential bias caused by lab manipulations, sequencing and downstream bioinformatic processing, we assembled an oomycete mock community of known composition from twenty species with staggered copy numbers of the ITS1 region. To do so, we followed the method of Bakker (2018) beginning with generating a standard curve of the copy numbers of the

ITS region from DNA extracted from *Pythium ultimum* strain 6358.Ba.B, generously provided by Dr. S. Chatterton (Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre). We then used this standard curve to estimate the ITS copy number from a diversity of oomycete DNA samples provided from the Ministry of Agriculture and Fisheries of Quebec, and Agriculture and Agri-Food Canada (Table S2).

A ~1 kb fragment containing the entirety of the ITS 1 region, the 5.8S gene, and the ITS 2 region, was PCR amplified from *P. ultimum* 6358.Ba.B using the ITS4 and ITS6 primers (Table 1; 52; Alpha DNA, Montréal, Canada). The ITS4/6 PCR reaction consisted of 11.5 µL dH_2O, 5.0 µL of 10X buffer (Qiagen, Canada), 2.5 µL of 10 µM ITS4 and ITS6 primers (Alpha DNA, Montréal, Canada), 1.0 µL of dNTPs (Qiagen, Canada), 0.5 µL of *T. aq* polymerase (Qiagen, Canada), and 2 µL of a 1:10 dilution of the template, for a total volume of 25 µL. The PCR was run in an Eppendorf Mastercycle ProS (Mississauga, ON, Canada) thermocycler and consisted of an initial denaturation of 2 minutes at 95°C, followed by 30 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 55°C, and 1 minute elongation at 72°C, before a final elongation of 5 minutes at 72°C (Sapkota & Nicolaisen, 2015). Four µL of the ITS PCR product was mixed with 1 µL of loading dye containing Gel Red (Biotium), and visualized on a 1% agarose gel after 60 minutes at 100 V. The amplified ITS fragment was quantified using the QuBit dsDNA High Sensitivity Kit (Invitrogen, USA), then serially diluted to 10^{-9}, where 1 µL of each dilution was then used as template in a 10 µL qPCR reaction.

The ITS1 region qPCR reactions amplified ~350 bp region using the oomycete-specific ITS6 forward and ITS7-a.e. reverse primers (Table 1; Taheri *et al*., 2017b). All qPCR reactions were set-up in triplicate in 96-well plates using the Freedom EVO100 robot (Tecan, Switzerland), with a triplicate no-template negative control included on each plate. The reactions consisted of

Table 1. ITS primers used in this study.

Name	Sequence (5'-3')	Reference
ITS6 F	GAAGGTGAAGTCGTAACAAGG	Cooke *et al*., 2000
ITS4 R	TCCTCCGCTTATTGATATGC	White *et al*., 1990
ITS7-a.e.	WGYGKTCTTCATCGATGTGC	Taheri *et al*., 2017

5.0 µL of Maxima SYBR Green/ROX qPCR Mix (ThermoFisher Scientific, Canada), 3.4 µL dH_2O, 0.3 µL of 10 µM ITS6 and ITS7-a.e. primers (Alpha DNA, Montréal, Canada), and were run in a ViiA 7 Real-Time PCR System (ThermoFisher Scientific, Canada). The cycling conditions consisted of an initial denaturation of 2 minutes at 94°C, followed by 30 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing at 59°C, and 1 minute elongation at 72°C, before a final elongation of 10 minutes at 72°C (Taheri *et al*., 2017b). The number of ITS1 region copies present in the serially diluted standards were calculated using the formula (Godornes *et al*., 2007).

$$\text{Number of ITS1 copies } \mu L^{-1} = \frac{\text{Avagadro's Constant x DNA (g } \mu L^{-1})}{\text{Number of base pairs x 600 Daltons}}$$

The standard curve was plotted, with an R^2 value of 0.9938 and an amplification efficiency of -3.389 (Fig. S4) falling within acceptable values (Fierer *et al*., 2005).

Copy numbers of the ITS1 region were then estimated for each oomycete sample (Table S2). One μL of a 1:10 dilution of extracted oomycete DNA was used as template for the ITS1 qPCR reaction, following the cycling conditions as described for generating the standard curve. Melt curves generated by 0.5°C increments at the end of the qPCR programme confirmed amplicon specificity. The mean cycle threshold was then calculated from the qPCR reactions for each oomycete sample, and the corresponding ITS1 copy number was estimated off the standard curve (Fig. S4; Bakker, 2018). Finally, the oomycete mock community was assembled with staggered ITS1 copy numbers, where different oomycete community members had different ranges of ITS1 copy numbers (Bakker, 2018).

ITS amplicon generation and sequencing to estimate the composition of the oomycete community

To estimate the composition of the oomycete communities in the rhizosphere and roots from the Test Phase *Brassicaceae* species, extracted DNA from all samples were used to prepare ITS amplicon libraries following Illumina's MiSeq protocols. First, all DNA samples were diluted 1:10 into 96-well plates using the Freedom EVO100 robot (Tecan, Switzerland). To assess potential bias caused by lab manipulations, sequencing and downstream bioinformatic processing, we included a no-template DNA extraction control, and mock community, on each plate.

The prepared plates of the Test Phase DNA samples were submitted to Génome Québec (Montréal, Québec) for ITS amplicon generation and sequencing (Bell *et al*., 2016; Lay *et al*., 2018). In order to preferentially target the oomycete community, and exclude other eukaryotes, as well as ensure sufficient quantity for detection, a semi-nested PCR strategy was used to generate

the ITS1 amplicons from each Test Phase DNA sample (Sapkota & Nicolaisen, 2015). Each sample was used as template in a PCR reaction consisting of 15 cycles using the ITS6 and ITS4 primers (Table 1; Cooke *et al*., 2000; Sapkota & Nicolaisen, 2015) to amplify a ~1 kbp fragment. A second round PCR reaction was then done using the oomycete-specific ITS6 and ITS7ae primers (Table 1; Sapkota & Nicolaisen, 2015, Taheri *et al*., 2017b) to amplify a ~350 bp fragment of the ITS1 region. This semi-nested PCR strategy has previously been shown to enrich for oomycete sequences and limit off-target amplification from other eukaryotes (Sapkota & Nicolaisen, 2015; Taheri *et al*., 2017b). The degenerate primer design has been suggested to allow for the wider capture of oomycete-specific ITS1 sequences, but not at the cost of being biased for a particular oomycete group (Sapkota & Nicolaisen, 2015; Taheri *et al*., 2017b).

These amplicons were then prepared for paired-end 250 bp sequencing using Illumina's MiSeq platform and the MiSeq Reagent Kits v3 (600-cycles) (Génome Québec, Montréal) (Bell *et al*., 2016; Lay *et al*., 2018). We estimated this should provide a mean of 40 000 reads per sample, which is in line with previous studies that describe microbial eukaryote communities (Bell *et al*., 2016; Lay *et al*., 2018).

Estimating ASVs from MiSeq ITS amplicons

The ITS amplicons generated by Illumina MiSeq sequencing were used to estimate the diversity and structure of the oomycete communities present in both the rhizosphere and roots of each Test Phase *Brassicaceae* sample. The integrity and totality of the ITS MiSeq data downloaded from Génome Québec, all 17 656 076 reads, was confirmed using their MD5 checksum protocol (Roy *et al*., 2018). Subsequently, all data were managed, and analyzed in R (R Core Team, 2020), and plotted using `ggplot2` (Wickham, 2016).

Instead of generating OTUs from the ITS amplicon data, we opted to use DADA2 for ASV inference, as it generates fewer false-positives than OTUs, reveals more low-abundant, or cryptic, microbes, and as ASVs are unique sequence identifiers, they are directly comparable between studies, unlike OTUs (Callahan *et al*., 2016a; Callahan *et al*., 2017; Fitzpatrick et al., 2018). ASV inference has also been successfully used with oomycete datasets to discriminate to the species level (Foster *et al*., 2020; Maciá-Vicente *et al*., 2020). Here, due to the variable length of the ITS region, we first used `cutadapt` (Martin, 2011) to carefully remove primer sequences from all the ITS reads generated from the control samples, the mock communities, and the experimental Test Phase *Brassicaceae* samples, including any primer sequences generated due to read-through. The `filterAndTrim` function from the `dada2` package (Callahan *et al*., 2016b) was then used for all reads, following the default settings, including, removing reads shorter than 50 nucleotides, or of low-quality (Q = < 20). Filtered and trimmed reads were then processed through DADA2 for ASV inference (Fig. S2 & S5). Default settings were used throughout the DADA2 pipeline, except the DADA inference functions `dadaF` and `dadaR` which used the `pool ='pseudo'` argument, to increase the likelihood of identifying rare taxa. Consequently, the chimera removal function `removeBimeraDenovo` included the `method ='pooled'` argument (Callahan *et al*., 2016b).

The unique ASVs inferred from the ITS amplicon data were assigned taxonomy using the UNITE database for all eukaryotes (Abarenkov *et al*., 2020). ASVs were assigned species when possible, though UNITE may not account for all the latest taxonomic revisions to individual groups between versions, such as Nguyen *et al* (2022). Moreover, it is important to keep in mind that species names reported here for each ASV are the closest designations after comparisons with reference sequences. Thus, even at 100% similarity, species names remain approximations. Data quality was assessed using the included controls (Fig. S5B), any off-target ASVs assigned to the

taxa *Viridiplantae, Alveolata, Fungi, Heterolobosa,* or *Metazoa* were subsequently removed, and the remaining oomycete ASVs were assigned functional lifestyles based on the FungalTraits database (Põlme *et al*., 2020). Rarefaction curves confirmed that we captured the majority of the oomycete communities in both root and rhizosphere samples from both field trials (Fig. S6). The oomycete-specific ITS sequencing data was subsequently re-analysed independently following the described protocol to avoid any biases from the four no-template negative controls, and the four mock communities. These are the Test Phase oomycete ASVs which are reported hereafter (Supplementary Materials).

Inferring phylogenetic trees

We assembled phylogenies for each compartment, from both trials, in order to infer phylogenetic diversity of the Test Phase *Brassicaceae* oomycete communities. Following the method described by Callahan *et al*., 2016b, the ITS region sequences for each ASV inferred from the Test Phase *Brassicaceae* data were aligned using a profile-to-profile algorithm (Wang *et al*., 2004), and a dendrogram guide tree, from the `decipher` package (Wright, 2016). With the `phangorn` package (Schliep, 2011), the maximum likelihood of each site was calculated using the `dist.ml` function using a JC69 equal base frequency model, before assembling phylogenies using the neighbour-joining method. An optimized general time reversible (GTR) nucleotide substitution model was fitted to the phylogeny using the `optim.pml` function. Phylogenies were subsequently added to each `phyloseq` object (McMurdie & Holmes, 2013).

α-diversity of the Test Phase *Brassicaceae* rhizosphere and root communities

First, to visualise taxonomic diversity, ASVs were plotted as taxa cluster maps using `heat_tree` from the `metacoder` package (Kembel *et al*., 2010) for the rhizosphere and roots of both experiments, where nodes represent class to genus: node colours represent the number of unique taxa, while node size indicates the relative abundance of each ASV. Taxa cluster maps facilitate visualizing abundance, as well as diversity across taxonomic hierarchies (Kembel *et al*., 2010).

Second, in order to estimate the coverage of the oomycete class, we incorporated the oomycete phylogenies into the `phyloseq` object following the method described by Callahan *et al* (2016b). Faith's phylogenetic diversity was calculated as an α-diversity index from the Test Phase *Brassicaceae* samples using the `pd` function from the `picante` package (sum of all branch lengths separating taxa in a community; Kembel *et al*., 2010). For comparison, Simpson and Shannon's α-diversity indices were also calculated (Fig. S7).

We assessed differences between the mean phylogenetic diversity between soil histories, and *Brassicaceae* hosts using the non-parametric Kruskal-Wallis rank sum test, `kruskal.test`, as the transformed data did not respect the assumptions for normality. Specific groups of statistical significance were identified with the *post hoc* pairwise Wilcoxon Rank Sum Tests, `pairwise.wilcox.test`, with the FDR correction on the p-values to account for multiple comparisons.

Identification of differentially abundant ASVs and specific indicator species

To refine our understanding of the abundance and composition of the Test Phase *Brassicaceae* oomycete communities, we used two complementary methods to identify taxa

specific to soil histories, or *Brassicaceae* hosts. First, taxa cluster maps were used to calculate the differential abundance of ASVs between experimental groups, including rhizosphere and root compartments, *Brassicaceae* host plants, and soil histories. Taxa cluster maps were generated using `compare_groups`, in the metacoder package (Foster *et al*., 2017), where the non-parametric Wilcoxon Rank Sum Tests determined if a randomly selected abundance from one group was greater on average than a randomly selected abundance from another group. As the statistical test was performed for each taxon, we used a false discovery rate (FDR) correction on the p-values to account for the multiple comparisons. When the comparison was between more than two groups, the differential abundances were plotted onto the taxa cluster map using `heat_tree_matrix` (Foster *et al*., 2017).

Second, indicator species analysis was used to detect ASVs that were preferentially abundant in pre-defined environmental groups (roots, or rhizosphere, soil histories, or *Brassicaceae* host). A significant indicator value is obtained if an ASV has a large mean abundance within a group, compared to another group (specificity), and has a presence in most samples of that group (fidelity) (Legendre & Legendre, 2012). The fidelity component complements the differential abundance approach between taxa clusters, which only considers abundance. We performed an indicator species analysis for the ASVs identified in the Test Phase of Trial 1, and then Trial 2. From the `indicspecies` package (De Cáceres & Legendre, 2009), we used the `multipatt` function with 9999 permutations. As the statistical test is performed for each ASV, we used the FDR correction on the p-values to account for multiple comparisons.

β-diversity of the Test Phase *Brassicaceae* rhizosphere and root communities

To test for significant community differences between both trials, compartments, soil histories and *Brassicaceae* hosts, we used the non-parametric permutational multivariate ANOVA (PERMANOVA), where any variation in the ordinated data distance matrix is divided among all the pairs of specified experimental factors. The PERMANOVA was calculated using the `adonis` function in the `vegan` package (Oksanen *et al*., 2020), with 9999 permutations, and the experimental blocks were included as "strata". Our PERMANOVA used a distance matrix calculated with the Bray-Curtis formula and tested the significance of the effects of soil history, *Brassicaceae* host, and compartment.

We used a variance partition, as a complement to the PERMANOVA, to model the explanatory power of soil history, *Brassicaceae* host, and soil chemistry in the structure of the Test Phase *Brassicaceae* oomycete communities. We then quantified how each significant factor (ie, the explanatory variables) impacted oomycete community structure with a distance-based redundancy analysis (db-RDA) (Legendre & Legendre, 2012). First, singleton ASVs were removed before the `phyloseq` data were transformed using Hellinger's method, such that ASVs with high abundances and few zeros are treated equivalently to those with low abundances and many zeros (Legendre & de Cáceres, 2013). With the `vegan` package (Oksanen *et al*., 2020), soil chemistry was standardized (Legendre & Legendre, 2012) using the `decostand` function. We modelled the explanatory power of each experimental factor in each compartment from both experiments with a variance partition of a partial RDA, using the `varpart` function, and a Bray-Curtis distance matrix (Borcard *et al*., 1992). Variation in the oomycete community data not described by the explanatory variables were quantified by the residuals. Finally, to quantify the amount of variation described by each explanatory factor, db-RDA were calculated using the `capscale` function. Colinear

variables were only identified in the soil chemistry from Trial 2, such that specific variables were removed without a loss of information. We subsequently removed total carbon from both the Trial 2 root and rhizosphere RDAs, as well as the zinc concentration from the root RDA. The final plots were generated using `phyloseq` (McMurdie & Holmes, 2013).

Co-inertia analysis of the relationship between oomycetes and bacterial communities

We used a co-inertia analysis (Dolédec & Chessel, 1994; Legendre & Legendre, 2012) to compare how each sample was influenced by different ASVs as a means to evaluate the strength of the soil history effect. Briefly, this analysis identifies the relationship between two datasets from a common sample by projecting that sample into a common multivariate space. For this analysis the two datasets were the oomycete ASVs identified in this study—and were structured by soil history—and the bacterial ASVs identified from the same experiment, and extracted DNA samples, but were not structured by soil history (Blakney *et al*., 2022). This type of analysis is appropriate for exploring relationships in species-rich datasets—for example, where there are more ASVs than sites—and it imposes no assumptions on the datasets, such as co-occurrence, or interactions (Legendre & Legendre, 2012).

The analysis identifies the axes of the common co-inertia space that represent the greatest inertia, or spread, of the common data. The analysis then compares how the positions of each sample in the new co-inertia space are influenced by particular bacterial or oomycete ASVs. The direction of the arrows indicates how a sample is influenced by bacterial ASVs (tail) compared to oomycete ASVs (head); samples with shorter arrows are more similar (Legendre & Legendre, 2012; Mamet *et al*., 2017). Co-inertia analysis is also evaluated with a RV co-efficient (R = correlation, V = vectorial); a multidimensional correlation coefficient equivalent to the Pearson

correlation coefficient. A higher RV indicates a stronger relationship between the oomycete and bacterial community matrices (Legendre & Legendre, 2012; Iffis *et al*. 2016).

Therefore, if soil history was particularly significant to the relationship between the bacterial and oomycete ASVs, the samples might be clustered by soil history on the common co-inertia space. Alternatively, a weaker soil history may only be reflected in the shift from bacterial ASVs to oomycete ASVs. This might be plotted by longer arrows oriented toward common oomycete ASVs, which represent the different by soil histories. Finally, if soil history has little to do with the relationship between the bacterial and oomycete ASVs, there may be no discernable pattern in how the sample are plotted, and the arrows between communities would be short.

First, to facilitate the analysis we reduced the bacterial `phyloseq` objects by removing any ASVs that occurred only once. The `phyloseq` objects for the oomycete and bacterial communities from the roots and rhizosphere of both field trials were transformed using Hellinger's transformation. Finally, each oomycete-bacterial sample pair were subjected to co-inertia analysis using the `coinertia` function from the `ade4` package (Dray & Dufour, 2007). The large number of ASVs identified here and from Blakney *et al* (2022), precluded us from plotting the ASVs onto the co-inertia plane. However as noted in Legendre & Legendre (2012) these plots are not essential to evaluating the co-inertia analysis.

Results

The *Brassicaceae* rhizospheres had significantly different oomycete communities compared to the roots.

To identify the composition of the oomycete rhizosphere and root communities from the Test Phase *Brassicaceae* crop species, we inferred ASVs from the retained oomycete-specific ITS amplicons using the DADA2 pipeline (Callahan *et al*., 2016a & 2017). The four replicates of the oomycete mock community were sequenced to an average of ~23 000 reads, and closely resembled each other in ASV composition (Fig. S5B & C). From the retained MiSeq reads of the mock community, DADA2 inferred 316 individual ASVs which were assigned taxa to at least the order level as 2 *Pythiales*, 2 *Peronosporales*, 1 *Saprolegniales*, and 1 *Albuginales* (Fig. S5B). Since the mock community was composed of 21 individuals from these four orders (Table S2), this provides some reassurance that our pipeline ought to be effective in identifying the oomycetes present in each experimental sample.

We retained 8 222 283 high-quality ITS MiSeq amplicons through the pipeline, with more reads retained in the rhizosphere samples of the Test *Brassicaceae* crop species in both Trials (Table 2; Fig. S5). The 1037 ASVs inferred from the retained reads, were subsequently filtered to 412 oomycete ASVs identified among the Test Phase samples. Differences between the rhizosphere and root Test Phase oomycete communities were highly significant in both field trials (Trial 1 PERM $R^2 = 0.1226$, $p < 0.0001$; Trial 2, PERM $R^2 = 0.0751$, $p < 0.0001$, Table 3). The majority of oomycete ASVs were found in the rhizosphere compared to their cognate root samples (Table 2). The oomycete rhizosphere communities were also consistently more phylogenetically diverse than the root communities (Fig. 1), which reflects the greater species richness observed in the rhizosphere (Fig. S7).

Table 2. The oomycete rhizosphere communities had more unique ASVs than the root communities of five *Brassicaceae* host plants in the Test Phase of a two-year crop rotation, harvested from two field trials (Trial 1, 2016; Trial 2, 2017) from Swift Current, Saskatchewan. 17 656 076 raw reads were produced via Illumina's MiSeq at Génome Québec, and processed through DADA2, where 8 068 013 reads were retained (ITS reads reported here) for ASV inference. A total of 1037 ASVs were identified across the entire dataset, which were filtered to 412 oomycete ASVs.

		Retained ITS Reads (mean ± SD / sample)	ASV Occurrence (mean ± SD / sample)	ASV Abundance (mean ± SD / sample)
Trial 1	Rhizosphere	2 178 736 (36 312 ± 12 084 / sample)	227 (69 ± 34 / sample)	1 353 787 (22 563 ± 15 585 / sample)
	Roots	1 785 905 (29 765 ± 11 416 / sample)	89 (63 ± 36 / sample)	81 396 (1357 ± 4627 / sample)
Trial 2	Rhizosphere	2 355 829 (39 263 ± 14 105 / sample)	218 (74 ± 40 / sample)	1 237 771 (20 629 ± 16 799 / sample)
	Roots	1 747 543 (29 126 ± 11 252 / sample)	43 (56 ± 39 / sample)	1820 (33 ± 147 / sample)

Table 3. PERMANOVA identified that the compartment and soil history were significant experimental factors in structuring the oomycete communities from the Test Phase of a two-year crop rotation, harvested in 2016 (Trial 1) and 2017 (Trial 2) from Swift Current, Saskatchewan. *Brassicaceae* host crops were only significant in the Test Phase oomycete communities of Trial 1, while the *Brassicaceae* host ~ soil history interaction was never significant. The PERMANOVA was calculated using a Bray-Curtis distance matrix, with 9999 permutations.

Experimental Factors	Trial 1[a]			Trial 2		
	F Model	R^2	Pr (> F)	F Model	R^2	Pr (> F)
Compartment[b]	17.8018	0.12264	**0.0001**	8.3717	0.07508	**0.0001**
Soil History[c]	2.1235	0.02926	**0.0015**	1.6600	0.02977	**0.0018**
Brassicaceae Host[d]	1.8876	0.05202	**0.0003**	0.9364	0.03359	0.6464
Compartment ~ Soil History	1.3196	0.01818	0.0941	1.6202	0.02906	**0.0020**
Compartment ~ *Brassicaceae* Host	1.7741	0.04889	**0.0008**	1.0426	0.03740	0.3031
Soil History ~ *Brassicaceae* Host	1.0734	0.05916	0.2367	0.8299	0.05954	0.9822
Compartment ~ Soil History ~ *Brassicaceae* Host	0.9040	0.04982	0.7576	0.8778	0.06298	0.9235

[a], Values in bold indicate significant factors, or interactions
[b], Rhizosphere or roots
[c], Fallow, lentil, or wheat
[d], *Brassica carinata*, *B. napus*, *B. juncea*, *Sinapis alba*, or *Camelina sativa*

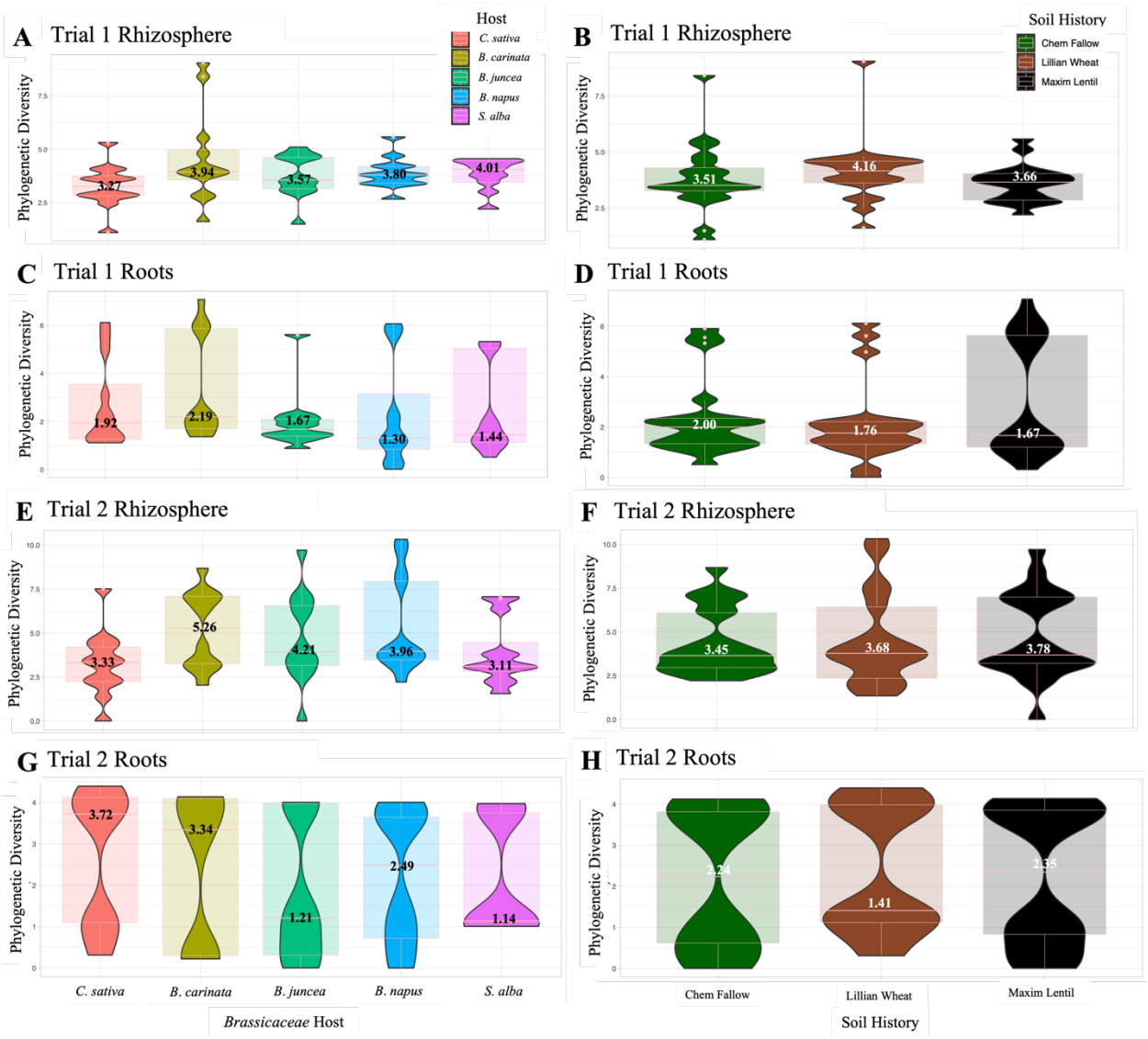
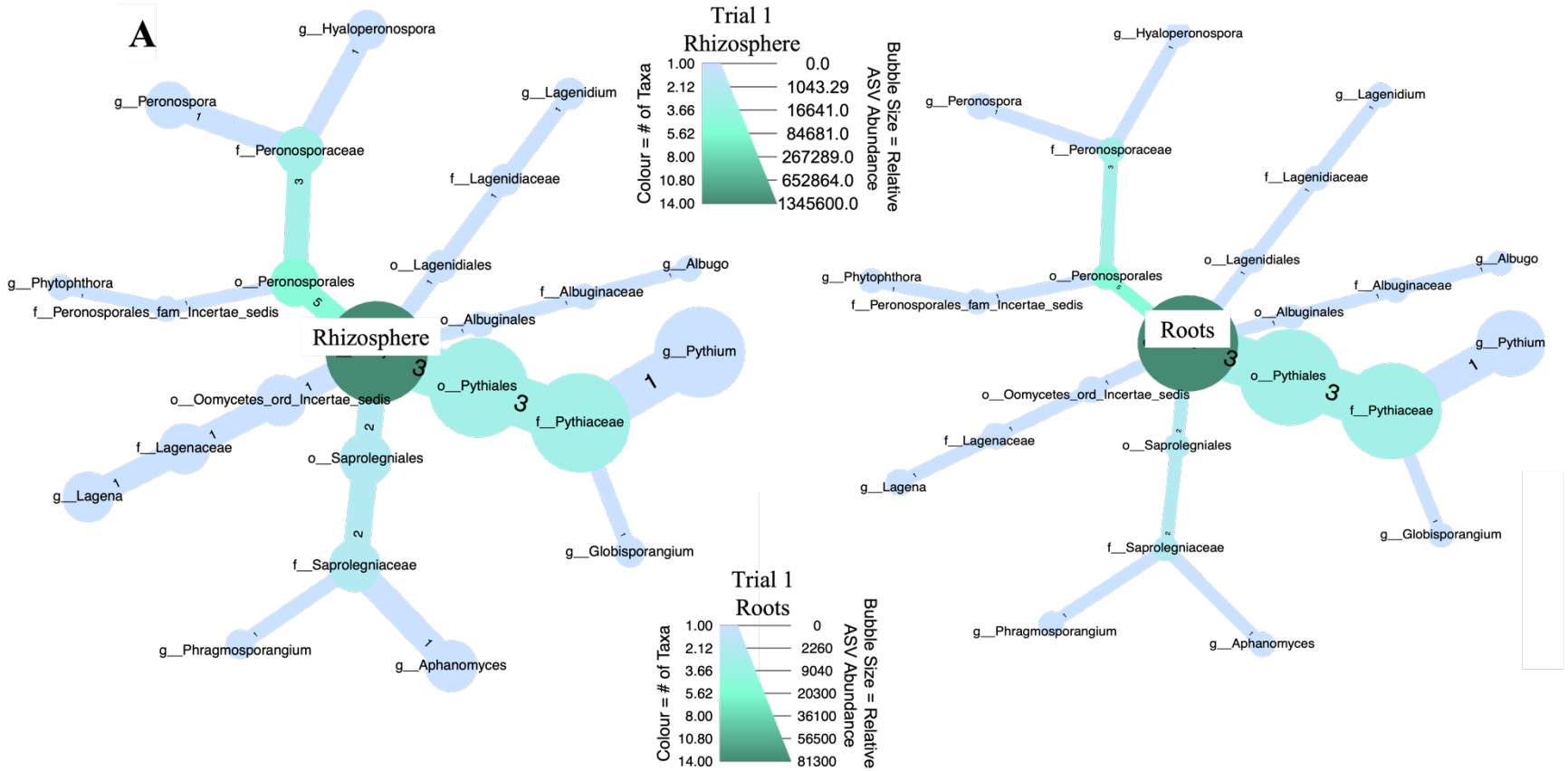

A
Trial 1 Rhizosphere
Host
C. sativa
B. carinata
B. juncea
B. napus
S. alba
Phylogenetic Diversity
3.27
3.94
3.57
3.80
4.01
B
Trial 1 Rhizosphere
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil
Phylogenetic Diversity
3.51
4.16
3.66
C
Trial 1 Roots
Phylogenetic Diversity
1.92
2.19
1.67
1.30
1.44
D
Trial 1 Roots
Phylogenetic Diversity
2.00
1.76
1.67
E
Trial 2 Rhizosphere
Phylogenetic Diversity
3.33
5.26
4.21
3.96
3.11
F
Trial 2 Rhizosphere
Phylogenetic Diversity
3.45
3.68
3.78
G
Trial 2 Roots
Phylogenetic Diversity
3.72
3.34
1.21
2.49
1.14
C. sativa
B. carinata
B. juncea
B. napus
S. alba
Brassicaceae Host
H
Trial 2 Roots
Phylogenetic Diversity
2.24
1.41
Chem Fallow
Lillian Wheat
Maxim Lentil
Soil History

Figure 1. Phylogenetic diversity tended to be higher in the oomycete rhizosphere communities (A, B, E, F) than in the root communities (C, D, G, H) from five *Brassicaceae* host plants in the Test Phase of a two-year rotation, harvested in 2016 (Trial 1, A-D) and 2017 (Trial 2, E-H) from Swift Current, Saskatchewan. Phylogenetic diversity also tended to be higher in the *Brassicaceae* host plants in Trial 2 (E, G) than Trial 1 (A, C). Diversity tended to be higher in the root communities in *Camelina sativa* compared to the corresponding rhizosphere communities only in Trial 2 (G vs E). As the transformed data did not adhere to assumptions of normality, the non-parametric Kruskal test was used to test for significance among the Test Phase oomycete communities grouped by *Brassicaceae* host crops, or by their Conditioning Phase soil histories; no significant differences were detected.

When the oomycete ASVs were plotted as taxa clusters, we observed similar taxonomic composition between the oomycete Test Phase rhizosphere and root communities from Trial 1 and 2 (Fig. 2). *Pythium* species dominated the roots and rhizosphere in both trials in terms of relative abundance, while the order *Peronosporales* consistently had the most taxa across each community (Fig. 2). In Trial 1, *Pythium* and *Peronospora* genera were significantly enriched ($p < 0.01$) in the Test Phase rhizosphere communities compared to the roots, while in Trial 2, only *Pythium* species were significantly more abundant in the rhizosphere (Fig. S8).

Indicator species analysis identified oomycete ASVs specific to the Test Phase rhizosphere communities in both trials, but none in the root communities. Forty-one ASVs were specific to the oomycete rhizosphere communities in Trial 1 ($p < 0.005$, Table 4), while no ASVs were specific to the root communities. Thirty-four ASVs belonged to the *Pythiaceae*, of which half were further identified as *Pythium* sp., two ASVs were *Lagena* sp., and one was *Aphanomyces* sp. (Table 4). The final four indicator ASVs were unknown oomycetes (Table 4). In Trial 2, indicator ASVs in the rhizosphere communities were similar to those from the rhizosphere of Trial 1; 45 ASVs were specific to the Test Phase rhizosphere communities in Trial 2 ($p < 0.05$, Table 4), whereas none were identified in the cognate root communities. The *Pythiaceae* accounted for 37 of these ASVs, of which 19 were further identified as *Pythium* sp. (Table 4). Two ASVs were recognized as *Lagena* sp., one was *Aphanomyces* sp., while the remaining five ASVs were unknown oomycetes (Table 4). All of the oomycete ASVs, including those identified by indicator species analysis as significant, were matched to plant pathogen functional lifestyles from the FungalTraits database (Põlme *et al*., 2020).


A
Trial 1
Rhizosphere
Colour = # of Taxa
1.00
2.12
3.66
5.62
8.00
10.80
14.00
Bubble Size = Relative ASV Abundance
0.0
1043.29
16641.0
84681.0
267289.0
652864.0
1345600.0
g__Hyaloperonospora
g__Peronospora
g__Lagenidium
f__Peronosporaceae
f__Lagenidiaceae
g__Phytophthora
o__Peronosporales
o__Lagenidiales
g__Albugo
f__Albuginaceae
f__Peronosporales_fam_Incertae_sedis
o__Albuginales
Rhizosphere
g__Pythium
o__Pythiales
o__Oomycetes_ord_Incertae_sedis
f__Pythiaceae
f__Lagenaceae
o__Saprolegniales
g__Lagena
g__Globisporangium
f__Saprolegniaceae
g__Phragmosporangium
g__Aphanomyces
Trial 1
Roots
Colour = # of Taxa
1.00
2.12
3.66
5.62
8.00
10.80
14.00
Bubble Size = Relative ASV Abundance
0
2260
9040
20300
36100
56500
81300
g__Hyaloperonospora
g__Peronospora
g__Lagenidium
f__Peronosporaceae
f__Lagenidiaceae
g__Phytophthora
o__Peronosporales
o__Lagenidiales
g__Albugo
f__Albuginaceae
f__Peronosporales_fam_Incertae_sedis
o__Albuginales
Roots
g__Pythium
o__Pythiales
o__Oomycetes_ord_Incertae_sedis
f__Pythiaceae
f__Lagenaceae
o__Saprolegniales
g__Lagena
g__Globisporangium
f__Saprolegniaceae
g__Phragmosporangium
g__Aphanomyces

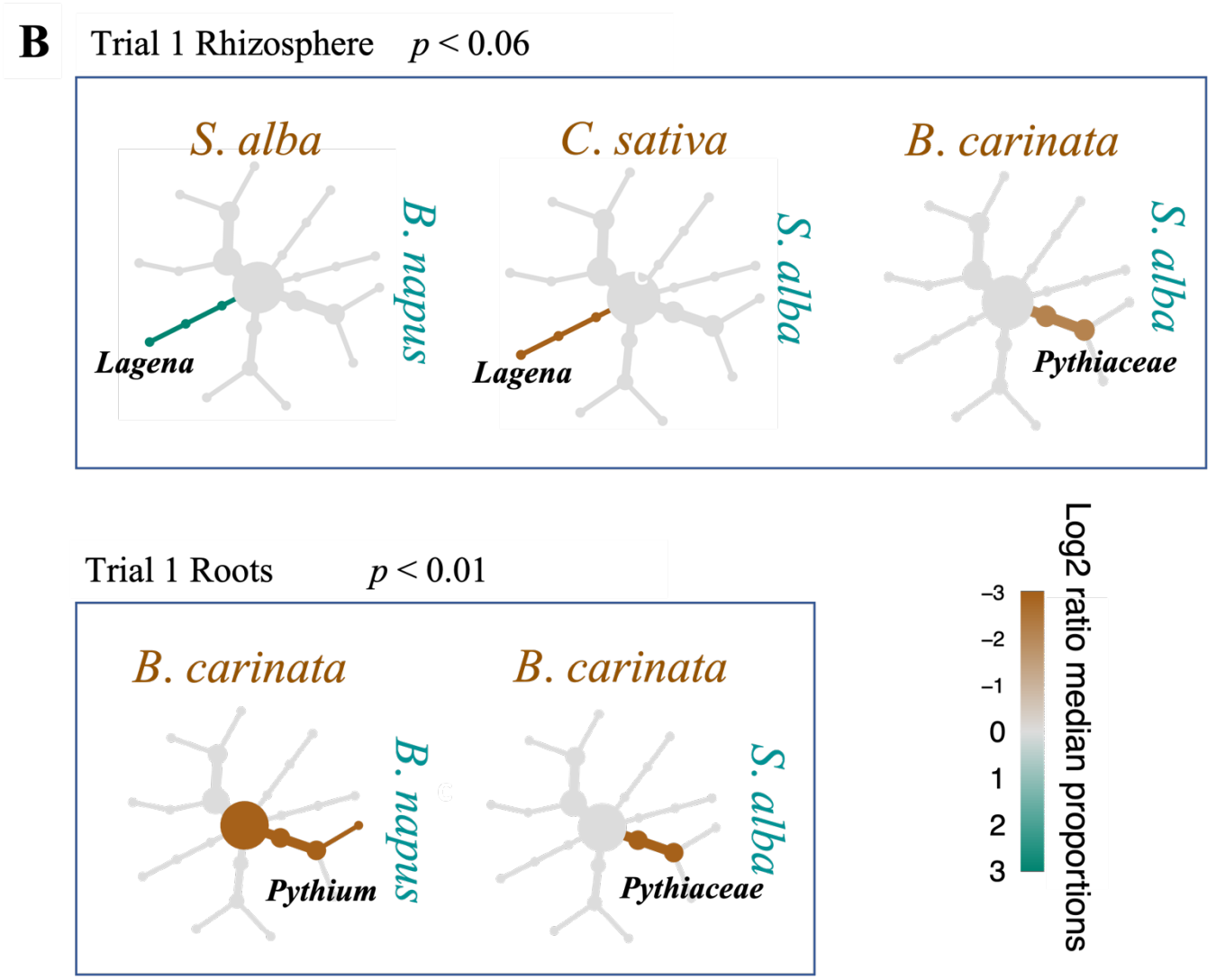

B
Trial 1 Rhizosphere $p < 0.06$
S. alba
B. napus
Lagena
C. sativa
S. alba
Lagena
B. carinata
S. alba
Pythiaceae
Trial 1 Roots $p < 0.01$
B. carinata
B. napus
Pythium
B. carinata
S. alba
Pythiaceae
Log2 ratio median proportions
−3
−2
−1
0
1
2
3

Figure 2. Taxa clusters of the oomycete ASVs inferred from among the rhizosphere and roots of five *Brassicaceae* host crops in the Test Phase of a two-year rotation from Swift Current, Saskatchewan. The abundance and composition of the oomycete communities are represented to the genus level, where the size of each taxonomic group (bubble) represents the abundance of inferred ASVs, and the colour scale represents the number of unique taxa. (A) In Trial 1 (harvested 2016), *Pythium* dominated in both the rhizosphere (left) and root (right) communities, while the genera *Aphanomyces*, *Lagena*, and *Peronospora*, were dramatically reduced between the rhizosphere and the root communities. (B) Significantly enriched taxa, labelled in bold, were identified between each pair of *Brassicaceae* host crops in Trial 1 rhizosphere (top panel), and root (bottom panel). Taxa that were significantly more abundant are highlighted brown or green, following the labels for each compared factor. Differential taxa clusters identified significantly enriched (ie, abundant) taxa, using the non-parametric Kruskal test, followed by the *post hoc* pairwise Wilcox test, with an FDR correction. No enrichment was detected for Trial 2, nor did soil histories enrich any taxa in either experiment. (C) In Trial 2 (harvested 2017), similar to A, *Pythium* dominates in both the rhizosphere and root communities, though there are very few oomycete ASVs detected in the *Brassicaceae* root communities.

Table 4. Indicator species were identified exclusively in the oomycete rhizosphere communities in the Test Phase of a two-year crop rotation, harvested in 2016 (Trial 1) and 2017 (Trial 2) from Swift Current, Saskatchewan. In Trial 1, 41 ASVs were identified as indicator species in the Test Phase rhizosphere communities, while 45 ASVs were identified in Trial 2, notably from the same taxonomic groups. ASVs from the rhizosphere communities were also significant in specific soil histories established in the previous Conditioning Phase: in Trial 1, five ASVs were associated with the lentil soil history, while in Trial 2, 11 ASVs were again associated with the lentil soil history, and one ASV was associated with wheat soil history. No indicator species were identified for any of the five *Brassicaceae* host crops. Indicator species analysis relies on abundance and site specificity to statistically test each ASV, which we report here as $p < 0.05$, with a FDR correction.

Most Closest Taxon	Trial 1	Trial 2
	Compartment[a] ($p < 0.05$)	Compartment ($p < 0.05$)
Aphanomyces sp.	Rhizosphere	Rhizosphere
Lagena sp.	Rhizosphere, 2x ASVs	Rhizosphere, 2x ASVs
Pythium sp.	Rhizosphere, 17x ASVs	Rhizosphere, 19x ASVs
Pythiaceae	Rhizosphere, 17x ASVs	Rhizosphere, 18x ASVs
Unknown Oomycetes	Rhizosphere, 4x ASVs	Rhizosphere, 5x ASVs
	Soil History[b] ($p < 0.05$)	Soil History ($p < 0.05$)

Pythium sp.	Rhizosphere/Lentil	-
Pythiaceae	Rhizosphere/Lentil, 4x ASVs	Rhizosphere/Lentil, 11x ASVs
Aphanomyces sp.	-	Rhizosphere/Wheat

[a], Rhizosphere communities, but never root communities
[b], Fallow, lentil, or wheat, grown the previous year

Soil history significantly structured the soil oomycete communities of *Brassicaceae* crops

Next, we tested if the three soil histories established by the previous crops structured significantly different oomycete communities. Soil history was significant in structuring the Test Phase oomycete communities in both field trials (Trial 1, PERM $R^2 = 0.0292$, $p < 0.0015$; Trial 2, PERM $R^2 = 0.0300$, $p < 0.0018$, Table 3), though the soil history ~ crop host interaction was not significant in either trial. We complemented the PERMANOVA by a variance partition to model the explanatory power of each factor (soil histories, soil chemistry, and the *Brassicaceae* host crops). Variance partitioning found that soil history explained similar amounts of the oomycete rhizosphere community data in both field trials (Trial 1 $R^2 = 0.0453$, $p < 0.001$, Fig. 3A; Trial 2 $R^2 = 0.0476$, $p < 0.001$, Fig. 3C). We also quantified how the experimental factors impacted the oomycete community structure with an RDA. Soil history was highly significant for the Test Phase oomycete rhizosphere communities in both field trails (Trial 1 adj. $R^2 = 0.0539$, $p < 0.001$, Fig. 4A; Trial 2 adj. $R^2 = 0.0727$, $p < 0.001$, Fig. 4B), where the communities were grouped by soil history (Fig. 4).

Soil history was less consistent in the oomycete root communities of both field trials. First, it explained a similar amount of the variance in the Test Phase root community data as in the rhizosphere communities in Trial 1 ($R^2 = 0.0418$, $p < 0.001$, Fig. 3B), but was not significant in the root community data of Trial 2 (Fig. 3D). Second, RDAs demonstrated the importance of soil history in the oomycete root communities of both trials (Trial 1 adj. $R^2 = 0.0429$, $p = 0.007$, Fig. S9A; Trial 2 adj. $R^2 = 0.0403$, $p = 0.005$, Fig. S9B). Though they were less significant compared to the rhizosphere (Fig. 4), they explained a similar amount of the data ($R^2 = \sim 0.04$, Fig. 4 & S9). A gradient separating the oomycete root communities based on their previous soil history was observed in Trial 2 (Fig. S9B), similar to the corresponding rhizosphere communities (Fig. 4), but

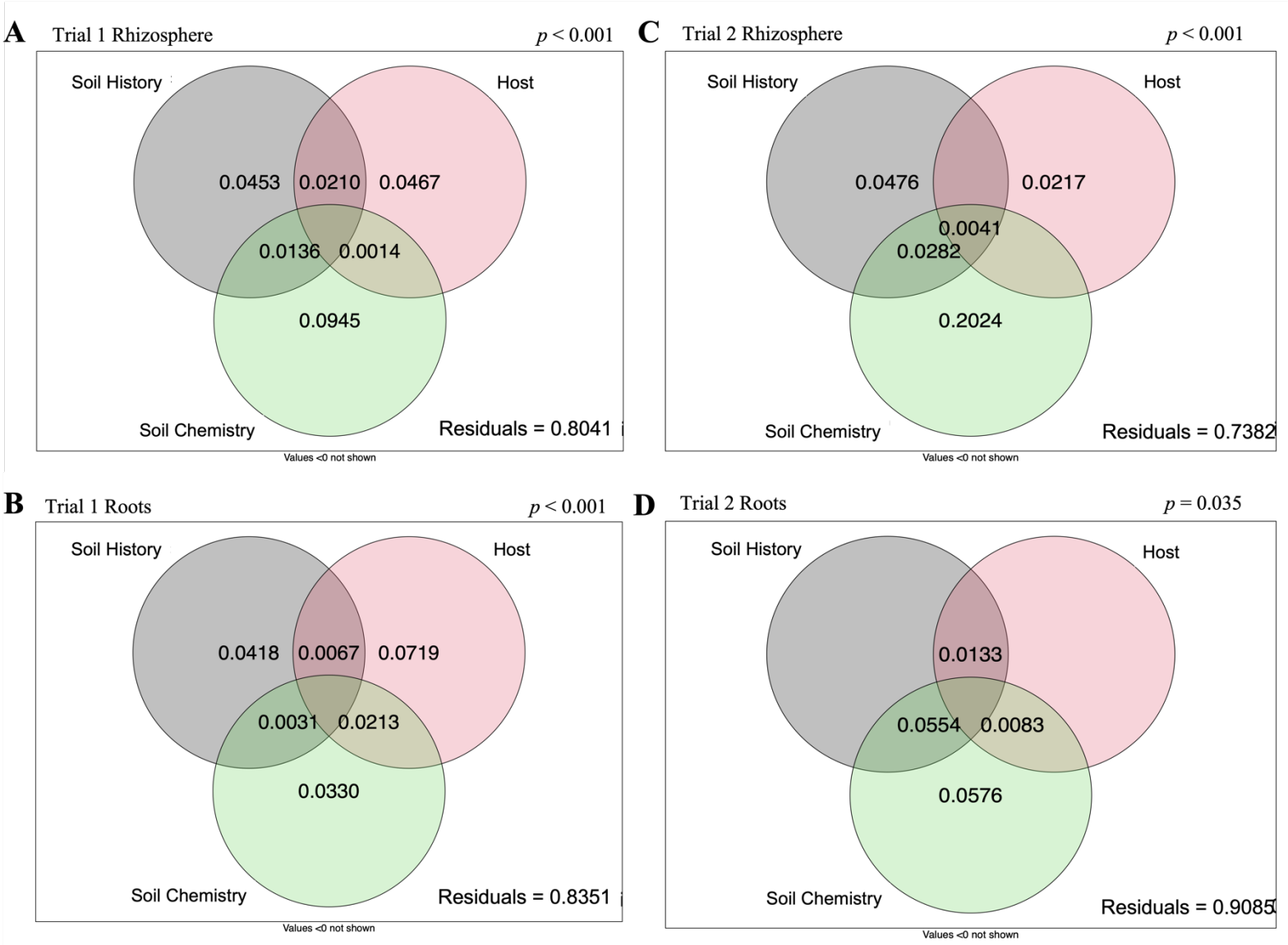

A
Trial 1 Rhizosphere
p < 0.001
Soil History
Host
0.0453
0.0210
0.0467
0.0136
0.0014
0.0945
Soil Chemistry
Residuals = 0.8041
Values <0 not shown
C
Trial 2 Rhizosphere
p < 0.001
Soil History
Host
0.0476
0.0217
0.0041
0.0282
0.2024
Soil Chemistry
Residuals = 0.7382
Values <0 not shown
B
Trial 1 Roots
p < 0.001
Soil History
Host
0.0418
0.0067
0.0719
0.0031
0.0213
0.0330
Soil Chemistry
Residuals = 0.8351
Values <0 not shown
D
Trial 2 Roots
p = 0.035
Soil History
Host
0.0133
0.0554
0.0083
0.0576
Soil Chemistry
Residuals = 0.9085
Values <0 not shown

Figure 3. Soil chemistry, soil history, and the *Brassicaceae* host crops were each influential in structuring the oomycetes communities in the rhizosphere and roots, from the Test Phase of a two-year crop rotation, harvested in 2016 (Trial 1) and 2017 (Trial 2) from Swift Current, Saskatchewan. Soil history explained a consistent amount of variance in the oomycete communities (A, B, & C). The current soil chemistry consistently explained the most variance in the oomycete communities (A, B, & D), except in the roots of Trial 1, where soil chemistry explained the least variance. In Trial 1 (A & B), the influence of the host plants increased between the rhizosphere communities (A) and the roots (B). Bray-Curtis distances were used in the variance partition.

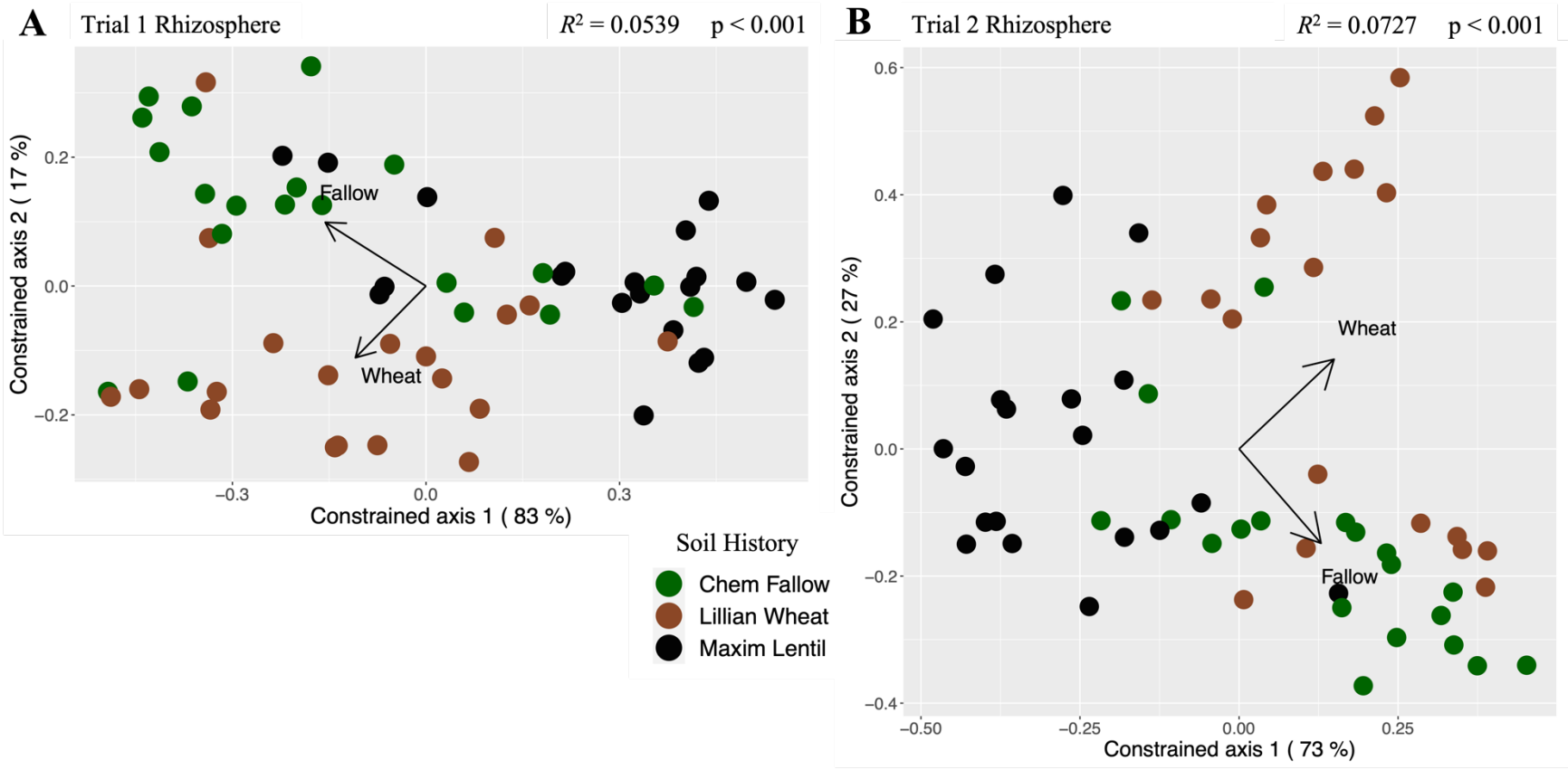

A
Trial 1 Rhizosphere
$R^2 = 0.0539$ p < 0.001
Constrained axis 2 (17 %)
Constrained axis 1 (83 %)
Fallow
Wheat
0.2
0.0
−0.2
−0.3
0.0
0.3
B
Trial 2 Rhizosphere
$R^2 = 0.0727$ p < 0.001
Constrained axis 2 (27 %)
Constrained axis 1 (73 %)
Wheat
Fallow
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.50
−0.25
0.00
0.25
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil

Figure 4. Soil history was significant in structuring the oomycete rhizosphere communities in both field trials of a two-year crop rotation, harvested in 2016 (Trial 1, A) and 2017 (Trial 2, B) from Swift Current, Saskatchewan. Distance-based redundancy analyses quantified how soil history impacted the oomycete community structure, where communities with similar composition appear closer together.

was less obvious in Trial 1 (Fig. S9A).

Soil history also determined indicator species identified in the Test Phase rhizosphere communities in both field trials. In Trial 1, five oomycete ASVs were specific to rhizosphere communities with lentil crop histories; four were recognized as *Pythiaceae* and the fifth as a *Pythium* sp. (Table 4). In Trial 2, 11 *Pythiaceae* ASVs were specific to rhizosphere communities with the lentil soil history ($p < 0.05$, Table 4), while another *Pythiaceae* ASV was specific to rhizosphere communities with the wheat soil history (Table 4).

The *Brassicaceae* crop hosts had limited influence on their oomycete communities

Brassicaceae hosts had a significant effect on oomycete community structure in Trial 1 ($R^2 = 0.0520$, $p < 0.0003$, Table 3), but not in the dry year of Trial 2. The variance partition illustrated that the *Brassicaceae* crop hosts accounted for 4.67% of variance of the Test Phase rhizosphere community data in Trial 1 (Fig. 3A). However, *Brassicaceae* crop hosts were not significant in the variance partition of Trial 2 (Fig. 3C & D). RDA also supported the significance of the *Brassicaceae* crop host in the oomycete rhizosphere communities in Trial 1 ($R^2 = 0.0454$, $p = 0.006$, Fig. S10A), but was not significant in Trial 2. The first RDA axis showed a gradient among oomycete communities between *C. sativa* and *S. alba*, with a notable amount of overlap. Interestingly, the second axis showed a gradient between soil histories, with the majority of communities from lentil sites clustered in the bottom left (Fig. S10A).

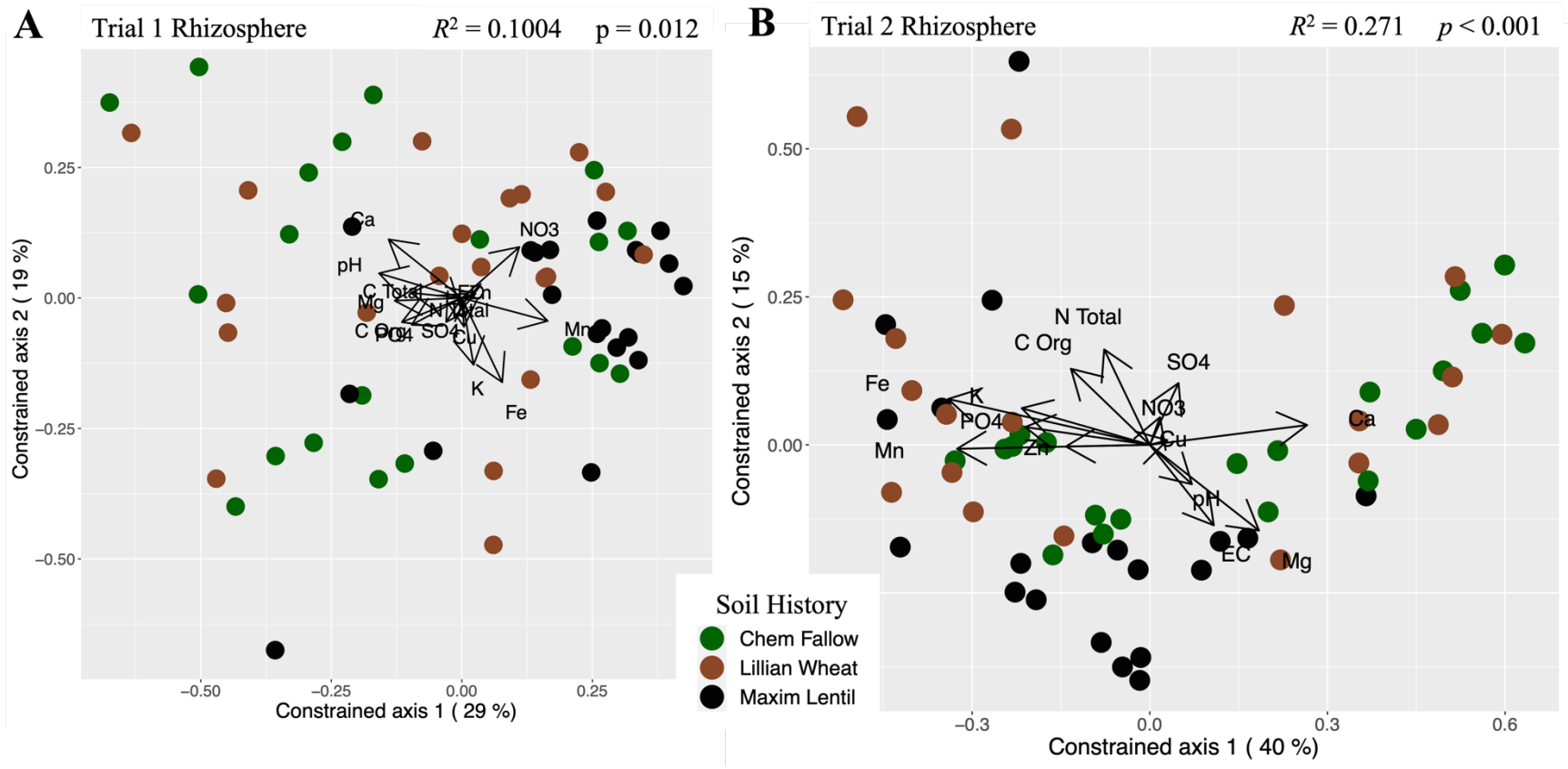
In the oomycete root communities, *Brassicaceae* crop hosts explained the most variation in Trial 1 ($R^2 = 0.0719$, $p < 0.001$, Fig. 3B), but were not significant in the variance partition of the root communities in Trial 2, similar to the rhizosphere in Trial 2. RDA also illustrated the importance of the *Brassicaceae* crop hosts in structuring the Test Phase oomycete root communities ($R^2 = 0.0961$, $p = 0.002$, Fig. S10B) in Trial 1, but not in Trial 2. Oomycete root

communities from *C. sativa* and *B. carinata* had more distinct clusters than the other three crop hosts in Trial 1 (Fig. S10A).

Differential taxa clusters from Trial 1 identified variations between *Brassicaceae* hosts in Trial 1, but not in the dry year of Trial 2. The Test Phase oomycete communities in *S. alba* rhizospheres were depleted in *Lagena* sp. ASVs relative to the rhizosphere communities of both *B. napus* and *C. sativa* ($p < 0.06$, Fig. 2B). *S. alba* root ($p < 0.01$) and rhizosphere ($p < 0.06$) communities were also depleted in *Pythiaceae* ASVs relative to the oomycete communities of *B. carinata* (Fig. 2B). Test Phase oomycete root communities from *B. carinata* were enriched in *Pythium* sp. ASVs, compared to *B. napus* ($p < 0.01$, Fig. 2B). Indicator species analysis did not identify any oomycete ASVs as specific to any of the five *Brassicaceae* host crops in either field trial.

Soil chemistry significantly influenced the oomycete rhizosphere and root communities

Variance partitioning revealed that the soil chemistry was the most significant factor in the oomycete rhizosphere communities in both field trials (Trial 1 $R^2 = 0.0945\%$, $p < 0.001$, Fig. 3A; Trial 2 $R^2 = 0.2024$, $p < 0.001$, Fig. 3C). RDA also supported that soil chemistry was the most explicative experimental factor of the Test Phase oomycete rhizosphere communities in both trials (Trial 1 adj. $R^2 = 0.1004$, $p = 0.012$, Fig. 5A; Trial 2 adj. $R^2 = 0.271$, $p < 0.001$, Fig. 5B). These data indicate that the Test Phase oomycete rhizosphere communities were strongly shaped by soil chemistry in both field trials. This effect was stronger in the rhizosphere during the dry year of Trial 2.


A
Trial 1 Rhizosphere
R^2 = 0.1004
p = 0.012
Constrained axis 2 (19 %)
Constrained axis 1 (29 %)
Ca
NO3
pH
Mg
Mn
K
Fe
Cu
SO4
B
Trial 2 Rhizosphere
R^2 = 0.271
p < 0.001
Constrained axis 2 (15 %)
Constrained axis 1 (40 %)
N Total
C Org
SO4
Fe
K
NO3
PO4
Ca
Mn
Zn
Cu
pH
EC
Mg
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil

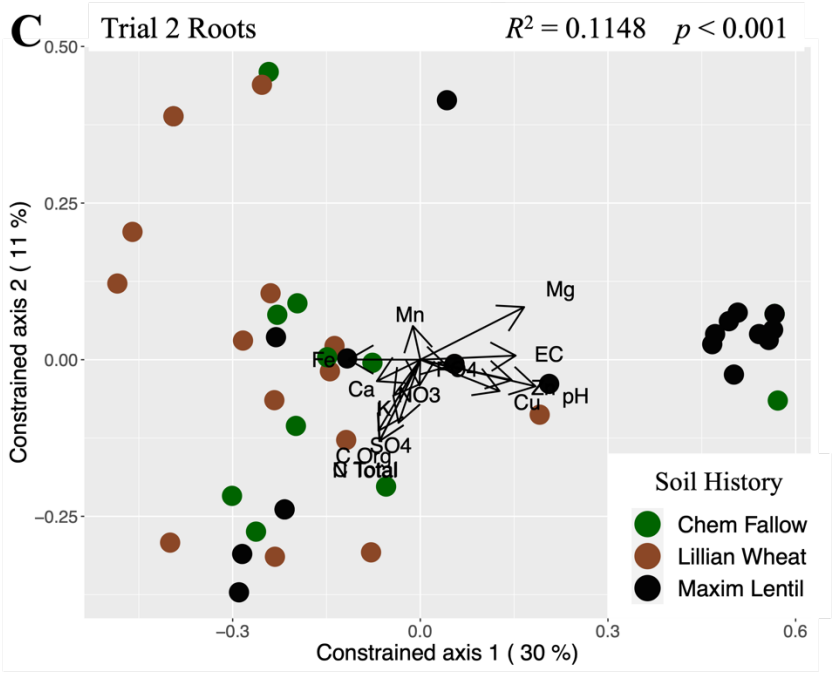

C
Trial 2 Roots
$R^2 = 0.1148$ $p < 0.001$
Constrained axis 2 (11 %)
Constrained axis 1 (30 %)
0.50
0.25
0.00
−0.25
−0.3
0.0
0.3
0.6
Mg
Mn
EC
pH
Cu
Ca
K
SO4
C Org
N Total
Soil History
Chem Fallow
Lillian Wheat
Maxim Lentil

Figure 5. Soil chemistry was the most significant factor structuring oomycete community structures in the rhizosphere (A, $R^2 = 0.1004$, p - 0.012; B, $R^2 = 0.271$, $p < 0.001$) from both field trials, as well as in the roots from five *Brassicaceae* crop hosts from Trial 2 (C, $R^2 = 0.1148$, $p < 0.001$) in the Test Phase of a two-year crop rotation, harvested in 2016 (Trial 1), and 2017 (Trial 2) from Swift Current, Saskatchewan. Distance-based redundancy analyses quantified how soil chemistry structured oomycete community structure, where communities with similar composition appear closer together. The largest factors in the rhizosphere were (A): iron, calcium, nitrate, and manganese, which contrasted with pH; and (B) calcium contrasting with manganese, and to a smaller extent zinc, magnesium opposed organic carbon, conductivity and pH contrasted total nitrogen; iron was also a strong factor. (C) the largest factor in the root communities were magnesium, which was weakly contrasted with calcium, while conductivity contrasted with iron. Note that in B and C total carbon was explained by organic carbon, while Zn was explained by Cu in C.

In the oomycete root communities, soil chemistry explained the least amount of variation in Trial 1 ($R^2 = 0.0330$, $p < 0.001$, Fig. 3B), but was the only significant factor during the dry year in Trial 2 ($R^2 = 0.0576$, $p < 0.035$, Fig. 3D). RDA also supported the significance of soil chemistry on the Test Phase oomycete root communities in Trial 2 (adj. $R^2 = 0.1148$, $p < 0.001$, Fig. 5C), but not in Trial 1. The data suggests that soil chemistry was only influential in the oomycete root communities during the dry year of Trial 2, when the effects of soil history and *Brassicaceae* crop host were reduced on structuring the communities.

Co-inertia analysis between the *Brassicaceae* oomycete and bacterial communities

We used a co-inertia test to investigate how influential soil history was on the relationship between the Test Phase oomycete communities investigated here and the previously identified bacterial communities (Blakney *et al*., 2022). In Trial 1, the root and rhizosphere samples had similar RV coefficients, 0.6291 and 0.7049, respectively, suggesting that the oomycete and bacterial communities did have significant relationships in both compartments. The rhizosphere samples were plotted on the first and second axes which represented 8.185% and 6.551% of the co-inertia. The low inertia suggests little influence of the two sets of ASVs on the samples. This is further illustrated in that the majority of the 60 samples in Trial 1 appeared quite similar, as they remained clustered together toward the centre of the plot. Rhizosphere samples from plots 17, 41, 46, and 51, were relatively more influenced by the presence of particular microbial ASVs, as they are further from the centre (Fig. S11A). Only samples 17 and 51 illustrated any noticeable divergence between which microbial ASVs influenced their structure, given their appreciable arrow lengths.

The root samples from Trial 1 captured 9.404% and 7.682% of the co-inertia in the first and second axes, respectively, which suggests minimal influence of the ASVs on each sample,

similar to their corresponding rhizosphere communities. Most of the root samples were also clustered together in the co-inertia plot; only samples 47 and 51 were noticeably influenced by the presence of particular microbial ASVs, given their less central positions. The arrow length of the root sample from plot 47 suggests it diverged between which microbial ASVs influenced their structure (Fig. S11B).

In Trial 2, the root and rhizosphere samples had less similar RV coefficients; the oomycete and bacterial communities had a significant relationship (RV = 0.8307) in the rhizosphere samples, but not in the root samples (RV = 0.5767). The first and second axes for the rhizosphere samples represented 12.776% and 4.578% of the co-inertia, respectively. Given the low inertia the rhizosphere samples appeared to be weakly influenced by the two datasets of microbial ASVs. Nonetheless, Trial 2 rhizosphere samples were more heterogenous as they were more dispersed compared to the rhizosphere samples from Trial 1, with samples 22 and 30 being distinctly different (Fig. S11C). Finally, the influence of the microbial ASVs only appeared to shift in samples 19 and 30, as shown by their arrow lengths.

The Trial 2 root samples captured 8.880% and 8.131% of the co-inertia in the first and second axes, respectively. Again, the low inertia suggests minimal influence of microbial ASVs on each sample, which is similar to their corresponding rhizosphere communities. Unlike the rhizosphere samples, however, the root samples were tightly clustered; only sample 51 was noticeably influenced by particular microbial ASVs. The arrow length of sample 51 also suggests the influence of the microbial ASVs shifted between the bacterial and oomycete communities (Fig. S10C).

Discussion

How soil history established by previous plant-soil microbial communities conditions future generations of oomycete communities remains relatively unknown. Oomycetes are vastly understudied compared to bacteria and fungi, yet are important microbial communities, especially for agriculture where many oomycetes are responsible for severe declines in yields. In this study, we investigated the impact of three different soil histories established by the previous year's crops on the soil oomycete communities associated with five *Brassicaceae* oilseed host crops. The semi-nested ITS amplicon strategy we incorporated with MiSeq metabarcoding specifically targeted oomycetes and has been previously shown to limit off-target amplification from the ITS region of other eukaryotes (Sapkota & Nicolaisen, 2015; Taheri *et al*., 2017b). The oomycete metabarcoding data illustrated that soil history had a greater influence on the communities than the *Brassicaceae* host crops, while soil chemistry structured the oomycete communities more during the dry field trial. Our results highlight the impact of edaphic factors over different growing seasons and the importance of monitoring and quantifying oomycete biodiversity.

Soil history significantly impacted the oomycete rhizosphere communities

The previous crops, and their agricultural treatments, impacted the subsequent oomycete communities through plant-soil microbial community feedback. We hypothesized that the three soil histories established by the previous crops would structure significantly different oomycete communities, regardless of their current *Brassicaceae* host, in both the roots and rhizosphere. Our data illustrated that this was largely sustained; we found consistent support for soil history influencing the structure of the oomycete rhizosphere communities of both field trials, as well as the root communities in Trial 2 (Table 3, Fig. 3). Moreover, gradient analysis (Fig. 4 & S9) highlighted how different oomycete communities tended to cluster according to the soil histories

established by the previous crops, especially among the rhizosphere communities. These are exciting results as they raise more questions about oomycete community dynamics and their interactions with different soil histories established through crop rotations.

In this study, we found that the oomycete communities were significantly structured by each of the three previously established soil histories. Conversely, we observed little effect from the current *Brassicaceae* crop hosts to re-structure the oomycete communities. Plant-microbial community feedback from new host plants has shown to be able to quickly erase the soil history established by a previous plant and modify bacterial communities (Kaisermann *et al*., 2017; Hannula *et al*., 2021; Blakney *et al*., 2022). Crop rotations, for example, have previously demonstrated to quickly adjust subsequent bacterial communities via plant-microbial community feedback mechanisms (Hamel *et al*., 2018; Blakney *et al*., 2022). Important fractions of bacterial rhizosphere communities tend to be fast-growing and have rapid turn-over, which may allow bacterial communities to be more responsive to the dynamic needs of their host plants (Mendes *et al*., 2011; Castrillo *et al*., 2017).

However, experimental evidence has suggested that such feedback mechanisms are not sufficient to alter fungal communities (Kaisermann *et al*., 2017; Hannula *et al*., 2021). One suggestion for why the influence of established soil history varies between bacterial and fungal communities has been due to their different growth rates (Semchenko *et al*., 2018; Hannula *et al*., 2021). Compared to the rapidly growing components of plant bacterial communities, fungal communities tend to remain more stable through time. Fungal growth appears more steady and less influenced by host plant feedback mechanisms, which limits how responsive fungal communities might be to the influence of new host crops (Kaisermann *et al*., 2017; Hannula *et al*., 2021).

Although oomycetes are not fungi, and have vastly different evolutionary origins (Fawke *et al*., 2015; Kamoun *et al*., 2015; Schwelm *et al*., 2017), our data illustrates a similar trend, where oomycetes, like fungi, remained relatively unaffected by changes in hosts, possibly due to their growth rate. Complementary to this idea is that oomycete oospores can persist from year to year and are constitutively dormant, such that not all oospores germinate at the same time even under optimal conditions (Martin & Loper, 1999; Fernández-Pavía *et al*., 2004; Kikway *et al*., 2022; Subila & Suseela, 2022). This could also help to account for why oomycete communities may appear less affected by the influence of the new *Brassicaceae* hosts feedback mechanisms (Kaisermann *et al*., 2017; Hannula *et al*., 2021).

In fact, our results illustrate that the soil history established by the previous lentil and wheat crops helped to structure distinct oomycete communities that were still detectable the following year (Fig. 4 & S9, Table 4). The lentil-specific oomycete rhizosphere community we detected in both field trials may be unsurprising, since legumes, including lentils, tend to retain more soil moisture compared to other crops, and soil moisture is a key factor for oomycete growth. Recent studies have also suggested that lentils have an increased vulnerability to oomycete outbreaks (Hwang *et al*., 2015; Rojas *et al*., 2017; Karppinen *et al*., 2020). Finally, *Pythiaceae* have previously been reported in Canadian pea fields (Taheri *et al*., 2017a), thus detecting a variety of *Pythiaceae* ASVs specific to the lentil soil history seems reasonable.

Somewhat more unexpected was the *Aphanomyces* ASV specific to the rhizosphere communities in Trial 2 with wheat soil history (Table 4). Most of the interest concerning the *Aphanomyces* focuses on *A. cochlioides* and *A. euteiches*, which are well described pathogens specific to sugar beets and legumes, respectively (Diéguez-Uribeondo *et al*., 2009). However, there is a divergent lineage that consists of saprotrophs and opportunistic plant pathogens that are not known to maintain specific hosts (Diéguez-Uribeondo *et al*., 2009). A saprotrophic oomycete

capable of degrading wheat residues might explain the *Aphanomyces* ASV identified among the rhizosphere communities with wheat soil history from Trial 2.

However, oomycete functional lifestyles can actually be rather diverse (Fiore-Donno & Bonkowski, 2021). Even though the putative lifestyles identified the ASVs we reported as plant pathogens—which is concordant with the number of *Pythium* and *Phytophthora* ASVs we identified, and with other metabarcoding surveys (Fiore-Donno & Bonkowski, 2021)—these lifestyle assignments can still contain a range of functions (Fiore-Donno & Bonkowski, 2021). For example, oomycete plant pathogens exist in an array of biotrophic and hemibiotrophic capacities (Fiore-Donno & Bonkowski, 2021) in terms of timing, specificity and duration (Fiore-Donno & Bonkowski, 2021). Furthermore, hemibiotrophs can also live saprotrophically in the soil in the absence of a plant host and have even been shown to play important roles in decomposition (Lifshitz & Hancock, 1983; Kramer *et al*., 2016). Thus, the wheat-specific *Aphanomyces* ASV we detected may hint at more diverse functions among this group of oomycetes.

Oomycete communities were not influenced by *Brassicaceae* crops during the drier field trial

Although our initial hypothesis concerning soil history was largely supported, we did nonetheless observe an influence of the *Brassicaceae* crop hosts on the oomycete communities, but only during Trial 1 (Fig. 3, Table 3), and particularly in their roots (Fig. S10). This is an interesting finding given that the root communities we identified were noticeably reduced and less diverse compared to their cognate rhizosphere communities. To reduce any rhizosphere-rhizoplane influence on the root communities, we used a rigorous protocol where we scrapped off attached soil from the roots, and repeatedly washed the roots to remove the rhizosphere. In theory, additional surface washes, or surface sterilization may have further reduced any residual rhizosphere-rhizoplane influence on the root communities.

Plant hosts ought to have the most influence to select for microbial communities in their roots, compared to the rhizosphere, or leaf surface (Gavrin *et al*., 2020; Maciá-Vicente *et al*., 2020). While our data illustrates that the *Brassicaceae* host crops were quite significant in the roots during Trial 1 (Fig. 3 & S9), we did not detect any oomycete ASVs as indicator species from any of the five hosts (Table 4). Furthermore, we observed a considerable amount of overlap among the oomycete root communities, notwithstanding the more distinct clusters of communities from *C*. *sativa* and *B*. *carinata* (Fig. S10). Our data may indicate that the influence of the *Brassicaceae* hosts on the oomycete root communities was insufficient to structure more distinct groups of oomycetes. Similar results were found for the communities from different cultivars of *Rhododendron* (Foster *et al*., 2020). This weaker effect of plant hosts could be due to other competing factors, such as the previous soil history, or current soil chemistry. Alternatively, the close genetic relationship of the host plants may preclude us from identifying more specific oomycete assemblages (Foster *et al*., 2020; Blakney *et al*., 2022).

Moreover, there was no influence of any of the *Brassicaceae* crops on the oomycete communities during Trial 2 that we observed, despite following identical experimental protocols, and the use of the same agricultural management practices and inputs. The disparate observations between the two field trials could be due to the environmental conditions being 6x drier during Trial 2: 55.0 mm of precipitation versus 328.4 mm in Trial 1 (Blakney *et al*., 2022). The *Brassicaceae* host plants appeared to be restricted in growth due to the dry conditions (Fig. S3), which would also constrain their nutrient uptake from the soil, and rhizodeposition (Fitzpatrick *et al*., 2018). Therefore, if the reciprocal plant-soil microbial community feedback was impaired due to the availability of water, it could account for the absent influence of the *Brassicaceae* hosts on the oomycete communities in Trial 2.

The drier field conditions of Trial 2 may also have had an impact on the oomycete community itself. Oomycetes prefer high soil moisture for motility, subsequent infection and growth, and the completion of their life cycle (Martin & Loper, 1999; Fawke *et al*., 2015; Martiny *et al*., 2015; Rojas & Huang, 2018). Our data demonstrates a similar community composition between both field trails, though with reductions in sequencing reads and diversity in Trial 2. This could be evidence for how the drier conditions impacted the community. Quantifying community sizes could help determine this in future experiments.

Nonetheless, the impact of the *Brassicaceae* crop hosts remained limited, as the structure of the oomycete communities remained significantly influenced by the previous crops. This could indicate that these specific crops may not be effective as a strategy to limit the accumulation of potentially pathogenic oomycetes in the soil over the short term. Various crop rotations, including those involving *Brassicaceae*, have been shown to help control phytopathogens by restructuring the microbial communities from one season to the next (Etesami & Alikhani, 2016; Yang *et al*., 2021). Such shifts generally occur through plant-soil feedback processes, such as rhizodeposition, or by producing anti-microbial compounds **(**Krasnow & Hausbeck, 2015; Lebeis *et al*., 2015; Revillini *et al*., 2016; Korenblum *et al*., 2020; Kawasaki *et al*., 2021; Yu *et al*., 2021). For example, *Brassicaceae* crops produce anti-microbial glucosinolates, which have been used to control phytopathogens, including oomycetes (Krasnow & Hausbeck, 2015). However, our data illustrates that the five *Brassicaceae* crops were unable to sufficiently alter the soil history established by the previous crops, given that the oomycete communities remained significantly structured by soil history. This could suggest that these crop rotations may be insufficient to control oomycete phytopathogens in the short term.

In addition, we observed that *B. carinata* crop hosts were significantly enriched in *Pythiaceae* ASVs in their root and rhizosphere communities compared to the other *Brassicaceae*

hosts (Fig. 2B). This could suggest that *B*. *carinata* may be more susceptible to oomycete accumulation. Conversely, we also noted that *S*. *alba* hosts were depleted in *Lagena* and *Pythiaceae* ASVs in their rhizosphere communities (Fig. 2B), compared to the other Brassicaceae crop hosts. This might demonstrate increased resistance to accumulating these oomycetes in their rhizosphere. These two examples warrant further study to help evaluate how effective these particular crop rotations may be at limiting oomycete infections.

Further to this, our study points out three recommendations needed to better understand the phytopathogenicity of oomycetes: first, biodiversity monitoring should inventory the oomycete communities established at the end of each growing season and observe which ASVs persisted in a given plot (Derevnina *et al*., 2016; Gómez *et al*., 2021). Second, quantifying the size of each community would help determine if crop rotations actually limit, or reduce, the growth of the oomycete communities. Third, since the impact of a crop may not be observed during the active growing season (Hamel *et al*., 2018), longer field trials with multiple timepoints could help confirm our findings. These additional steps may provide a more nuanced understanding of the dynamics within oomycete communities and help determine the utility of crop rotations as a strategy to limit the accumulation of oomycete phytopathogens in agricultural soil.

Soil chemistry constrained the oomycete community structure

Although we initially sought to test the influence of soil history on structuring oomycete communities, our data revealed that the soil chemistry had the strongest influence in the rhizosphere during both field trials, and among the root communities during the dry season in Trial 2 (Fig. 3 & 5). In our agricultural setting, soil chemistry was largely a synthesis of the previous soil history, current agricultural management practices, and the plant-microbial community

feedback mechanisms (Bouffaud *et al*., 2014). These processes interact to yield a number of edaphic conditions previously identified to promote oomycete growth.

For example, soils with excessive or insufficient nutrients for their local microbial communities are prone to outbreaks of oomycete infections, as soil nutrient imbalance provides niche space for them (Löbmann *et al*., 2016; Rojas *et al*., 2017). Indicators of soil nutrient balance may include conductivity, cation exchange capacity (EC), total nitrogen, and total carbon (Löbmann *et al*., 2016; Rojas *et al*., 2017; Karppinen *et al*., 2020). Although none of the measured edaphic factors were particularly related to the communities observed in Trial 1, oomycete rhizosphere and roots communities with lentil soil histories were strongly associated with EC in Trial 2 (Fig. 3). Soil moisture is another key factor in promoting oomycete growth and is compounded by seeding into cool (< 16°C) soils (Hwang *et al*., 2015; Rojas *et al*., 2017; Karppinen *et al*., 2020). These conditions favour the release and chemotaxis of oomycete zoospores (Martin & Loper, 1999; Fawke *et al*., 2015). Therefore, we might have expected to observe a more dramatic change in the oomycete rhizosphere community between the wetter season of Trial 1 and the dry season of Trial 2.

The importance of soil chemistry may also be reflected in the results of our co-inertia analysis. This analysis illustrated that although the oomycete and bacterial data tended to have a significant relationship, neither community was particularly vital in the roots nor the rhizosphere, and nor did any of the three soil histories influence their relationship (S11). Given that both of these microbial communities were derived from the same soil samples, they were more likely to experience the same edaphic factors. Therefore, the lack of obvious influences in the co-inertia analysis could be due to the oomycete and bacterial communities being similarly constrained by their common soil chemistry.

Microbes largely share basic biological reactions to abiotic factors, such as changes to pH, temperature, or water availability (Martiny *et al*., 2015). For example, bacteria, fungi, oomycetes, among others, require water for chemotaxis and locomotion, as well for maintaining turgor pressure (Martiny *et al*., 2015; Rojas & Huang, 2018). Cellular function requires the correct regulation of pressure, without which cells are unable to grow, divide, or move (Rojas & Huang, 2018). Although microbes have evolved a number of specialized tactics to regulate osmolarity, water stress, among other limitations imposed by soil chemistry, remain common constraints (Martiny *et al*., 2015). Therefore, the homogeneity we observed from the co-inertia analysis could reasonably be due to the oomycete and bacterial communities being similarly constrained by their common soil chemistry.

Conclusion

Oomycetes are major global phytopathogens, yet are understudied compared to other microbes. Here, we have shown for the first time the important role of soil history in structuring oomycete rhizosphere and root communities. We tested three different soil histories and found that none of the five planted *Brassicaceae* oilseed crops were able to restructure the oomycete communities the following year. We also took a novel approach in investigating how oomycete and bacterial communities may have structured one another. To our knowledge this is the first demonstration of the weak impact between the two microbial communities. Rather, the similarities between the two microbial communities may be due to being constrained by common edaphic factors. This study advances our understanding of how different agricultural practices can impact future microbial communities differently. Our results also highlight the need for continued monitoring of oomycete biodiversity and quantification.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada, CRD Fund (Grant Number: CRDPJ 500507-16), Canola Council of Canada and Saskatchewan Pulse Growers, which are gratefully acknowledged. We thank Yantai Gan and Lee Poppy for setting up and managing field experiments at Swift Current, Antoine Dionne (Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec), Syama Chatterton and Hossein Borhan (Agriculture and Agri-Food Canada), for generously providing a diversity of oomycete DNA samples for the mock community. We also thank Stéphane Daigle for assistance in statistical analysis, Chih-Ying Lay, Jacynthe Masse, Chantal Hamel, and Simon Joly and for their helpful comments and discussions. Finally, AB would like to thank Morgan Botrel for her talents in collaborating on the graphical abstract, as well as Simon Morvan, Alexis Carteron, and the Quebec Centre for Biodiversity Science for their support and encouragement.

Author Contributions

AJCB performed the qPCR experiment and assembled the mock community, prepared the samples for sequencing, analyzed the data, and wrote the manuscript with input from all co-authors. LB conducted field trials 1 and 2 and collected data; MSA & MH designed the experiment, supervised the work, contributed reagents, analytical tools, and revised the manuscript.

Data Accessibility

Sequencing data and metadata are available at NCBI Bioproject under accession number: PRJNA849532.

Supplementary Materials

Table S1. Nitrogen (N), phosphorous (P), potassium (K), and sulfur (S), available in the soil upon establishing the Test Phase, and the fertilizer that was subsequently applied during the Test Phase for the experiment at Swift Current, Saskatchewan. Adapted from Hossain *et al*., 2019.

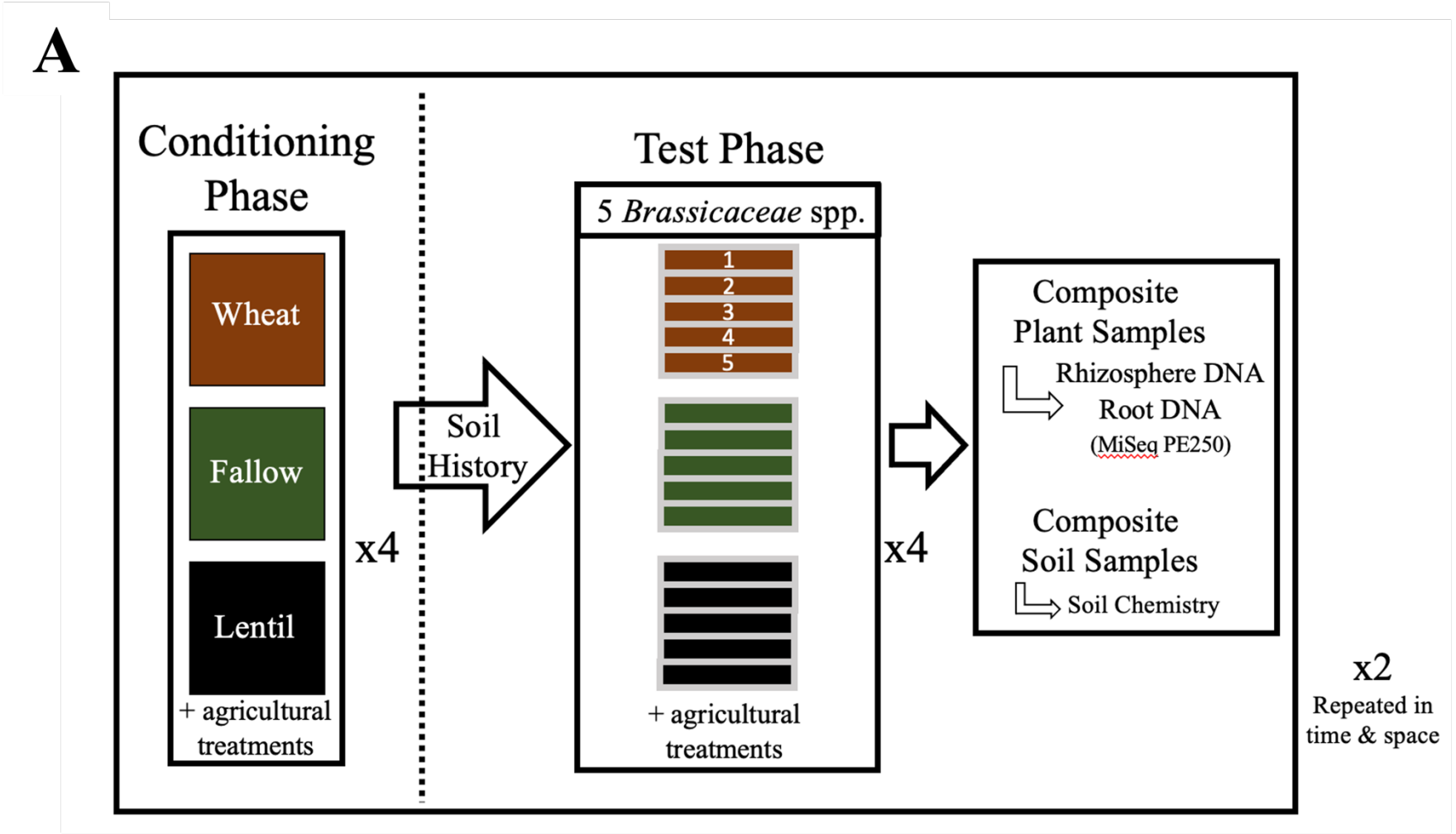
Swift Current Test Phase Plot Fertilization (N-P_2O_5-K_2O-S kg ha $^{-1}$)			
	Soil History	Nutrients Available[a]	Fertilizer Applied
Trial 1	Chem-fallow	37-34-646-22	48-7-0-10
	Lentil	20-33-578-28	65-7-0-10
	Wheat	18-31-511-19	68-7-0-10
Trial 2	Chem-fallow	42-34-446-83	55-7-0-10
	Lentil	30-43-488-83	43-7-0-10
	Wheat	18-26-482-82	67-7-0-10

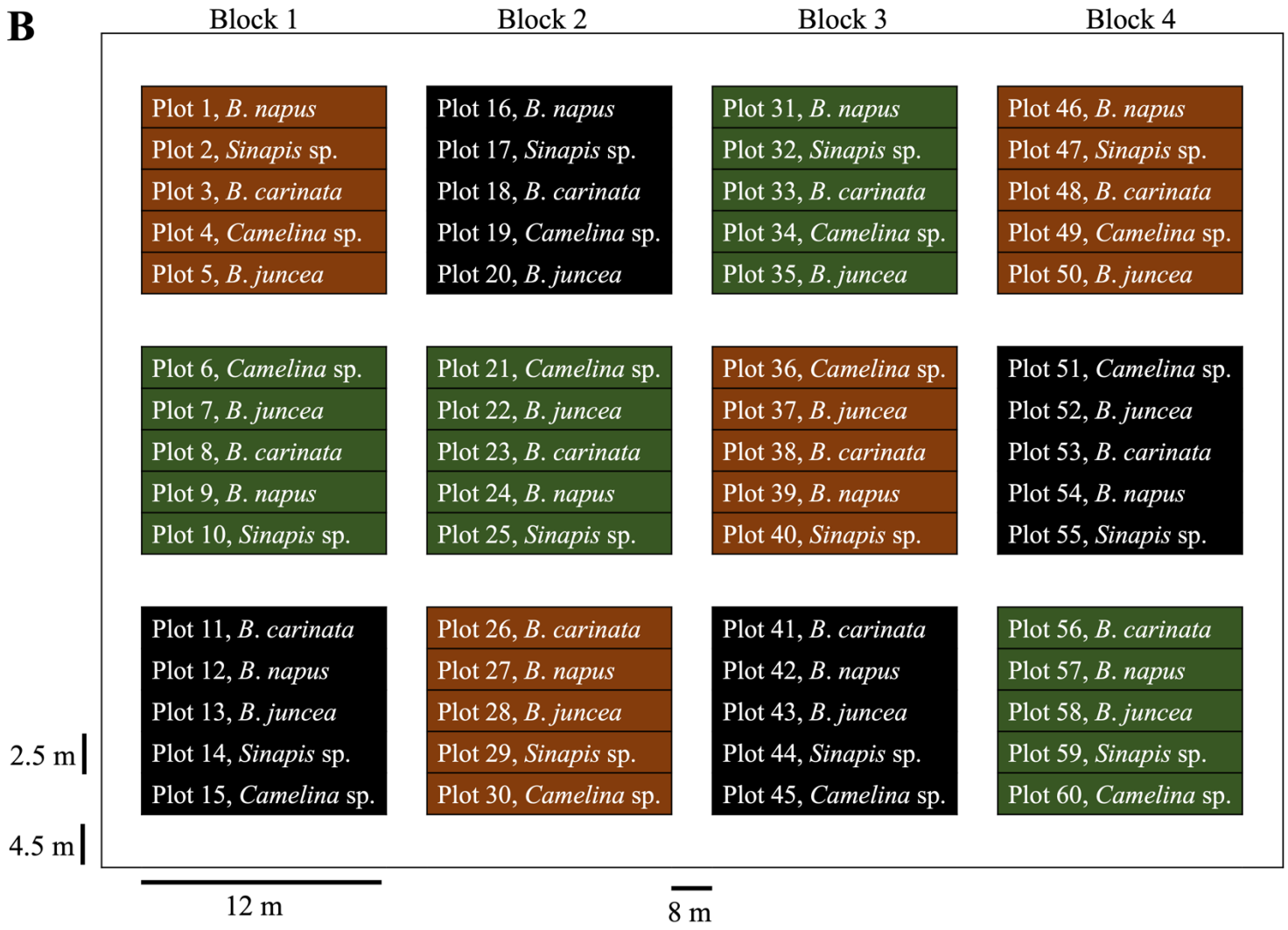
[a], Measurements taken at *Brassicaceae* planting prior to fertilizing, with available N and S measured at 0–60 cm depth, P and K at 0–15 cm depth.

Table S2. Oomycete strains that composed our mock community, which were included on each plate (Figure 2). The oomycete mock community we assembled contained DNA from these 21 oomycete taxa in staggard copies / μL of ITS1 sequences, as per Bakker (2018). A copy of the mock community was included on each 96-well plate submitted for sequencing. Taxa are provided below to illustrate the level of comparison.

Oomycetes		Copies of ITS
Orders		
Albuginales	*Albugo candida* 2v	~ 100
Saprolegniales	*Aphanomyces euteiches* 2	10 000
	Aphanomyces euteiches 206C	1000
Peronosporales	*Phytophthora sojae* 31594R	1 000 000
	Phytophthora sojae 31461	500 000
	Phytophthora capsici 31710	250 000
	Phytophthora capsici 31598C	100 000
Pythiales	*Pythium dissotocum* 31309R	20 000 000
	Pythium sylvaticum 31411R	1 000 000
	Pythium sylvaticum 31392R	1 000 000
	Pythium vanterpoolii 31799	750 000
	Pythium irregulare 30717	500 000
	Pythium sylvaticum 31019R	500 000
	Pythium torulosum 31800	250 000
	Pythium conidiophorum 30918R	100 000
	Pythium sylvaticum 30623	100 000

Pythium arrhenomanes 30886	75 000
Pythium ultimum	50 000
Pythium heterothallicum 30538	50 000
Pythium irregulare 30717	25 000
Pythium irregulare	1000


A
Conditioning Phase
Wheat
Fallow
Lentil
+ agricultural treatments
x4
Soil History
Test Phase
5 Brassicaceae spp.
1
2
3
4
5
+ agricultural treatments
x4
Composite Plant Samples
Rhizosphere DNA
Root DNA
(MiSeq PE250)
Composite Soil Samples
Soil Chemistry
x2
Repeated in time & space


B
Block 1
Block 2
Block 3
Block 4
Plot 1, B. napus
Plot 2, Sinapis sp.
Plot 3, B. carinata
Plot 4, Camelina sp.
Plot 5, B. juncea
Plot 16, B. napus
Plot 17, Sinapis sp.
Plot 18, B. carinata
Plot 19, Camelina sp.
Plot 20, B. juncea
Plot 31, B. napus
Plot 32, Sinapis sp.
Plot 33, B. carinata
Plot 34, Camelina sp.
Plot 35, B. juncea
Plot 46, B. napus
Plot 47, Sinapis sp.
Plot 48, B. carinata
Plot 49, Camelina sp.
Plot 50, B. juncea
Plot 6, Camelina sp.
Plot 7, B. juncea
Plot 8, B. carinata
Plot 9, B. napus
Plot 10, Sinapis sp.
Plot 21, Camelina sp.
Plot 22, B. juncea
Plot 23, B. carinata
Plot 24, B. napus
Plot 25, Sinapis sp.
Plot 36, Camelina sp.
Plot 37, B. juncea
Plot 38, B. carinata
Plot 39, B. napus
Plot 40, Sinapis sp.
Plot 51, Camelina sp.
Plot 52, B. juncea
Plot 53, B. carinata
Plot 54, B. napus
Plot 55, Sinapis sp.
Plot 11, B. carinata
Plot 12, B. napus
Plot 13, B. juncea
Plot 14, Sinapis sp.
Plot 15, Camelina sp.
Plot 26, B. carinata
Plot 27, B. napus
Plot 28, B. juncea
Plot 29, Sinapis sp.
Plot 30, Camelina sp.
Plot 41, B. carinata
Plot 42, B. napus
Plot 43, B. juncea
Plot 44, Sinapis sp.
Plot 45, Camelina sp.
Plot 56, B. carinata
Plot 57, B. napus
Plot 58, B. juncea
Plot 59, Sinapis sp.
Plot 60, Camelina sp.
2.5 m
4.5 m
12 m
8 m

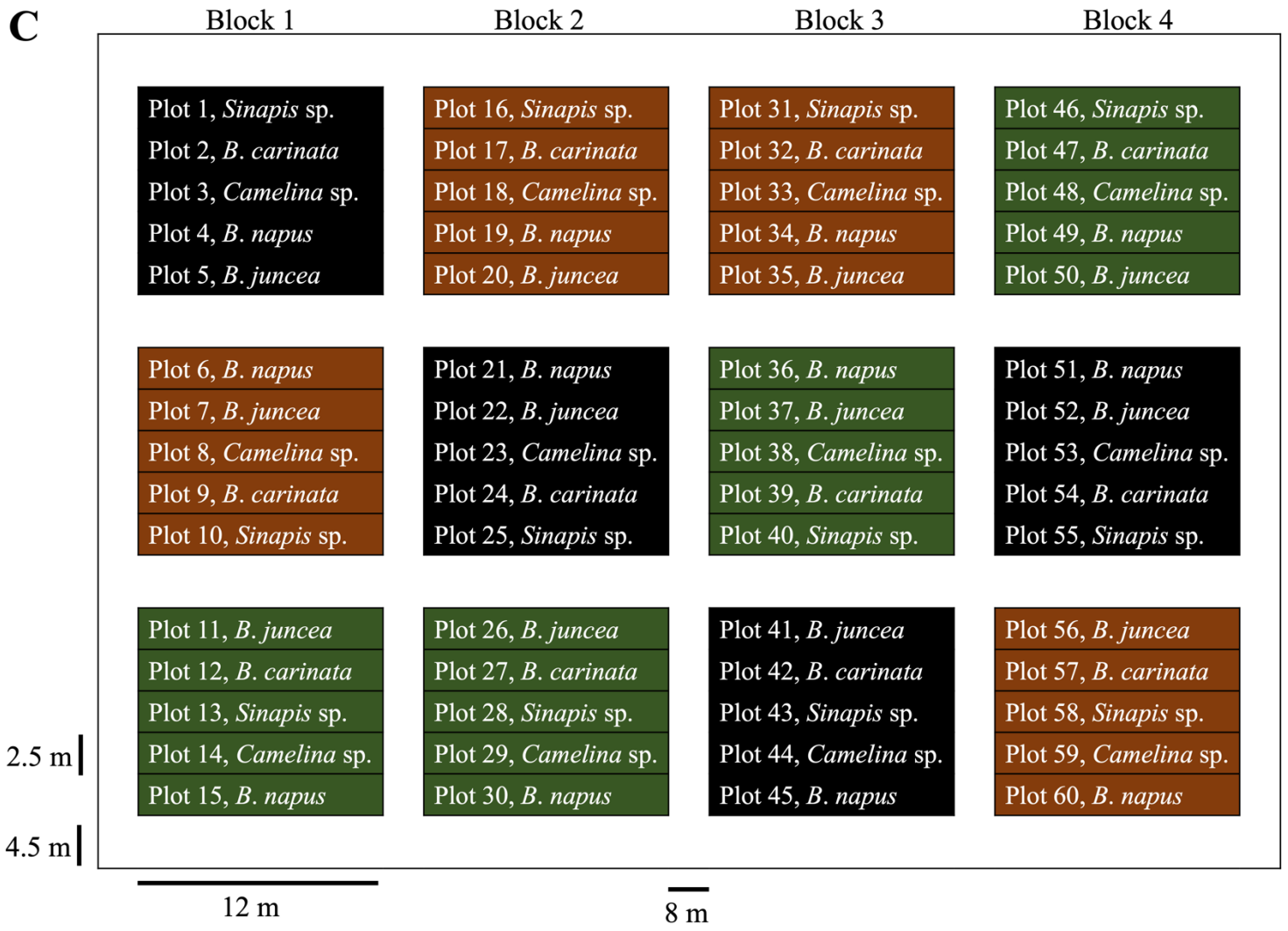

C
Block 1
Block 2
Block 3
Block 4
Plot 1, Sinapis sp.
Plot 2, B. carinata
Plot 3, Camelina sp.
Plot 4, B. napus
Plot 5, B. juncea
Plot 16, Sinapis sp.
Plot 17, B. carinata
Plot 18, Camelina sp.
Plot 19, B. napus
Plot 20, B. juncea
Plot 31, Sinapis sp.
Plot 32, B. carinata
Plot 33, Camelina sp.
Plot 34, B. napus
Plot 35, B. juncea
Plot 46, Sinapis sp.
Plot 47, B. carinata
Plot 48, Camelina sp.
Plot 49, B. napus
Plot 50, B. juncea
Plot 6, B. napus
Plot 7, B. juncea
Plot 8, Camelina sp.
Plot 9, B. carinata
Plot 10, Sinapis sp.
Plot 21, B. napus
Plot 22, B. juncea
Plot 23, Camelina sp.
Plot 24, B. carinata
Plot 25, Sinapis sp.
Plot 36, B. napus
Plot 37, B. juncea
Plot 38, Camelina sp.
Plot 39, B. carinata
Plot 40, Sinapis sp.
Plot 51, B. napus
Plot 52, B. juncea
Plot 53, Camelina sp.
Plot 54, B. carinata
Plot 55, Sinapis sp.
Plot 11, B. juncea
Plot 12, B. carinata
Plot 13, Sinapis sp.
Plot 14, Camelina sp.
Plot 15, B. napus
Plot 26, B. juncea
Plot 27, B. carinata
Plot 28, Sinapis sp.
Plot 29, Camelina sp.
Plot 30, B. napus
Plot 41, B. juncea
Plot 42, B. carinata
Plot 43, Sinapis sp.
Plot 44, Camelina sp.
Plot 45, B. napus
Plot 56, B. juncea
Plot 57, B. carinata
Plot 58, Sinapis sp.
Plot 59, Camelina sp.
Plot 60, B. napus
2.5 m
4.5 m
12 m
8 m

Figure S1. Field plans for the experiment. A two-phase cropping sequence—consisting of a Conditioning Phase the first year, and a Test Phase in the second year—was repeated in two field trials, Trial 1, 2015-2016, and Trial 2, 2016-2017, on adjacent sites in a field previously growing spring wheat (*Triticum aestivum* cultivar AC Lillian). The experimental design was a split-plot replicated in four complete blocks. In the 'Conditioning Phase', three soil history treatments were randomly assigned, consisting of spring wheat (*Triticum aestivum*, cv. AC Lillian), red lentil (*Lens culinaris* cv. CDC Maxim CL), or left fallow (brown, black, green, respectively). In the 'Test Phase', the conditioned plots were each subdivided and five *Brassicaceae* oilseed crop species were randomly assigned to one of these five subplots. Thus, each experiment had 60 subplots to sample. (A) Trial 1 field plan for the *Brassicaceae* crops, which were Ethiopian mustard (*Brassica carinata* L., cv. ACC110), canola (*B. napus* L., cv. L252LL), oriental mustard (*B. juncea* L., cv. Cutlass), yellow mustard (*Sinapis alba* L., cv. Andante), and camelia (*Camelina sativa* L., cv. Midas). Boarder space between plots and blocks is in white. (B) Trial 2 field plan for the same *Brassicaceae* crops. For further details of this well-described experiment and its design, see Hossain *et al*. (2019), Liu *et al*. (2019), and Wang *et al*. (2020).

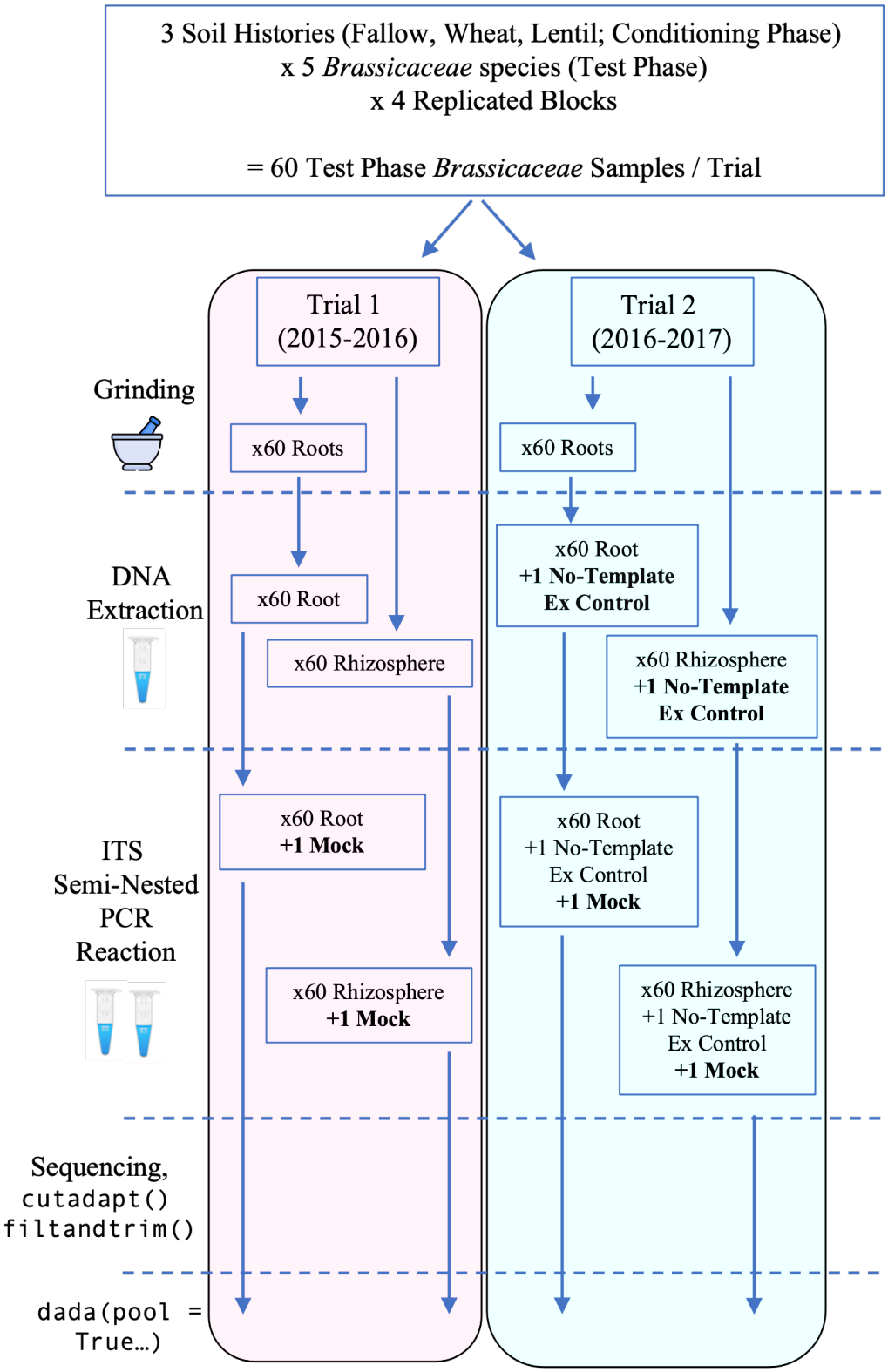

3 Soil Histories (Fallow, Wheat, Lentil; Conditioning Phase)
x 5 *Brassicaceae* species (Test Phase)
x 4 Replicated Blocks
= 60 Test Phase *Brassicaceae* Samples / Trial
Trial 1 (2015-2016)
Trial 2 (2016-2017)
Grinding
x60 Roots
x60 Roots
DNA Extraction
x60 Root
x60 Root +1 No-Template Ex Control
x60 Rhizosphere
x60 Rhizosphere +1 No-Template Ex Control
ITS Semi-Nested PCR Reaction
x60 Root +1 Mock
x60 Root +1 No-Template Ex Control +1 Mock
x60 Rhizosphere +1 Mock
x60 Rhizosphere +1 No-Template Ex Control +1 Mock
Sequencing, cutadapt() filtandtrim()
dada(pool = True…)

Figure S2. Organization of our lab workflow for the Test Phase *Brassicaceae* samples from harvest to generating amplicon sequence variants (ASVs). The Test Phase *Brassicaceae* samples were harvested in mid-late July. Four plants from two different locations within each of the 60 subplots were excavated and pooled together as a composite sample (Hossain *et al*., 2019; Liu *et al*., 2019, Wang *et al*., 2020). In the field, each plant had its rhizosphere soil divided from the root material, both portions were immediately flash-frozen in liquid nitrogen, and kept on ice. In the lab, roots were ground in liquid nitrogen, and DNA was extracted from all the Test Phase *Brassicaceae* root and rhizosphere portions. No-template extraction controls were included to assess what contaminates, or biases, the extraction kits might impart. We confirmed by gel electrophoresis that the no-template extraction controls contained DNA prior to sequencing. All DNA samples were submitted to Génome Québec for semi-nested ITS PCR amplification, library preparation, and paired-end 250 bp Illumina MiSeq sequencing. All reads were subsequently trimmed using cutadapt and processed through the DADA2 pipeline for ASV inference.

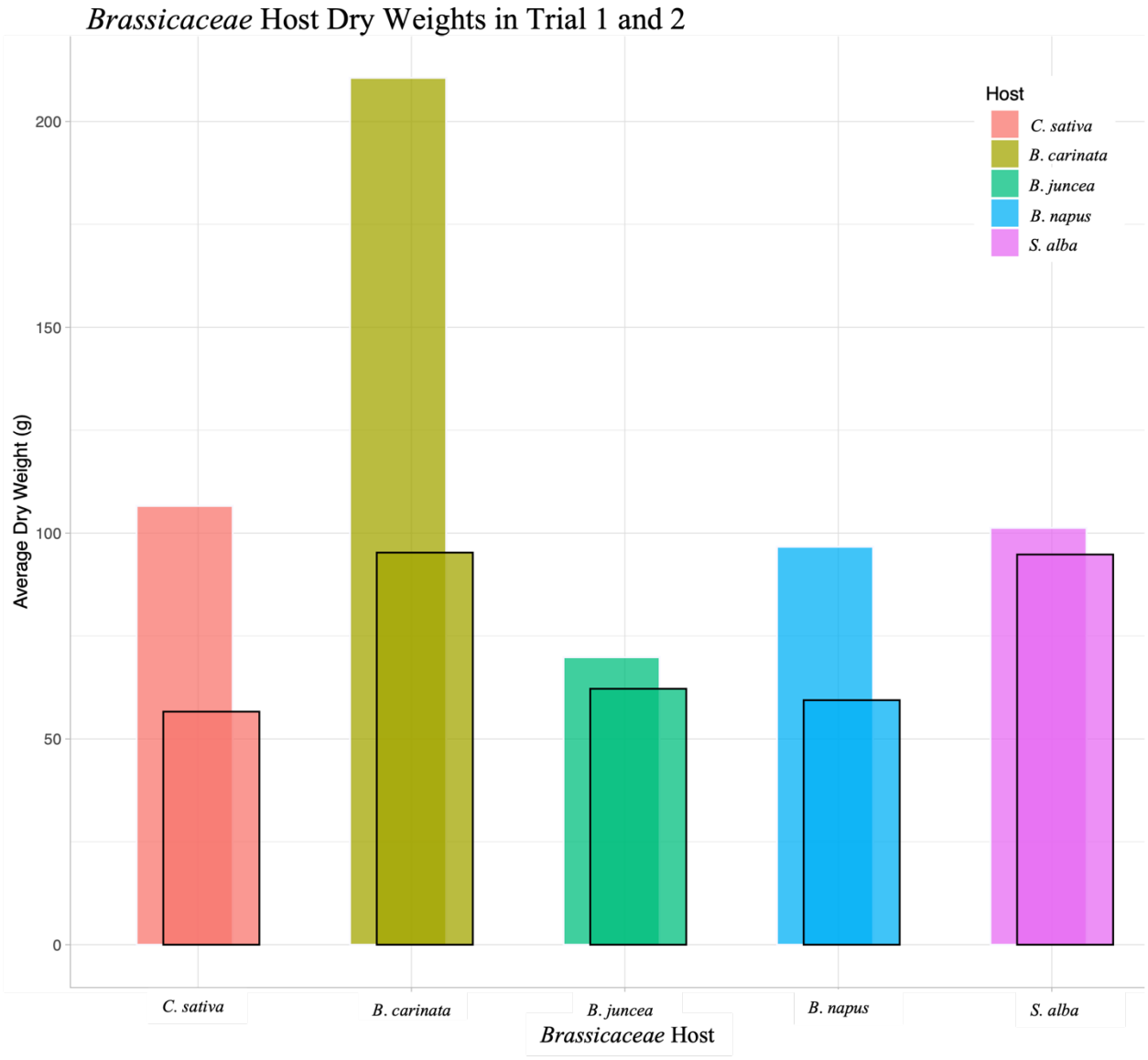


Figure S3. *Brassicaceae* host dry weights (g) decreased in Trial 2 (black outlines), compared to Trial 1 (no outlines). The Test Phase *Brassicaceae* samples were harvested in mid-late July, at Swift Current, Saskatchewan. The aerial portions were retained and dried to determine their weight.

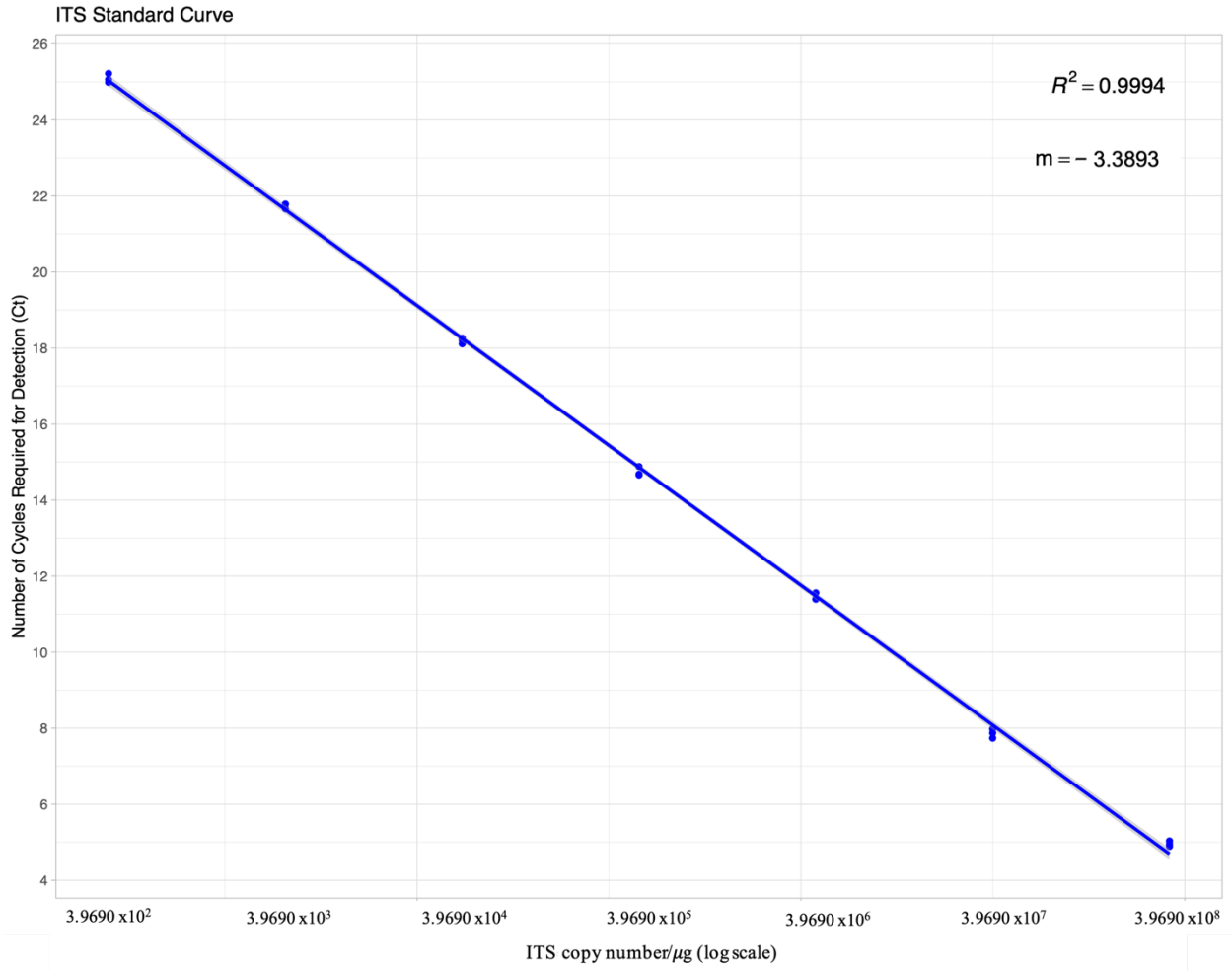
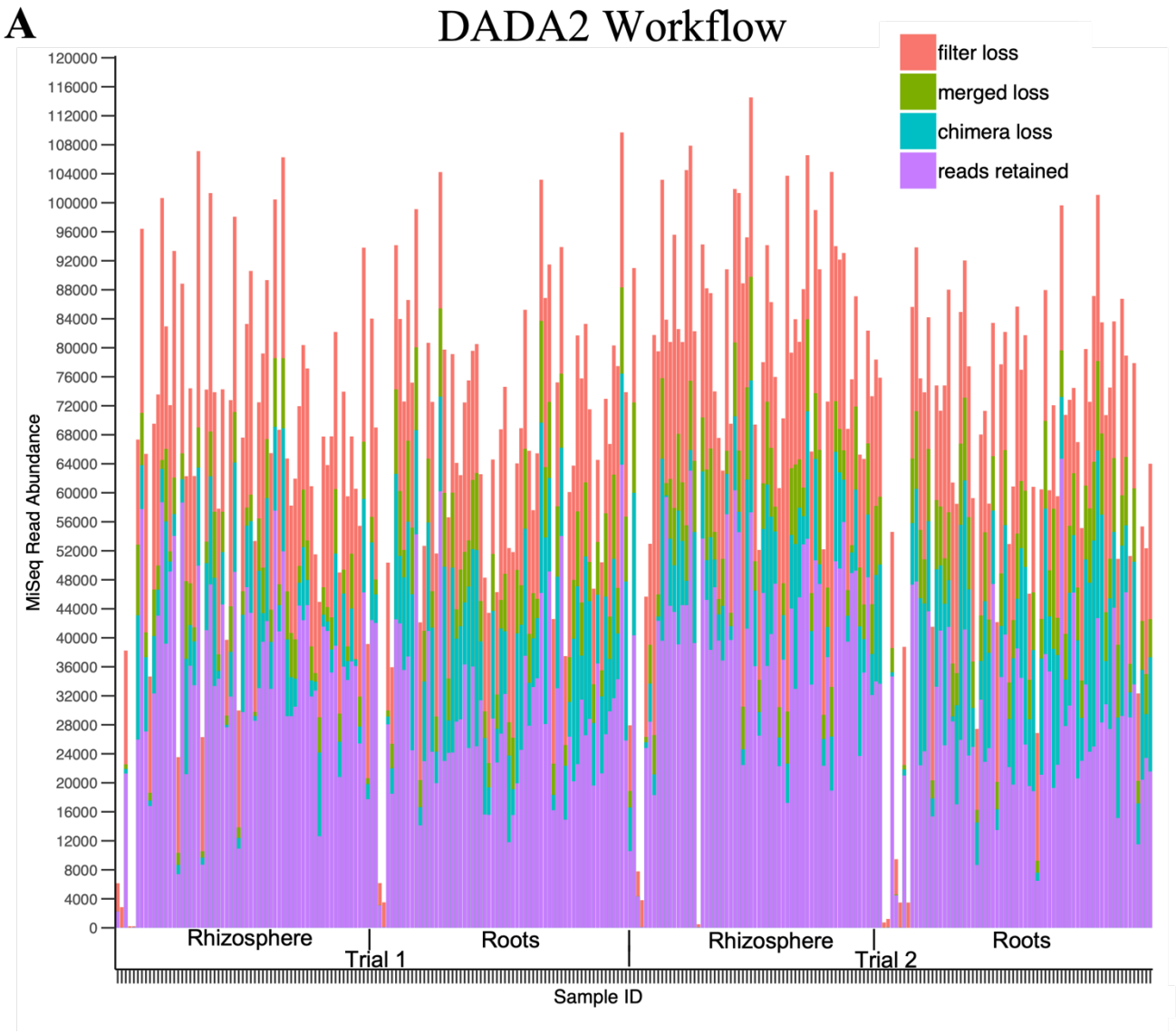


Figure S4. A standard curve of the ITS copy numbers (X-axis) versus the number of cycles required for detection (cycle threshold, Ct, Y-axis), as determined from the serial dilution of a quantified ITS amplicon from concentrated *Pythium ultimum*.


A
DADA2 Workflow
filter loss
merged loss
chimera loss
reads retained
MiSeq Read Abundance
120000
116000
112000
108000
104000
100000
96000
92000
88000
84000
80000
76000
72000
68000
64000
60000
56000
52000
48000
44000
40000
36000
32000
28000
24000
20000
16000
12000
8000
4000
0
Rhizosphere
Trial 1
Roots
Rhizosphere
Trial 2
Roots
Sample ID

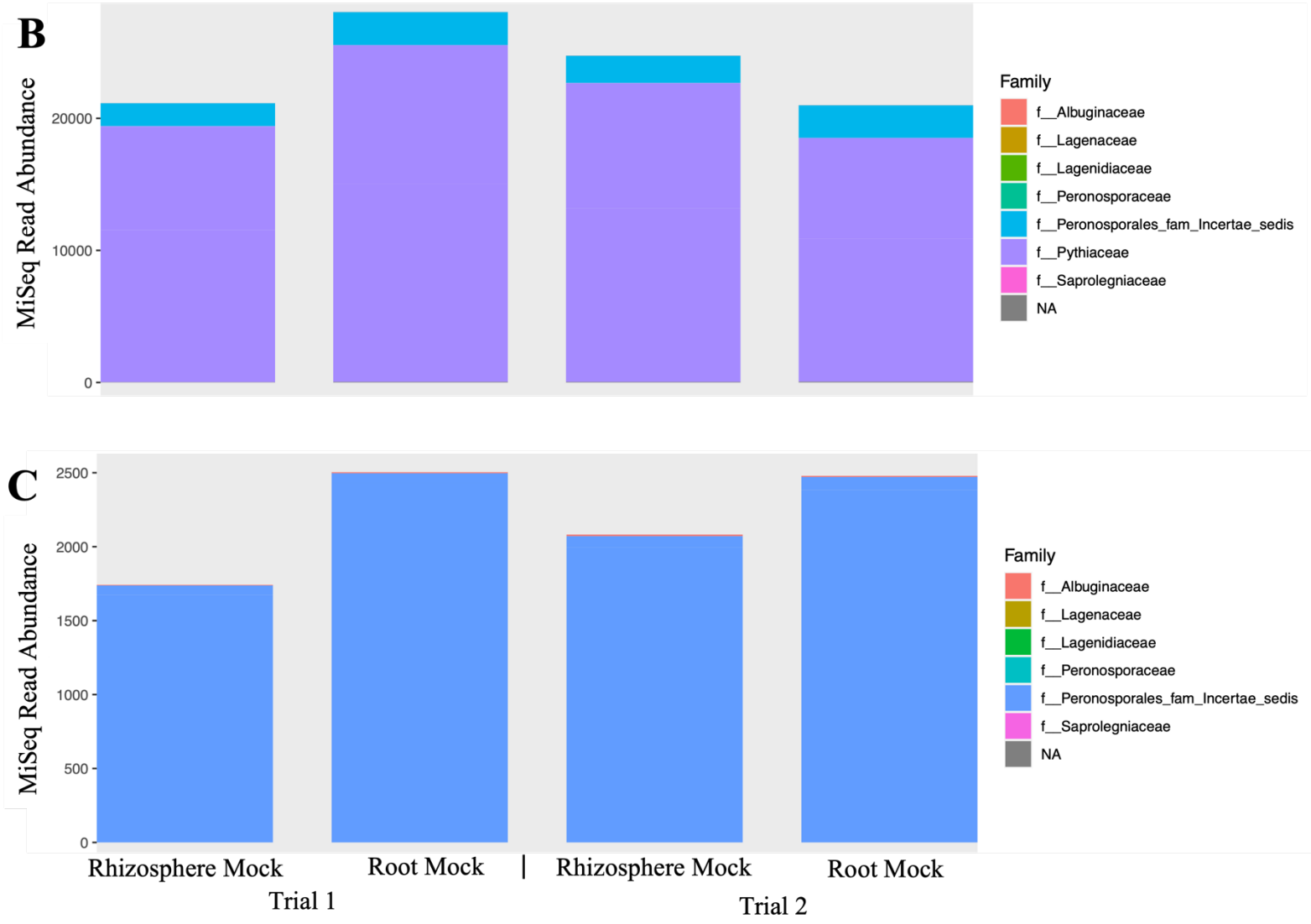

B
MiSeq Read Abundance
20000
10000
0
Family
f__Albuginaceae
f__Lagenaceae
f__Lagenidiaceae
f__Peronosporaceae
f__Peronosporales_fam_Incertae_sedis
f__Pythiaceae
f__Saprolegniaceae
NA
C
MiSeq Read Abundance
2500
2000
1500
1000
500
0
Family
f__Albuginaceae
f__Lagenaceae
f__Lagenidiaceae
f__Peronosporaceae
f__Peronosporales_fam_Incertae_sedis
f__Saprolegniaceae
NA
Rhizosphere Mock
Root Mock
Rhizosphere Mock
Root Mock
Trial 1
Trial 2

Figure S5. (A) The DADA2 workflow processed 17 656 076 raw reads produced from one lane of sequencing via Illumina's MiSeq at Génome Québec in order to infer amplicon sequence variants (ASVs). Reads were produced in each of the no-template negative controls, although no DNA was detected in these samples post-extraction. The stringency of the `filterAndTrim` step eliminated nearly all the reads from these samples. 8 222 283 high-quality reads were retained among the Test Phase *Brassicaceae* samples. Among the Test Phase *Brassicaceae* samples, the rhizosphere samples retained noticeably more reads than their root partners. (B) Mock communities were assembled from known oomycete DNA and sequenced with experimental samples to confirm that various taxonomic groups of oomycetes were detectable with our pipeline. The mock communities were dominated by *Pythiaceae*, which is reflected in the composition of the mock sequences. (C) With the *Pythiaceae* sequences are removed, the number of *Peronosporales* and other groups become more evident.

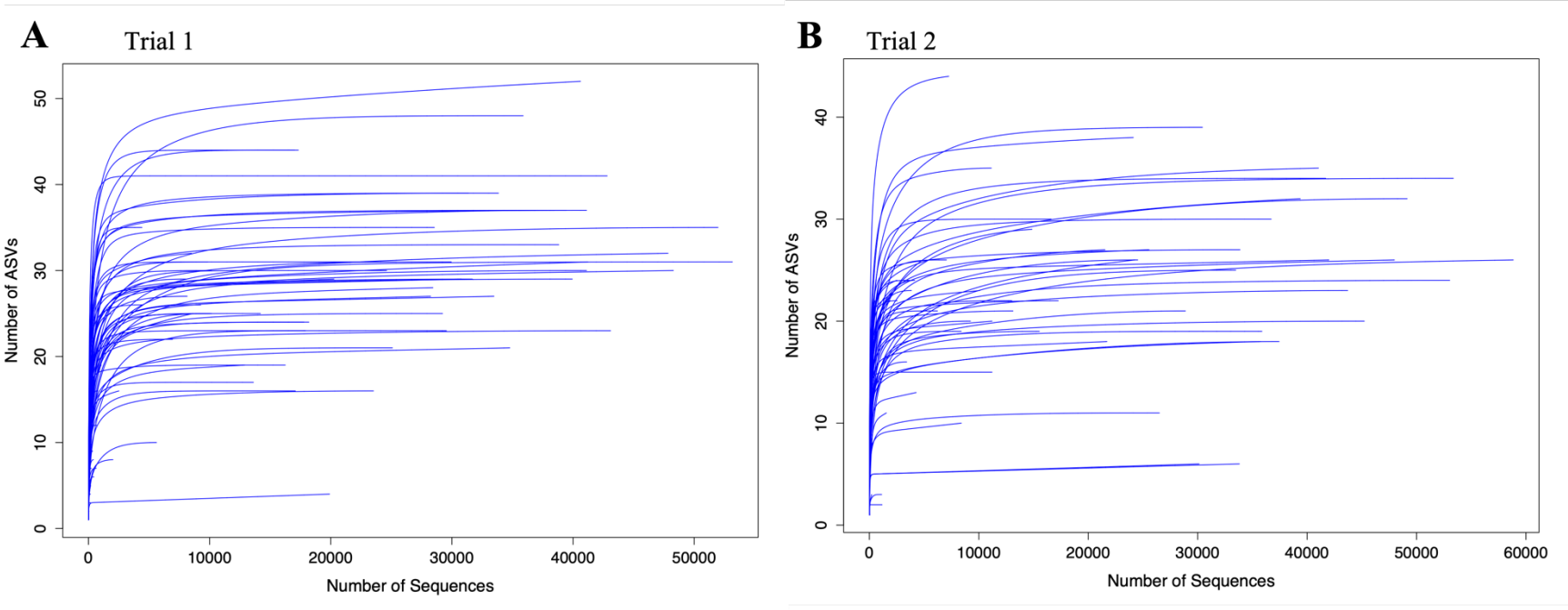
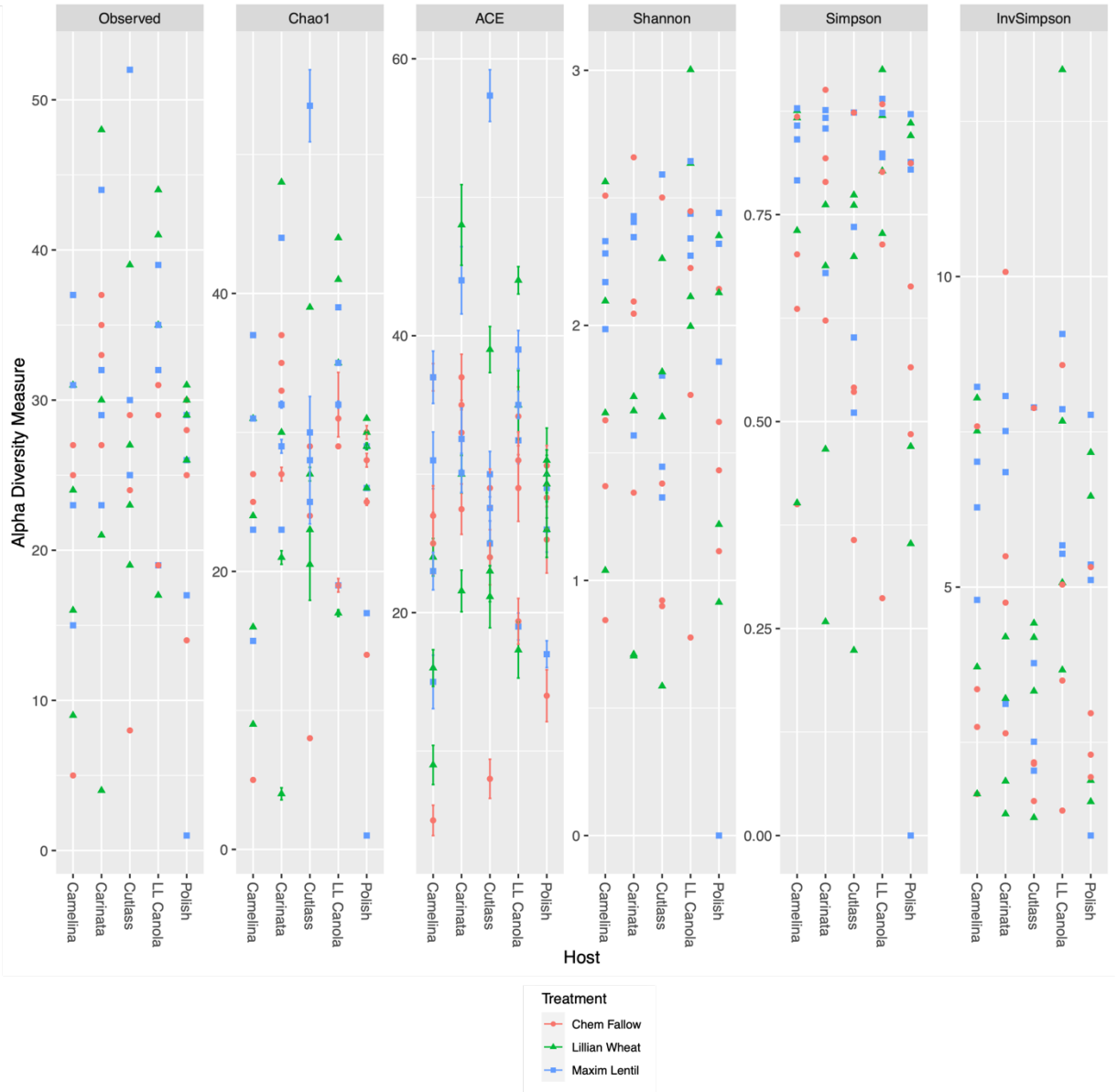
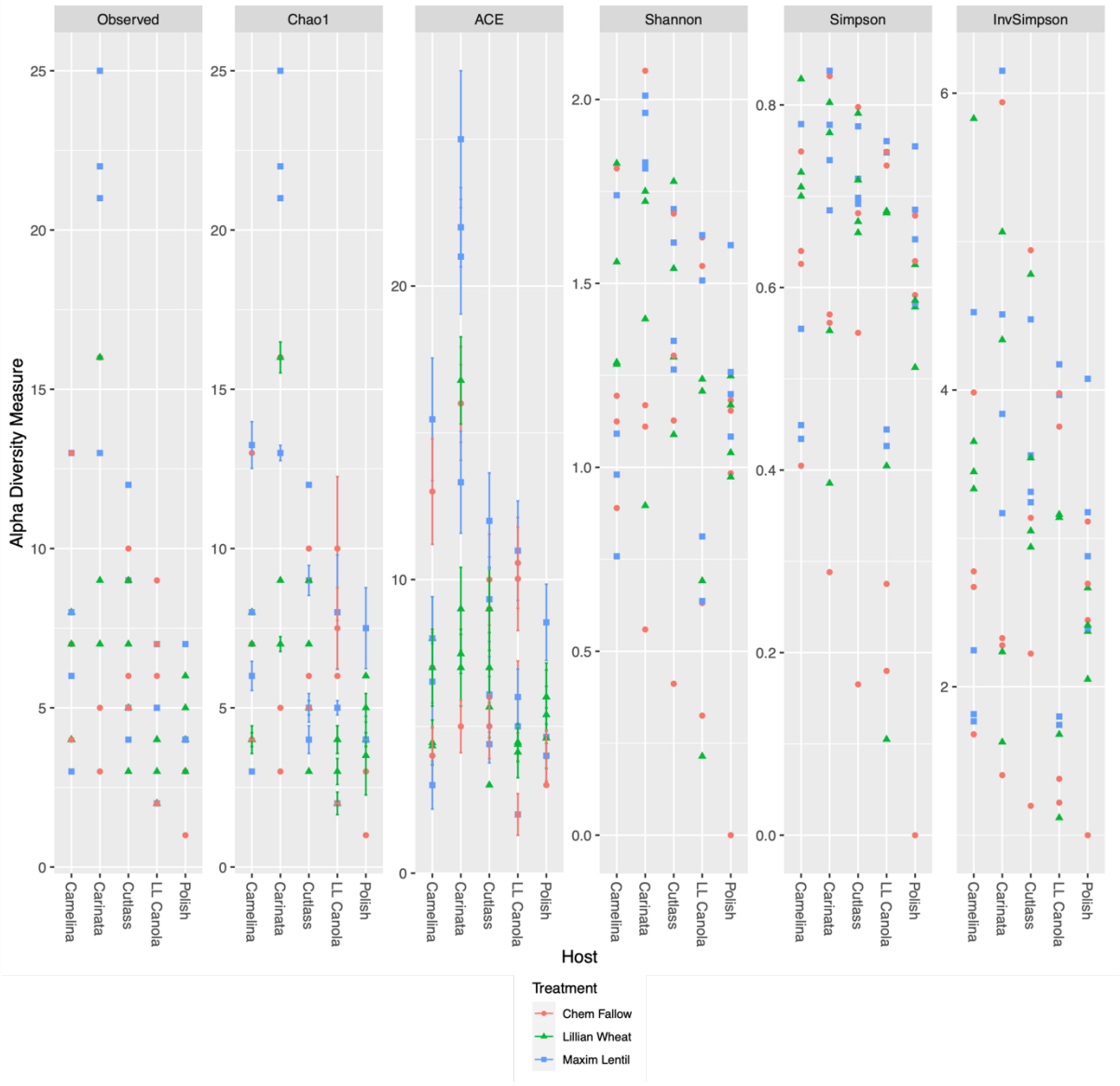


Figure S6. Rarefaction curves illustrated that the majority of the oomycete communities were identified in (A) Trial 1, and (B) Trial 2. The samples were harvested from two field trials during the Test Phase of a two-year crop rotation, in Swift Current, Saskatchewan.

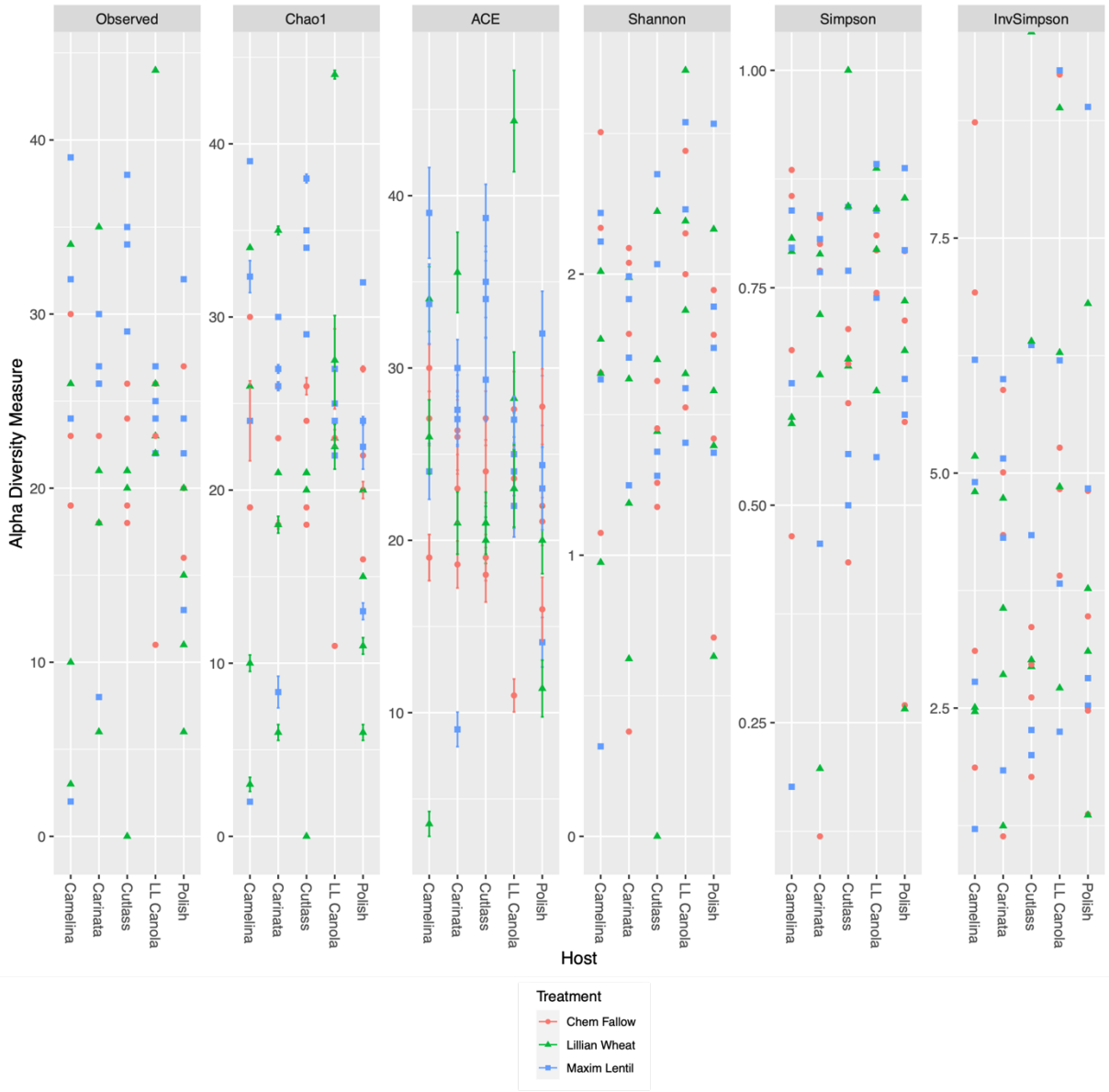
A Trial 1 Rhizosphere



B Trial 1 Roots



C Trial 2 Rhizosphere



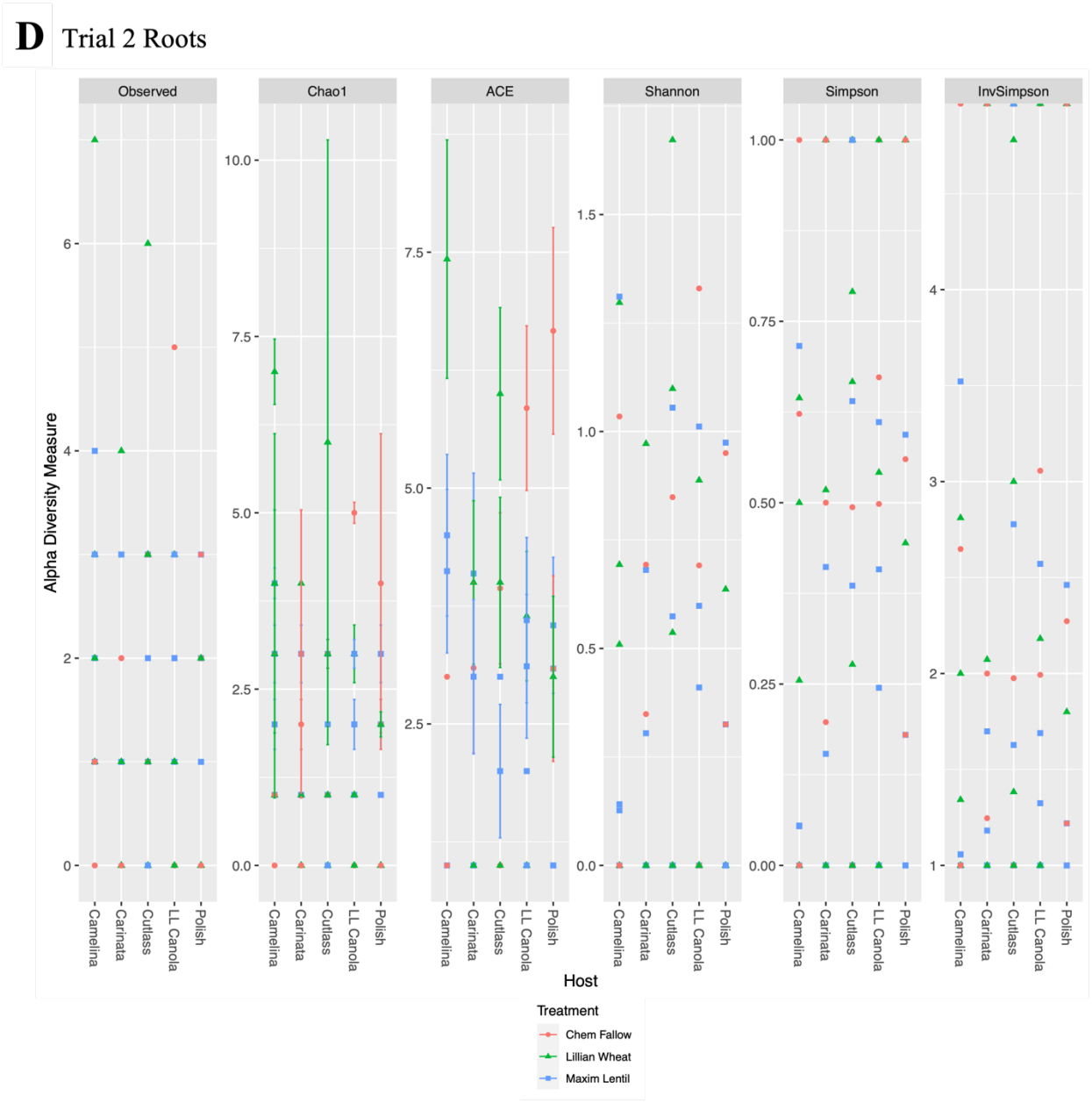


Figure S7. Taxa-based α-diversity indices (y-axis) for the rhizosphere (A & C) and root (B & D) communities from field trial 1, harvested 2016, and trial 2, harvest 2017. Each α-diversity index was grouped by *Brassicaceae* host, and reflect the phylogenetic diversity observed (Fig. 1), where communities are broadly similar across hosts, and soil histories.

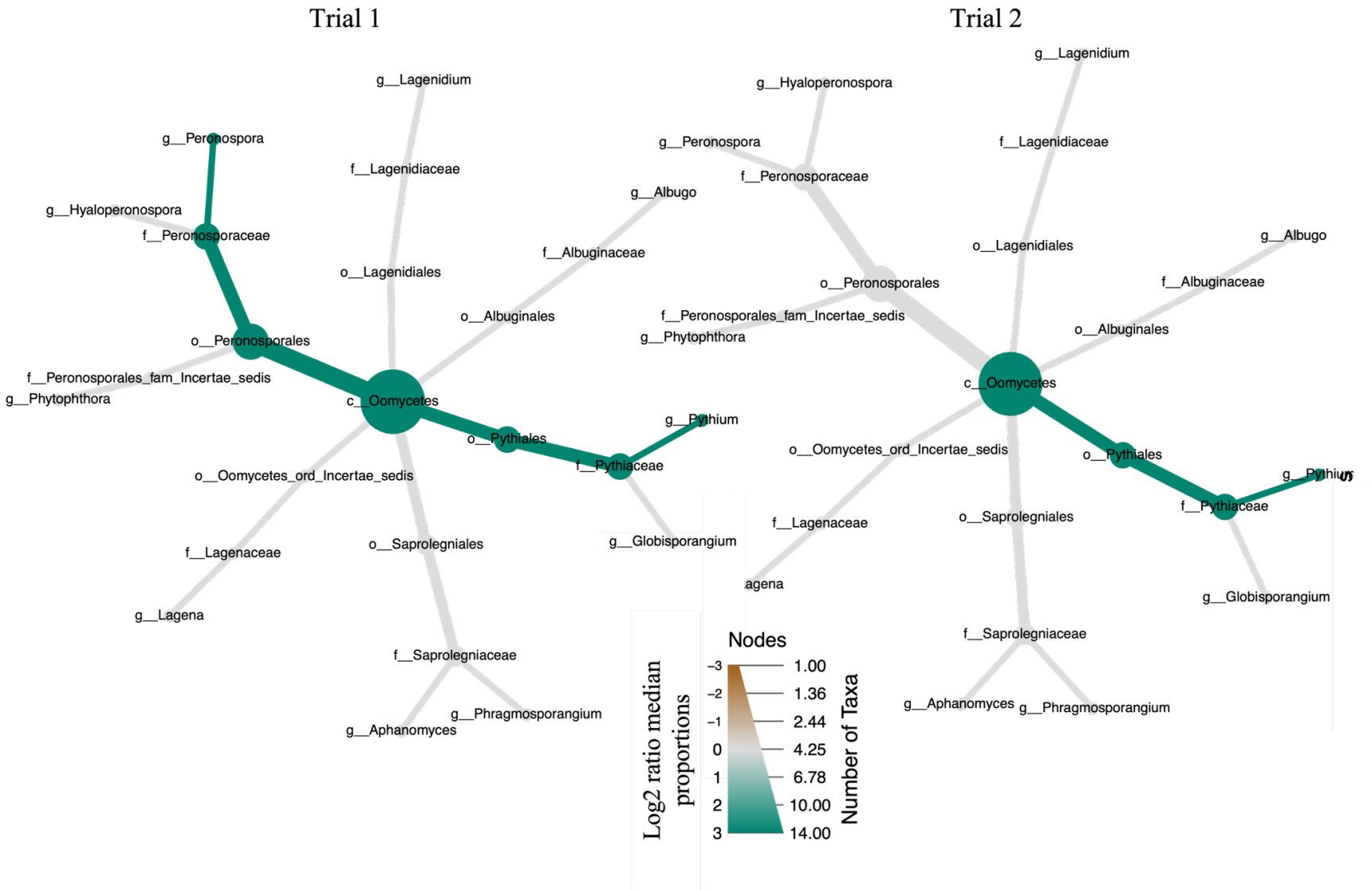

Trial 1
g__Lagenidium
g__Peronospora
f__Lagenidiaceae
g__Hyaloperonospora
f__Peronosporaceae
g__Albugo
f__Albuginaceae
o__Lagenidiales
o__Albuginales
o__Peronosporales
f__Peronosporales_fam_Incertae_sedis
g__Phytophthora
c__Oomycetes
g__Pythium
o__Pythiales
f__Pythiaceae
o__Oomycetes_ord_Incertae_sedis
f__Lagenaceae
o__Saprolegniales
g__Globisporangium
g__Lagena
f__Saprolegniaceae
g__Phragmosporangium
g__Aphanomyces
Trial 2
g__Lagenidium
g__Hyaloperonospora
g__Peronospora
f__Lagenidiaceae
f__Peronosporaceae
o__Lagenidiales
g__Albugo
f__Albuginaceae
o__Peronosporales
f__Peronosporales_fam_Incertae_sedis
o__Albuginales
g__Phytophthora
c__Oomycetes
o__Oomycetes_ord_Incertae_sedis
o__Pythiales
f__Pythiaceae
f__Lagenaceae
o__Saprolegniales
agena
g__Globisporangium
f__Saprolegniaceae
g__Aphanomyces
g__Phragmosporangium
Nodes
−3
−2
−1
0
1
2
3
1.00
1.36
2.44
4.25
6.78
10.00
14.00
Log2 ratio median proportions
Number of Taxa

Figure S8. Differential taxa clusters of oomycete ASVs illustrated significantly more *Pythium* species in the rhizosphere communities compared to the roots in both field trials harvested from the Test Phase of a two-year rotation from Swift Current, Saskatchewan. *Peronospora* were also enriched in the rhizosphere communities in Trial 1, but not in Trial 2. Here, the size of the taxonomic groups (bubbles) represents the number of taxa, and the colour scale represents the proportion of each group, where the abundance of each taxonomic group in the cluster is compared between each compartment, using the using the non-parametric Kruskal test and the *post hoc* pairwise Wilcox test, with the FDR correction. Taxa that are significantly (p. adj < 0.01) more abundant in the rhizosphere are highlighted in green.

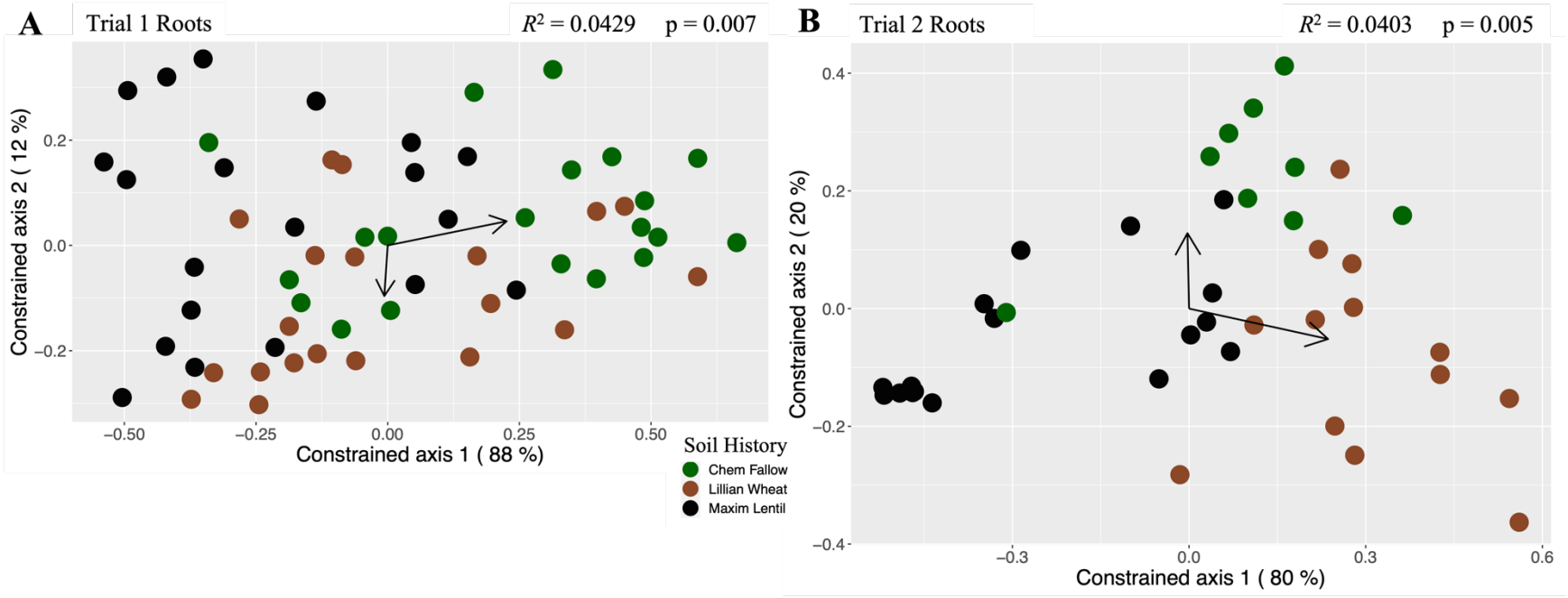


Figure S9. RDA illustrated that soil history structures the oomycete root communities in two field trials, Trial 1 (A, adj. R^2 = 0.0429, p = 0.007), and Trial 2 (B, adj. R^2 = 0.0403, p = 0.005), harvested from the Test Phase of a two-year rotation from Swift Current, Saskatchewan.

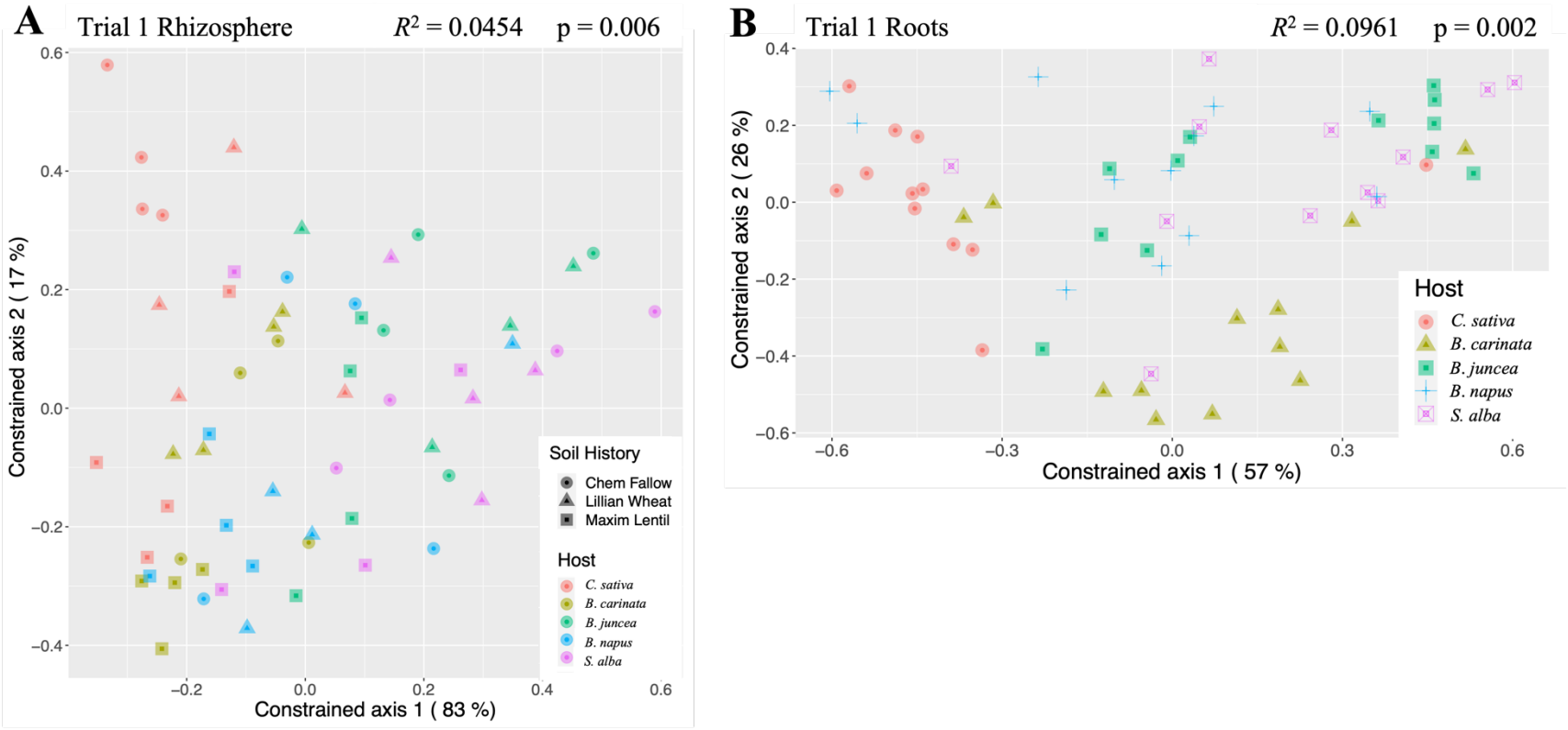
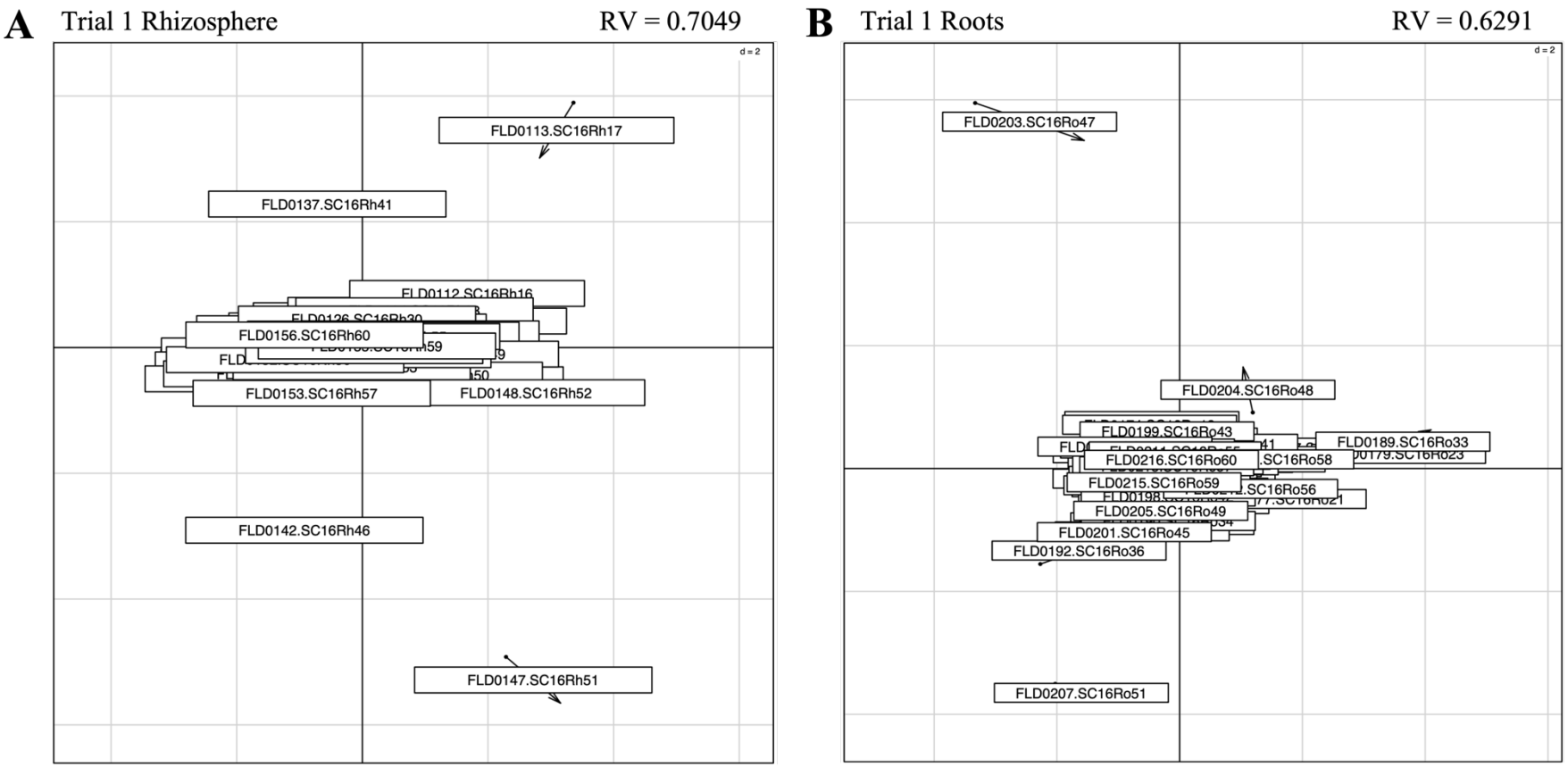
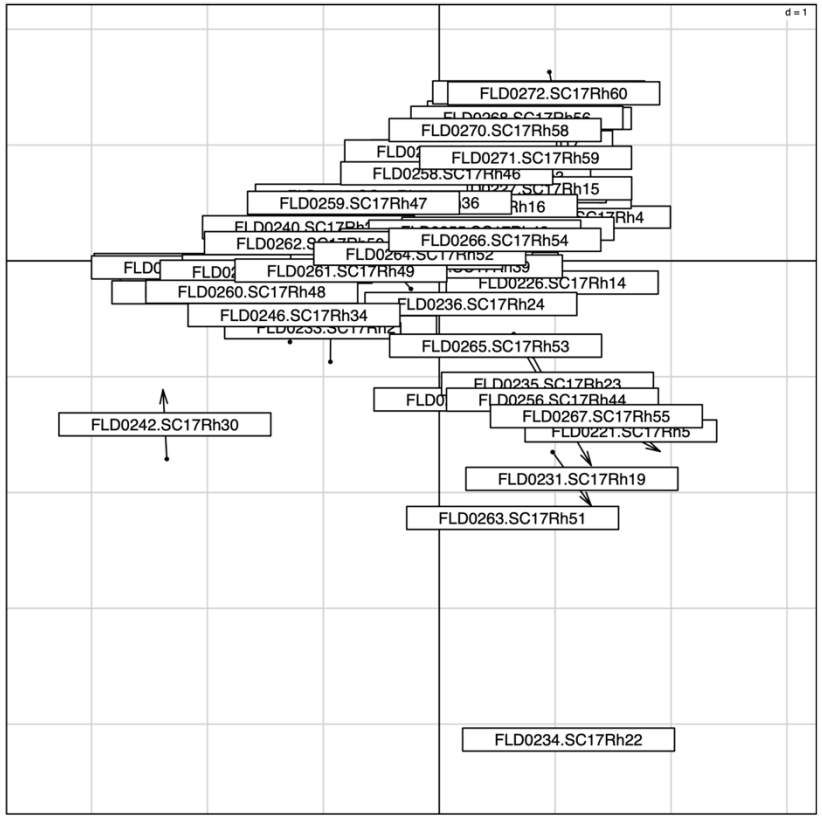


Figure S10. RDA illustrated the influence of *Brassicaceae* crop plants on the oomycete rhizosphere (A, adj. R^2 = 0.0454, p = 0.006) and root (B, adj. R^2 = 0.0961, p = 0.002) communities harvested from the Test Phase of field trial 1, as part of a two-year rotation from Swift Current, Saskatchewan.


A
Trial 1 Rhizosphere
RV = 0.7049
d = 2
FLD0113.SC16Rh17
FLD0137.SC16Rh41
FLD0112.SC16Rh16
FLD0156.SC16Rh60
FLD0153.SC16Rh57
FLD0148.SC16Rh52
FLD0142.SC16Rh46
FLD0147.SC16Rh51
B
Trial 1 Roots
RV = 0.6291
d = 2
FLD0203.SC16Ro47
FLD0204.SC16Ro48
FLD0199.SC16Ro43
FLD0189.SC16Ro33
FLD0216.SC16Ro60
FLD0215.SC16Ro59
FLD0205.SC16Ro49
FLD0201.SC16Ro45
FLD0192.SC16Ro36
FLD0207.SC16Ro51

C Trial 2 Rhizosphere RV = 0.8307



D Trial 2 Roots RV = 0.5767

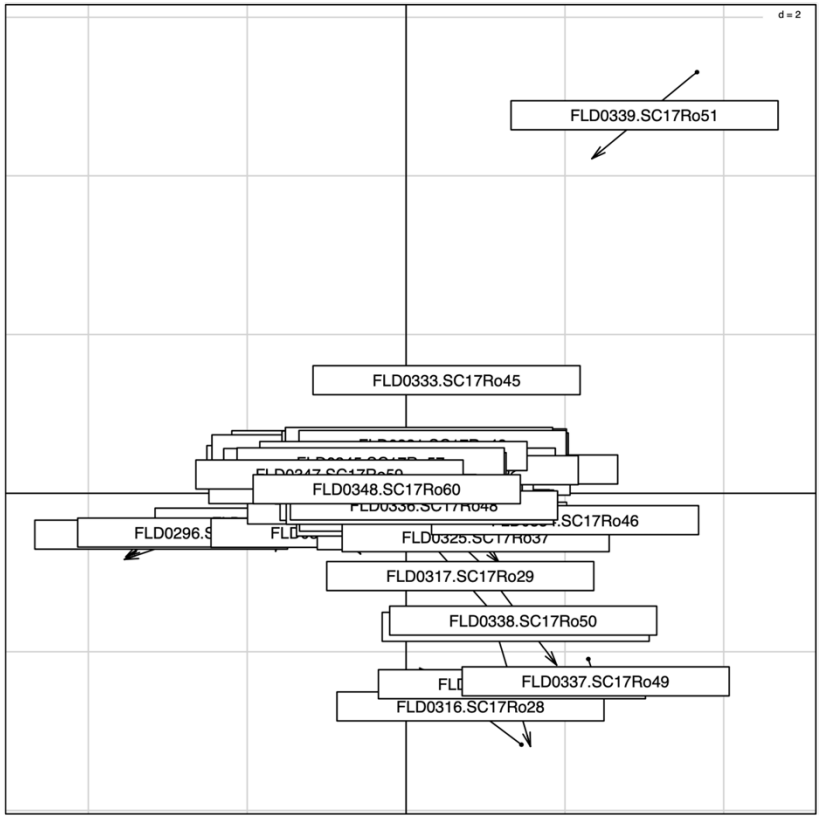


Figure S11. Co-inertia analysis illustrated that oomycete and bacterial communities were more significantly related in the rhizosphere (A, RV = 0.7049; C, RV = 0.8307) communities than their cognate root (B, RV = 0.6291; D, RV = 0.5767) samples harvested from the Test Phase of *Brassicaceae* field trial 1 (A & B), or trial 2 (C & D), as part of a two-year rotation from Swift Current, Saskatchewan. Moreover, the communities were largely similar, with very little divergence driven by any particular oomycete or bacterial ASV.

Chapter 3: Soil history continues to structure the bacterial communities of *Brassica napus* host plants across growth stages

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Keywords: *Brassicaceae*, bacterial communities, soil history, growth stages

Abstract

Soil history has been shown to condition future plant-soil microbial communities up to a year after being established (Kaisermann *et al*., 2017; Bakker *et al*., 2021; Hannula *et al*., 2021). However, previous experiments have also illustrated that adult plants can "re-write", or mask, different soil histories through host plant-soil microbial community feedbacks. This leaves a knowledge gap concerning how soil history influences microbial community structure across different growth stages. Therefore, in this experiment we tested the hypothesis that previously established soil histories would decrease in influencing the structure of *Brassica napus* bacterial communities specifically over the growing season. We used an on-going agricultural field experiment to establish three different soil histories, plots of monocrop canola (*B*. *napus*), or rotations of wheat-canola, or pea-barley-canola. During the following season, we repeatedly sampled the surrounding bulk soil, rhizosphere and roots of *B*. *napus* at different growth stages—the initial seeding conditions, seedling, rosette, bolting, and flower—from all three soil history plots. We compared the taxonomic composition and diversity of bacterial communities, as estimated using 16S rRNA metabarcoding, to identify any changes associated with soil history and growth stages on the different *B. napus* soil bacterial communities. We found that soil history remained significant across each growth stage in structuring the bulk soil and rhizosphere communities, but not the roots. This suggests that the host plant's capacity to "re-write" different soil histories may be quite limited as key components that constitute the soil history's identity remain present and continue to impact bacterial communities. For agricultural, this highlights how previously established soil histories persist and may have important long-term consequences on future plant-microbial communities, such as bacteria.

Introduction

Microbes are ubiquitous in interacting with plants across growth stages (GS), hence the critical impact microbes have on plant metabolism, growth, and survival (Vandenkoornhuyse *et al*., 2015). For instance, soil microbes increase access to nutrients (Richardson *et al*., 2009; Weidner *et al*., 2015; Yu *et al*., 2021), temper environmental changes (Lau & Lennon, 2012), or stress (Marasco *et al*., 2012; Hou *et al*., 2021), protect against pathogens (Sikes et al., 2009; Mendes *et al*., 2011), and cue each plant developmental stage (Lau & Lennon, 2011 & 2012; Chaney & Baucom, 2020; O'Brien *et al*., 2021). Soil microbial communities help integrate these diverse signals and modulate the plant's responses (Castrillo *et al*., 2017; Hou *et al*., 2021). Given their close, perpetual interactions, plants and their microbial communities can be considered as a holistic entity, or holobiont (Zilber-Rosenberg & Rosenberg, 2008; Vandenkoornhuyse *et al*., 2015; Puginier *et al*., 2022).

In the context of a holobiont, plants interact with similar cohorts of microbial traits across generations. Establishing such a long-term relationship highlights a host plants capacity to tailor the structure of their microbial communities, and particularly the bacterial fraction, in response to variable conditions and the plant's needs through time (Lebeis *et al*., 2015; Korenblum *et al*., 2020; Kawasaki *et al*., 2021; Yu *et al*., 2021). One mechanism host plants use to positively shape bacterial communities is by altering the local soil chemistry, since the soil is the source of the majority of plant-associated bacteria (Grady *et al*., 2019). Host plants have the capacity to alter the local soil chemistry through two concurrent processes; first, the host plant's growth, development, and homeostasis is determined by its capacity to uptake nutrients from the soil, which will alter the soil chemistry (Hu *et al*., 2021). Second, through rhizodeposition the host plant can vary the quantity and array of compounds released into the rhizosphere as required, which also changes the local soil

chemistry (Lebeis *et al*., 2015; Korenblum *et al*., 2020; Kawasaki *et al*., 2021; Song *et al*., 2021; Yu *et al*., 2021).

As such, plant-soil microbial communities generate a reciprocal feedback process that incorporates various biotic and abiotic factors for the benefit of the current community (Hwang *et al*., 2015; Yang *et al*., 2021; Liu *et al*., 2022), and which will impact future plant-soil microbial generations and their composition (Bever *et al*., 2010; Kaisermann *et al*., 2017; Berendsen *et al*., 2018; Fitzpatrick *et al*., 2018). Thus, information from one plant-soil microbial community is transmitted through time to impact subsequent plant-microbial generations, i.e., that the soil history, also referred to as soil legacy, of previous plant-soil microbial communities' condition future ones (Kaisermann *et al*., 2017; Bakker *et al*., 2021; Hannula *et al*., 2021). Future host plants can then alter the soil bacterial communities for their own purposes, in a phylogenetic-dependent manner (Fitzpatrick *et al*., 2018; Blakney *et al*., 2022). However, several questions remain concerning the duration of different soil histories, including what their impact on future plant-microbial communities may be, or how quickly plant-soil microbial community feedbacks may alter, or "re-write" different soil histories.

Furthermore, soil history will dictate how future plant-soil microbial communities form their microbial communities not only by establishing the biotic and abiotic context, but also through priority effects (Meisner *et al*., 2021; Chase *et al*., 2021; Debray *et al*., 2022). This temporal mechanic refers to how the order of arrival of microbial species into an environment (i.e. species A then B then C vs C then A) will regulate future community composition differently (Debray *et al*., 2022). Therefore, as a new plant host develops not only could its potential microbial community be constrained according to the soil history, but also according to the composition at an earlier growth stage. For example, a given bacterial taxa recruited to the rhizosphere could be beneficial

to the plant host during an early GS, but it may preclude other taxa from the rhizosphere at a later GS, such as through niche exclusion, or competition (Debray *et al*., 2022). Unfortunately, however, most experiments testing soil history have only focused on the microbial communities of adult plants (Fitzpatrick *et al*., 2018; Hannula *et al*., 2021; Blakney *et al*., 2022; Blakney *et al*., 2023), and have thus been unable to tease apart more of the variation among the microbial communities. Consequently, not incorporating temporal sampling has explicitly ignored the different roles of time on the assembly of microbial communities (Chung *et al*., 2022; De Long *et al*., 2023; de Vries *et al*., 2023).

This has left a severe knowledge gap of how soil history impacts microbial communities—and particularly the bacterial component—at different growth stages (GS) of the host plant, as well as how these communities develop throughout the growing season (Walsh *et al*., 2021). For example, our previous experiment illustrated how different soil histories could be "re-written" by host plant-soil microbial community feedback mechanisms, or persist for up to a year (Blakney *et al*., 2022; Blakney *et al*., 2023). However, since we only sampled adult host plants, we were unable to explore the strength or variation of different soil histories on the microbial communities throughout the growing season. Moreover, there is growing evidence of the importance of microbial regulation on different plant growth stages, which can impact the ecology and evolution of their host plants (O'Brien *et al*., 2021; Gu *et al*., 2022). For example, microbial communities can shift the timing or transition to different GS through nutritional or phytohormone pathways (O'Brien *et al*., 2021). More recently, increased attention has been paid to seed (Links *et al*., 2014; Nelson *et al*., 2018; Rezki *et al*., 2018; Eldridge *et al*., 2021; Shao *et al*., 2021) and flower (Shade *et al*., 2013b; Wagner *et al*., 2014; Cui *et al*., 2021) microbial communities. This research focuses on how microbes are vertically transmitted, subsequently establish new communities, and cue

germination (O'Brien *et al*., 2021). In fact, microbes may be critical at early life stages; seed and seedling GS are precarious periods where the plant is most vulnerable to environmental stress and infection from phytopathogens (Hwang *et al*., 2015; Walsh *et al*., 2021). Thus, experiments are needed to test the influence and dynamics of soil microbial communities at a variety of GS.

In this study, we investigated how different soil histories impacted soil bacterial communities at five developmental GS throughout the season. We partnered with an on-going agricultural field experiment, as crop rotations and their agricultural inputs provide a ready-made model for how a previous plant-soil community feedback establishes a soil history that impacts the future plant-bacterial community (Yang *et al*., 2021; Liu *et al*., 2022). This addresses the lack of field experiments in studying soil history (Revillini *et al*., 2016). Here, the three established soil histories were rotated plots of monocrop canola (*Brassica napus*), wheat-canola (WC), and pea-barley-canola (PBC). During the following season, we repeatedly sampled *B*. *napus* and surrounding soil at different GS— the initial seeding conditions, seedling, rosette, bolting, and flower—from all three soil history plots. This design permitted us to test the hypothesis that the previously established soil histories—monocrop, WC, PBC, and their respective microbial communities and agricultural treatments—would decrease in influencing the structure of the *B*. *napus* bacterial root and rhizosphere communities over the growing season. We predict i) that the bacterial bulk soil communities will remain stable, and continue to be structured by their soil history throughout the experiment, ii) that the bacterial bulk soil and rhizosphere communities will remain similar at the seed and seedling GS according to their soil history, and iii) that the bacterial rhizosphere communities will converge in similarity over the growing season, regardless of their soil history, as will the different root communities. To test our "fading soil history through time" hypothesis, we estimated the bacterial communities from the bulk soil, rhizosphere and roots, of *B*.

napus plants throughout the growing season using a 16S rRNA metabarcoding approach to infer amplicon sequence variants (ASVs). We compared the taxonomic composition and diversity of the bacterial communities to identify any changes associated with different soil histories and GS on the *B. napus* soil bacterial communities.

Materials and Methods

Site and experimental design

A field experiment was conducted at the experimental farm of Agriculture and Agri-Food Canada's Research and Development Centre, in Lacombe, Alberta (52°28′06″N, 113°44′13″W). The site is located in the semi-arid region of the Canadian Prairies; according to the weather station at the research farm, the 2019 growing season (May, June and July) had 197.8 mm of precipitation; compared to the 30-year average [1981-2010] of 216.3 mm. The daily temperature average for the 2019 season was 12.6 °C, while the 30-year average was 14.5°C. The farm has a loam texture (46% sand, 33% silt, and 21% clay), and has been well-described previously (Harker *et al*., 2015).

Our experiment piggybacked on a long-term crop rotation experiment (Harker *et al*., 2015), however, for the purposes of our experiment we will only discuss the 2018 and 2019 growing seasons, which we used as a two-phase cropping sequence; the Conditioning Phase in 2018, and the Test Phase in the second year, 2019 (Fig. S1A). The experimental design was a split-plot replicated in four complete blocks (Fig. S1B). For the Conditioning Phase, we selected three soil history treatments that consisted of i) monocrop canola (*Brassica napus* L., cv. L252LL), ii) a two-year crop rotation between spring wheat (*Triticum aestivum* cv. AAC Brandon) and *B. napus*, referred to as WC, and iii) a three-year rotation between pea (*Pisum sativum* L. cv AAC Lacombe), barley (*Hordeum vulgare* cv. Canmore), and *B. napus*, referred to as PBC (Fig. S1B). Thus, the

2018 Conditioning Phase established a three different soil histories composed of either canola, wheat (WC), or barley (PBC), plus their respective management plans as described below (Harker *et al*., 2015). In the Test Phase, the 12 Conditioning Phase plots were all seeded with *B*. *napus* (Fig. S1). The Test Phase established the *B*. *napus* host plant-soil microbial community feedback, composed of the *B*. *napus* genotype, their soil bacterial community, their management plan, and previous soil history.

Crop management and sampling

Crops were grown and maintained according to standard management practices, as previously described by Harker *et al* (2015). A pre-seed 'burn off' herbicide treatment using glyphosate (Roundup, 900 g acid equivalent per hectare, a. e. ha^{-1}) and bromoxynil (Pardner, 280-330 g active ingredient per hectare, a.i. ha^{-1}) was applied to all plots each year to ensure a clean starting field prior to seeding. The herbicide Liberty was applied to *B*. *napus*, while Pixxaro A & B with Axial were applied to wheat and barley plots, for in-season weed control, while fungicides were only applied as needed. Soil tests were used to determine the rates of in-season nitrogen, phosphorus, and potassium application. Crops were harvested between late August and early October, depending on the crop and year.

The subsequent Test Phase *B*. *napus* plant hosts were subjected to the same standard management practices as the Conditioning Phase, including pre-seed 'burn off', in-season herbicide and fungicide treatments as needed, and fertilized as recommended by soil tests. We accounted for the use of the various agricultural treatments in the downstream amplicon data by considering each plant sample and their total complement of particular agricultural treatments as a unit.

Test phase *B. napus* plants were sampled at specific growth stages, seed, seedling, rosette, bolting, and flower, as described by the Canola Council of Canada (Canola Encyclopedia: Canola Growth Stages, 2017). First, at the seed stage (GS00, May 10th, 2019), we took a 25 mL sample of the *B. napus* seeds to be seeded, as well an equivalent amount of soil from each plots. At the seedling stage, post-emergence, when only the cotyledons were visible (GS10, May 27th, 2019), five seedlings and their accompanying soil were pooled together for each sample. Composite samples were taken only at the seedling stage in order to have enough root material for our subsequent DNA extractions. At the rosette, bolting and flower stages, individual plants and their associated soil were harvested from each plot. The rosette stage (GS19, June 18th, 2019) was harvested when nine leaves were visible, followed by the bolting stage (GS34, July 2nd, 2019) when a 20 cm stem was present. Finally, the flower stage (GS65, July 15th, 2019) was harvested when 50% of the flowers on the raceme were open (Canola Encyclopedia: Canola Growth Stages, 2017).

At each growth stage we sampled three compartments: bulk soil, the rhizosphere, and the roots (Fig. S2). Within each plot, bulk soil was sampled from between the seeded rows, at least 10 cm from any seeds, or plants. Note that at the seed stage the only material collected was bulk soil and the seeds, as described. In the field, each plant had its aerial portions removed and its roots and accompanying soil stored in coolers on ice. Based on the sampling strategy, in this study we define the bulk soil microbiome as the soil bacterial community not influenced by the resident host plant, the rhizosphere microbiome as the bacterial community in the soil in close contact with the roots (Hannula *et al*., 2021), and the root microbiome as the bacterial community attached to, and within, the roots (Berendsen *et al*., 2018). In the field, all samples were kept on ice in coolers, then stored in the lab at -80°C before being shipped to Université de Montréal's Biodiversity Centre, Montréal (QC, Canada) on dry ice for further processing (Delavaux *et al*., 2020; Lay *et al*., 2018).

DNA extraction from Test Phase *Brassicaceae* root and rhizosphere samples

Total DNA was extracted from all compartments (bulk soil, seeds, rhizosphere, and roots) of the Test Phase field trial samples. Roots were first separated out from the total soil sample and gently scraped it off using sterilized utensils into fresh collection trays. Seeds and root samples were ground separately in liquid nitrogen via sterile mortar and pestles (Fig. S2). For bulk soil and rhizosphere samples, ~500 mg was used for the NucleoSpin Soil gDNA Extraction Kit (Macherey-Nagel, Germany), while ~130 mg of seeds and roots were used with the DNeasy Plant DNA Extraction Kit (Qiagen, Germany) (Lay *et al*., 2018; Blakney *et al*., 2022). To assess the influence of the extraction kits on our sequencing results, and the efficacy of our lab preparation on the Test Phase samples, we included a no-template extraction negative control with the bulk soil, rhizosphere and root extractions (Fig. S2). All extracted DNA samples were quantified using the Qubit dsDNA High Sensitivity Kit (Invitrogen, USA), and qualitatively evaluated by mixing ~2 μL of each sample with 1 μL of loading dye containing Gel Red (Biotium) and running it on a 0.7 % agarose gel for 30 minutes at 150 V. The no-template extraction negative controls were confirmed to not contain DNA after extraction, where the detection limit was > 0.1 ng (Qubit, Invitrogen, USA). We failed to extract DNA from nine of the seedling root samples due to a lack of root material; those samples were subsequently excluded hereafter (Fig. S2).

16S rRNA gene amplicon generation and sequencing to estimate the bacterial community

To estimate the composition of the bacterial communities in the bulk soil, seed, rhizosphere and roots from across the Test Phase *B*. *napus* growth stages, extracted DNA from all samples were used to prepare 16S rRNA gene amplicon libraries following Illumina's MiSeq protocols (Bell *et*

al., 2016; Lay *et al*., 2018; Blakney *et al*., 2022). First, all DNA samples were diluted 1:10 into 96-well plates using the Freedom EVO100 robot (Tecan, Switzerland). To assess potential bias caused by lab manipulations, sequencing and downstream bioinformatic processing, a commercially available 16S rRNA mock community of known composition (Table S1) was included on each plate (Fig. S2) following the manufacturer's instructions (BEI Resources, USA). The mock community contained DNA of 20 bacterial species (Table S1) in equimolar counts (10^6 copies/μL) of 16S rRNA gene sequences. A no-template PCR negative control was also included on each plate, to assess the influence of the PCR reaction, and the efficacy of lab preparation on sequencing (Fig. S2). Four μL of each negative control was mixed with 1 μL of loading dye containing Gel Red (Biotium) and visualized on a 1% agarose gel after 60 minutes at 100 V. None of the negative controls for the DNA extractions, nor the PCR reactions, contained detectable amounts of DNA prior to submission.

The prepared plates of the Test Phase DNA samples were submitted to Génome Québec (Montréal, Québec) for 16S rRNA amplicon generation and sequencing (Bell *et al*., 2016; Lay *et al*., 2018; Blakney *et al*., 2022). PCR amplification used the S-D-Bact-0341-b-S-17 forward and S-D-Bact-0785-a-A-21 reverse primers, commonly referred to as 341F and 805R, respectively, to generate a 416 bp fragment from the V3-V4 region of the 16S rRNA gene (Klindworth *et al*., 2012). These amplicons were then prepared for paired-end 250 bp sequencing using Illumina's MiSeq platform (Génome Québec, Montréal) (Bell *et al*., 2016; Lay *et al*., 2018; Blakney *et al*., 2022). We estimated this should provide a mean of 60 000 reads per sample, which is in line with previous studies that describe bacterial communities (Bell *et al*., 2016; Lay *et al*., 2018; Blakney *et al*., 2022).

Estimating ASVs from MiSeq 16S rRNA gene amplicons

The 16S rRNA gene amplicons generated by Illumina MiSeq were used to estimate the diversity and composition of the bacterial communities present in the bulk soil, seed, rhizosphere and roots from across the Test Phase *B. napus* growth stages. The integrity and totality of the 16S MiSeq data downloaded from Génome Québec was confirmed using their MD5 checksum protocol (Roy *et al*., 2018). Subsequently, all data were managed, and analyzed in R (4.0.3 R Core Team, 2020), and plotted using `ggplot2` (Wickham, 2016).

The `dada2` package (Callahan *et al*., 2016a) was first used to filter and trim all 11 010 728 raw reads, forward and reverse, from the 16S rRNA gene amplicon data generated from the control samples, the mock communities, and the Test Phase *B. napus* samples, using the `filterAndTrim` function (Fig. S2), as described in Blakney *et al*., 2022. Filtered and trimmed reads were then processed through DADA2 for ASV inference (Fig. S3). Default settings were used throughout the DADA2 pipeline, except the DADA inference functions `dadaF` and `dadaR` which used the `pool ='pseudo'` argument, to increase the likelihood of identifying rare taxa. Consequently, the chimera removal function `removeBimeraDenovo` included the `method ='pooled'` argument (Callahan *et al*., 2016b).

ASVs identified from the 16S rRNA gene amplicon data were assigned taxonomy—to the species level where possible—using the Silva database release 138, which adopts the standardized Genome Taxonomy Database taxonomy structure (Yilmaz *et al*., 2013; Parks *et al*., 2018). The quality of the data was assessed using the included controls (Fig. S4); any ASVs identified as chloroplasts, or mitochondria were subsequently removed from the data, as were off-target archaeal ASVs. Rarefaction curves confirmed that we captured the majority of the bacterial communities in both the bulk soil, seed, rhizosphere, and roots (Fig. S5). Test Phase *Brassicaceae* 16S rRNA

sequencing data was subsequently re-analysed independently following the described protocol to avoid any biases from the no-template negative controls and the mock communities. These are the Test Phase *B*. *napus* ASVs which are reported hereafter.

Estimating absolute abundance of bacterial communities by quantitative PCR

To identify any changes in abundance of the bacterial ASVs within the Test Phase *B*. *napus* growth stages, we estimated the absolute abundance, or size, of the bacterial communities in each Test Phase DNA sample by qPCR (Azarbad *et al*., 2018; Blakney *et al*., 2022). Given the technical-limitations of high-throughput sequencing in assessing abundance, estimating the absolute abundance can provide complementary data to better interpret the bacterial communities (Gloor *et al*., 2017; Props *et al*., 2017; Harrison *et al*, 2020; Jian *et al*., 2020). As such, we quantified the number of 16S rRNA gene sequences present in each DNA sample via qPCR (as cycle threshold, Ct, values) and then estimated the corresponding community size as the 16S rRNA gene copy number/μg from a standard curve (Zhang *et al*., 2017; Azarbad *et al*., 2018, Fig. S6).

First, a standard curve of 16S rRNA gene copy numbers was constructed. Near full-length 1.5 kb 16S rRNA gene fragments were PCR amplified using the primers PA-27F-YM and PH-R (Bruce *et al*., 1992; Table S2) from DNA extracted from previously used soil samples (Lay *et al*., 2018). The 16S PCR reactions consisted of 11.5 μL dH_2O, 5.0 μL of 10X Buffer (Qiagen, Canada), 2.5 μL of 10 μM PA-27F-YM forward and PH-R reverse primers (Alpha DNA, Montréal, Canada; Klindworth *et al*., 2012), 1.0 μL of dNTPs (Qiagen, Canada), 0.5 μL of *T*. *aq* polymerase (Qiagen, Canada), and 2 μL of template DNA, for a total volume of 25 μL. PCR amplification was run in an Eppendorf Mastercycle ProS (Germany) thermocycler, and consisted of an initial denaturation of 2 minutes at 95°C, followed by 30 cycles of 30 seconds denaturation at 95°C, 30 seconds

annealing at 55°C, and 1 minute elongation at 72°C, before a final elongation of 5 minutes at 72°C (Bell *et al*., 2016; Lay *et al*., 2018; Blakney *et al*., 2022a). The amplified 1.5 kb 16S rRNA gene was visualized by a 1% gel electrophoresis, as described above, quantified using the QuBit dsDNA High Sensitivity Kit (Invitrogen, USA), and then serially diluted to 10^{-9}. One µL of each dilution was then used as template in a 10 µL qPCR reaction.

The 16S rRNA gene qPCR reactions consisted of 5.0 µL of Maxima SYBR Green/ROX qPCR Mix (ThermoFisher Scientific, Canada), 3.4 µL dH_2O, 0.3 µL of 10 µM Eub 338 forward and Eub 518 reverse primers (Alpha DNA, Montréal, Canada; Fierer *et al*., 2005). All qPCR reactions were set-up in triplicate in 96-well plates using the Freedom EVO100 robot (Tecan, Switzerland), with a no-template negative control included on each plate. Reactions were run in a ViiA 7 Real-Time PCR System (Life Technologies, Canada) following the same cycling conditions as described previously for the 16S rRNA PCR amplification. The Eub338/Eub518 qPCR reaction amplified a 200 bp region of the V3 region (Muyzer *et al*., 1993; Nogales *et al*., 1999; Bathe & Hausner, 2006; Davis *et al*., 2009). The number of 16S rRNA gene copies present in the serially diluted standard were calculated using the formula (Godornes *et al*., 2007):

$$\text{Number of 16S rRNA gene copies } \mu L^{-1} = \frac{\text{Avogadro's Constant x DNA (g } \mu L^{-1})}{\text{Number of base pairs x 600 Daltons}}$$

The standard curve for the serial diluted samples was plotted, with an R^2 value of 0.9938 and an amplification efficiency of -3.2013 (Fig. S6), falling within acceptable values (Fierer *et al*., 2005).

16S rRNA gene copy numbers were then estimated for each Test Phase sample by using 1 µL of a 1:10 dilution of DNA as template in the same 16S rRNA qPCR reaction and cycling

conditions as described above for the standard curve. Melt curves generated by 0.5°C increments at the end of the qPCR programme confirmed amplicon specificity, and the 16S rRNA gene copy number was then determined from the standard curve. A correction to determine the absolute abundance of each ASVs inferred from the Test Phase samples was achieved by multiplying the total 16S rRNA gene copy number per μg, as estimated from the qPCR reaction, by the relative abundance matrix of ASVs identified (Azarbad *et al*., 2018; Bakker, 2018).

Generating phylogenetic trees

In order to employ phylogeny-based analysis methods, including phylogenetic diversity (PD), and UniFrac distances, we assembled phylogenies for the Test Phase *B*. *napus* bacterial communities. Following the method described by Callahan *et al*., 2016b, 16S rRNA gene sequences for each ASV inferred from the Test Phase data were aligned using a profile-to-profile algorithm (Wang & Dunbrack, 2004) with a dendrogram guide tree using the `decipher` package (Wright, 2016). With the `phangorn` package (Schliep, 2011), the maximum likelihood of each site was calculated using the `dist.ml` function using a JC69 equal base frequency model, before assembling phylogenies using the neighbour-joining method. An optimized GTR nucleotide substitution model was fitted to the phylogeny using the `optim.pml` function. Phylogenies were subsequently added to the `phyloseq` object (McMurdie & Holmes, 2013).

α-diversity of the Test Phase Brassicaceae rhizosphere and root communities

In order to estimate the coverage of the bacterial domain of life, we incorporated phylogenies into the phyloseq object following the method described by Callahan *et al*., 2016b. Faith's PD was calculated as an α-diversity index from the Test Phase *B*. *napus* samples using the

`pd` function from the `picante` package (Kembel *et al*., 2010; sum of all branch lengths separating taxa in a community). Log transformed PD indices were confirmed to respect normality.

We assessed differences of the mean PD between GS for each soil history, and their interactions using a Multi-Factor ANOVA and Tukey's *post hoc* test for significant groups that respected the assumptions of normality (Azarbad *et al*., 2020, Blakney *et al*., 2022). Normality of the residuals was established with a Shapiro-Wilk test, `shapiro.test`, while the heteroscedascity of residuals was confirmed with using a Bartlett test, `bartlett.test`. For significant ANOVAs, a *post hoc* Tukey's Honest Significant Difference test, TukeyHSD, was used to determine which groups were statistically different.

Identification of differentially abundant ASVs and specific indicator species

To refine our understanding of the abundance and composition of the Test Phase *B*. *napus* bacterial communities, we used two complementary methods to identify taxa specific to GS and soil histories. First, taxa cluster maps were used to calculate the differential abundance of ASVs between experimental groups. Second, indicator species analysis was used to detect ASVs that were preferentially abundant in pre-defined environmental groups (compartments, GS, soil histories). A significant indicator value is obtained if an ASV has a large mean abundance within a group, compared to another group (specificity), and has a presence in most samples of that group (fidelity) (De Cáceres & Legendre; Legendre & Legendre, 2012). The fidelity component complements the differential abundance approach between taxa clusters, which only considers abundance. Moreover, given the large number of taxa in our study, it was not practical to view taxa clusters as matrices below class, whereas indicator species analysis pinpoints specific ASVs of interest.

β-diversity of the Brassica napus bacterial communities

To test for significant differences between the Test Phase *B*. *napus* bacterial communities from different GS and soil histories, we used the non-parametric permutational multivariate ANOVA (PERMANOVA), where any variation in the ordinated data distance matrix is divided among all the pairs of specified experimental factors. The PERMANOVA was calculated using the `adonis` function in the `vegan` package (Oksanen *et al*., 2020), with 9999 permutations, and the experimental blocks were included as "strata". This was complemented with a PERMANOVA for each compartment (bulk soil, rhizosphere and roots) that specifically tested GS and soil histories as experimental factors, and used a weighted Unifrac distance matrix (Lozupone & Knight, 2005; Lozupone *et al*., 2007).

This distance index incorporates the phylogenetic relationship of each ASV and their absolute abundance, as estimated by qPCR of the 16S rRNA gene (Lozupone & Knight, 2008). Determining community distances based only on the number of shared taxa does not account for evolutionary distances between taxa, which are often extremely diverse among microbes (Fitzpatrick *et al*., 2018; Walters *et al*., 2018). Conversely, using a UniFrac index illustrates how bacterial community composition varies by phylogeny, which provides insight into how different community assembly mechanisms, including dispersal, drift, selection, and speciation, may be at work (Vellend, 2010). The weighted UniFrac distance matrix was calculated using the `distance` function in `phyloseq` (McMurdie & Holmes, 2013).

Distance-based redundancy analyses, using UniFrac distances weighted by absolute abundance, were used to quantified the amount of variation described by each experimental factors in the bacterial communities from the soils (bulk soil and rhizosphere), or root compartments (i.e.

how much of the phylogenetic change between communities was due to the compartment, soil history, or GS). Model accuracy was assessed with an adjusted R^2 value and tested for significance using an ANOVA (Carteron *et al*., 2021). Results were similar to Bray-Curtis and unweighted Unifrac distances, but tended to be more explicative using weighted Unifrac.

Results

Bulk soil and rhizosphere samples were more similar than to root or seed samples.

Illumina MiSeq produced 11 010 728 raw reads for the whole dataset, which were then processed through DADA2 (Callahan *et al*., 2016 & 2017). We retained 2 770 390 reads from all the experimental samples, which inferred a total of 33 392 ASVs (Table 1 & Fig. S3). As a positive control for our experiment, we included two replicates of the bacterial mock community, which retained 7430 and 9537 16S rRNA reads (Fig. S4). The mock communities closely resembled each other by ASV composition (Fig. S4), where our pipeline correctly identified all 20 of the bacterial species included in the communities (Fig. S4 & Table S1). These results provide some reassurance that our pipeline ought to be effective in identifying a range of bacterial ASVs present in the experimental samples.

Similar counts of 16S rRNA reads were retained in the bulk soil and rhizosphere samples; means of ~25 429 and 22 467 reads, respectively (Table 1). We identified ~1300 to 1700 ASVs in the bulk soil samples, while the rhizosphere samples had ~800 to 1800 ASVs (Table 1). In contrast, an average of ~3098 16S rRNA reads were retained in the root samples, where only ~100 to 450 ASVs were inferred (Table 1). Finally, we estimated the absolute abundance, or size, of each bacterial community by qPCR amplification of the 16S rRNA gene, where total community sizes ranged from ~200 000 to ~18 000 000 16S rRNA gene copies (Table 1).

Table 1. The bacterial bulk soil and rhizosphere communities had more unique ASVs than the root communities at each growth stage of their host plant *Brassica napus*. Samples were harvested throughout the 2019 growing season during the Test Phase of a multi-year crop rotation in Lacombe, Alberta. 11 010 728 raw reads were produced via Illumina's MiSeq platform at Génome Québec, and processed through DADA2, where 2 770 390 reads were retained (16S rRNA Reads reported here) for ASV inference. A total of 33 392 ASVs were identified across the dataset. Bacterial community size was estimated by qPCR as the number of 16S rRNA gene copies.

Growth Stage[a]	Compartment[b]	16S rRNA Reads[c]	ASV Occurrence[d]	16S rRNA Gene Copies[e]
Seed	Bulk (n = 12)	21 213 ±1842	1497 ±128	2 360 322 ±1 610 500
	Seed (n = 1)	7	6	2 251 078
Seedling	Bulk (n = 12)	22 886 ±1516	1526 ±72	1 224 464 ±1 083 033
	Rhizosphere (n = 12)	19 817 ±3941	1355 ±143	6 722 767 ±1 929 973
	Root (n = 3)	2519 ±117	432 ±28	1 153 665 ±738 663
Rosette	Bulk (n = 12)	23 020 ±4472	1448 ±128	1 415 797 ±1 088 160
	Rhizosphere (n = 12)	19 984 ±7475	1295 ±444	2 131 129 ±833 941
	Root (n = 12)	3058 ±1099	361 S±67	13 105 921 ±5 757 254
Bolting	Bulk (n = 12)	26 811 ±6475	1567 ±209	3 266 621 ±1 896 126

	Rhizosphere (n = 12)	29 286 ±6090	1611 ±219	2 305 561 ±1 249 093
	Root (n = 12)	2502 ±3362	231 ±116	8 711 539 ±4 380 029
Flowering	Bulk (n = 12)	28 704 ±6599	1649 ±150	3 049 801 ±3 738 808
	Rhizosphere (n = 12)	23 263 ±4147	1552 ±132	10 044 431 ±5 541 865
	Root (n = 12)	4353 ±3784	352 ±105	3 159 861 ±1 327 309

[a], Test phase growth stages
[b], Presented with the number of samples (n) retained
[c], Values are presented as mean ± SD
[d], Values are presented as mean ± SD
[e], Values are presented as mean ± SD

Globally, we found that the bacterial communities from the bulk soil, seeds, rhizosphere, roots were significantly different (PERM $R^2 = 0.60135$, $p < 0.001$; Table S3). β-diversity analysis highlighted the difference between the bulk soil and rhizosphere communities from the root and seed communities (Fig. S8A). These differences were further reflected by significantly different levels of phylogenetic diversity (PD), where the bulk soil and rhizosphere communities remained the most diverse throughout the growing season (Fig. S7, $p < 0.001$). Comparatively, the root and seed communities remained consistently less diverse (Fig. S7). Indicator species analysis did not identify any specific ASVs, according to compartment, growth stage (GS), or soil history.

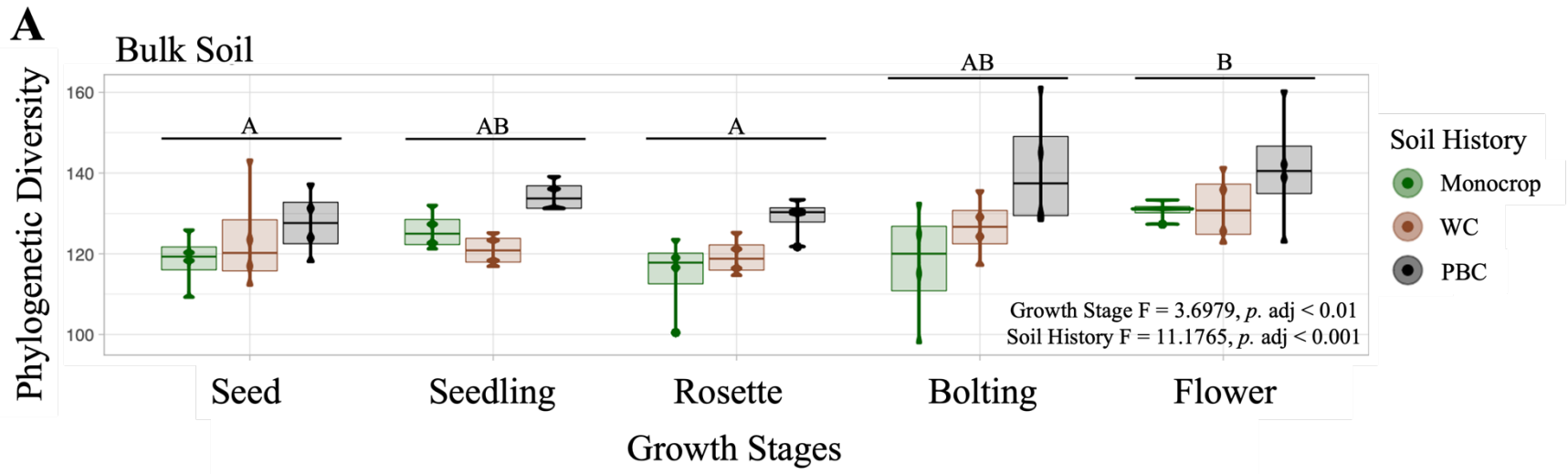
Bacterial bulk soil communities were significantly impacted by soil history than time

To test our hypothesis that the previously established soil history would decrease in influencing the structure of the *B. napus* bacterial communities over the growing season, we first examined how the bacterial bulk soil communities changed through time. Note that for the bulk soil communities GS was a proxy for time through the season, as we would not expect bulk soil to be influenced by the growth stages of the plant hosts. Soil history and GS (i.e. time) were both significant in the bulk soil communities, thought the interaction was not (PERM $R^2 = 0.08770$, $p < 0.001$; $R^2 = 0.08596$, $p < 0.016$, respectively; Table 2). PD remained stable across the growing season, except at the flower stage where diversity was significantly higher ($p.$ adj < 0.01; Fig. 1A). PD was also significantly higher at each GS time point the in bulk soil communities with a soil history of pea-barley-canola (PBC), compared to the communities from monocrop or wheat-canola (WC) plots ($p.$ adj < 0.001; Fig. 1A). Monocrop bulk soil communities were also globally depleted in *Fibrobacteria*, compared to WC or PBC bulk soils ($p. < 0.05$; Fig. 1B). The bacterial bulk soil communities were enriched in class ABY1 (phylum *Patescibacteria*) at the rosette stage ($p. < 0.05$;

Table 2. PERMANOVA identified growth stage and soil history as significant experimental factors for the bacterial bulk soil and rhizosphere communities harvested in 2019 from *Brassica napus* in the Test Phase of a multi-year crop rotation in Lacombe, Alberta. Soil history established the previous year was not significant for the bacterial communities from the root communities. PERMANOVA was calculated using a weighted Unifrac distance matrix, with 9999 permutations.

Experimental Factors	Test Phase Compartments[a]								
	Bulk Soil			Rhizosphere			Roots		
	F Model	R^2	Pr (> F)	F Model	R^2	Pr (> F)	F Model	R^2	Pr (> F)
Growth Stage[b]	1.31843	0.08596	**0.016**	2.04117	0.12043	**0.001**	2.33385	0.16454	**0.006**
Soil History[c]	2.69030	0.08770	**0.001**	2.73273	0.10749	**0.001**	1.40096	0.06585	0.163
Soil History ~ Growth Stage	0.71201	0.09285	0.928	0.70968	0.08374	0.812	0.95793	0.13508	0.548

[a], Values in bold indicate significant factors or interactions
[b], Test Phase growth stages: seed, seedling, rosette, bolting, or flower
[c], Soil history established the previous year: monocrop canola (*B. napus*), wheat-canola rotation (WC), or pea-barley-canola rotation (PBC)


A
Bulk Soil
Phylogenetic Diversity
160
140
120
100
A
AB
A
AB
B
Growth Stage F = 3.6979, p. adj < 0.01
Soil History F = 11.1765, p. adj < 0.001
Seed
Seedling
Rosette
Bolting
Flower
Growth Stages
Soil History
Monocrop
WC
PBC

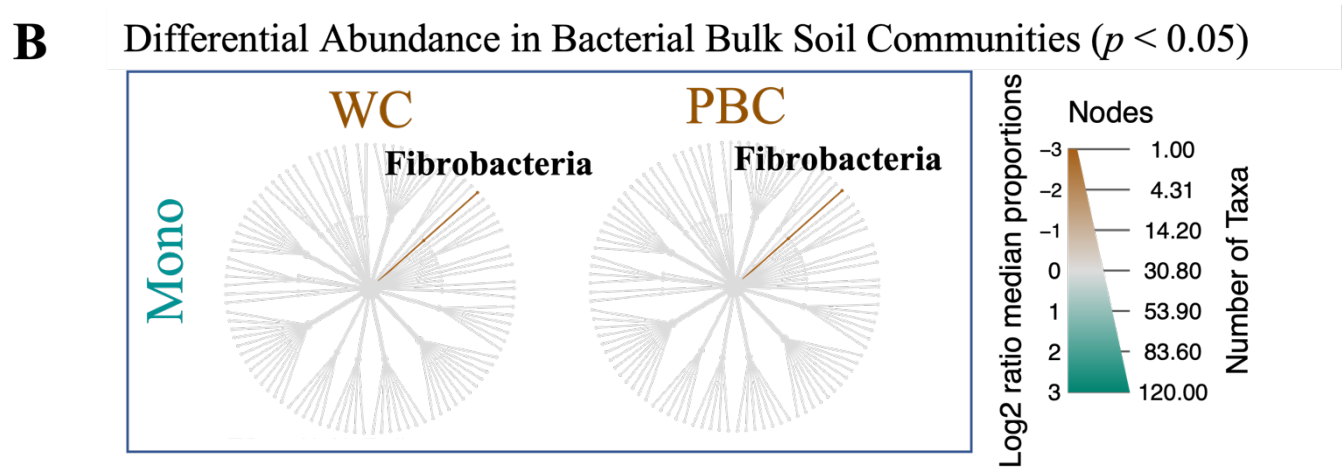

B
Differential Abundance in Bacterial Bulk Soil Communities (p < 0.05)
WC
PBC
Fibrobacteria
Fibrobacteria
Mono
Nodes
Log2 ratio median proportions
−3
−2
−1
0
1
2
3
1.00
4.31
14.20
30.80
53.90
83.60
120.00
Number of Taxa

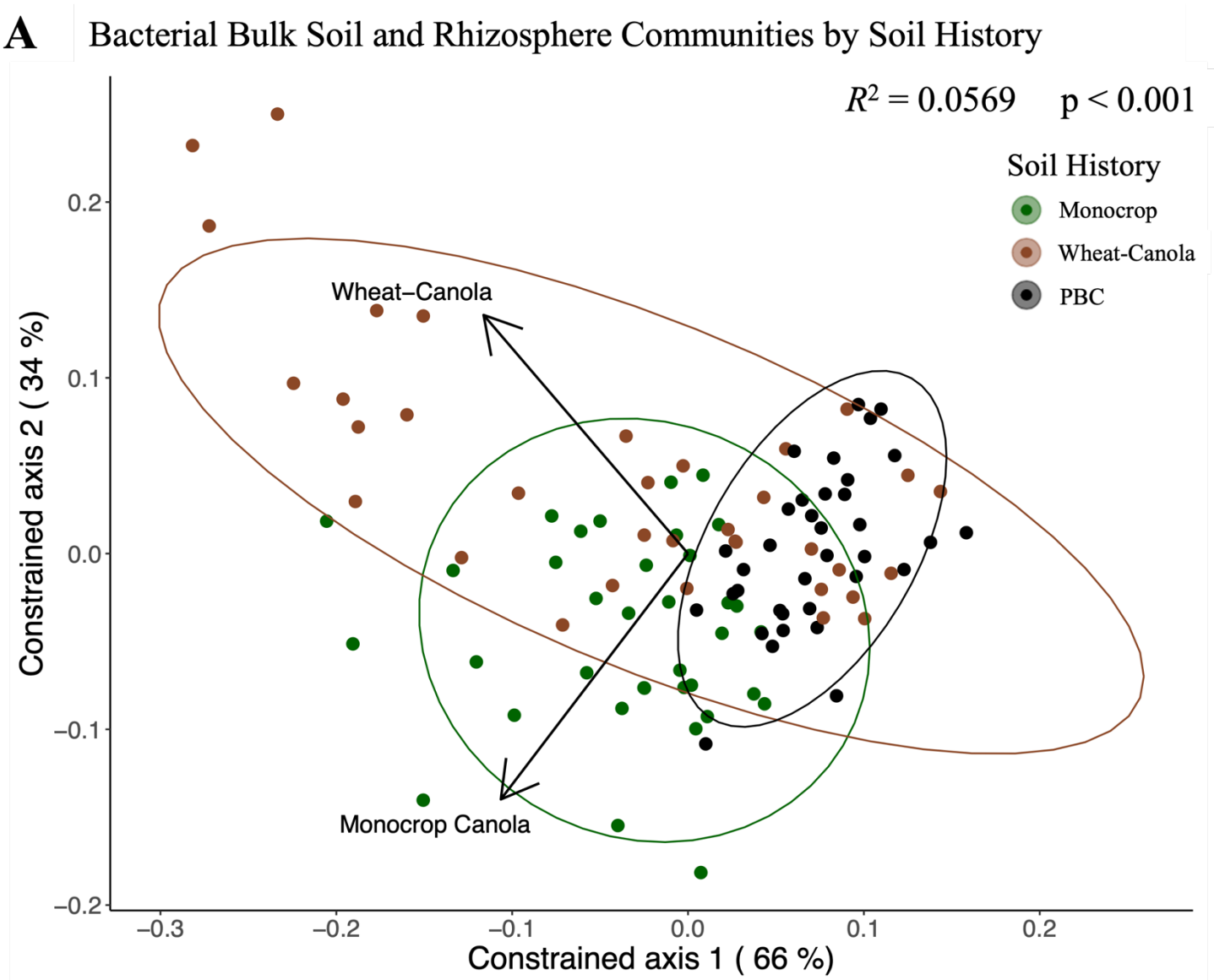
Figure 1. Bacterial communities identified from bulk soil samples were largely stable across different growth stages and soil histories. Samples were harvested throughout the 2019 *Brassica napus* growing season during the Test Phase of a multi-year crop rotation in Lacombe, Alberta. (A) Phylogenetic diversity was significantly higher during the flower growth stage than at the seed or rosette stages (A vs B, $p < 0.01$)). Bacterial bulk soil communities with the pea-barley-canola (PBC) soil history were also more diverse at each growth stage than the monocrop or wheat-canola (WC) communities ($p < 0.001$). Diversity across growth stages and soil histories was tested with a Multi-Factor ANOVA, which confirmed the previously established soil histories and the Test Phase *B*. *napus* growth stages did not interact. Statistically significant groups were identified using Tukey's *post hoc* test. (B) *Fibrobacteria* were significantly enriched ($p < 0.05$) in bacterial bulk soil communities with wheat-canola (WC) and pea-barley-canola (PBC) soil histories, compared to communities from monocrop canola (Mono) plots. Significantly enriched taxa, labelled in bold, were tested between each pair of growth stages and soil history. Taxa that were significantly more abundant are highlighted brown or green, following the labels for each compared host. These differential taxa clusters identified significantly enriched (ie, abundant), using the non-parametric Kruskal test, followed by the *post hoc* pairwise Wilcox test, with an FDR correction.

Fig. S9B), and in class *Rhodothermia* (phylum *Bacteroidota*) at the bolting stage ($p. < 0.05$; Fig. S9C), when compared to their cognate rhizosphere communities. The β-diversity of the bulk soil communities also showed that the PBC communities were more phylogenetically consistent to each other across the growing seasons than to either the monocrop, or WC (Fig. 2A), as all the PBC soil communities tended to remain more closely clustered. However, we also observed that the bulk soil communities at the seed and seedling communities tended to be more phylogenetically similar, while the remaining time points (rosette, bolting and flowere) were more diverse (Fig. 2B).

Rhizosphere communities were highly structured by growth stages and soil history

Next, we compared the dynamics of the bacterial rhizosphere soil communities. Similar to the bulk soil communities, soil history and GS were also both significant for the rhizosphere communities, while the interaction was not (PERM $R^2 = 0.10749$, $p < 0.001$; $R^2 = 0.12043$, $p < 0.016$, respectively; Table 2). PD increased across GS, regardless of previous soil history, such that the bolting and flower rhizosphere communities were more diverse than those from the seedling and rosette GS ($p.$ adj < 0.001; Fig. 3A). Furthermore, PBC communities were significantly more diverse at each GS, than the rhizosphere communities from either the monocrop or WC soil histories ($p.$ adj < 0.001; Fig. 3A). Nonetheless, unlike the bulk soil communities, there were no ASVs that were significantly differential enriched, or depleted, according to soil history.

However, we did observe a number of significant changes to taxonomic abundances in the rhizosphere at several GS. First, we noted widespread taxonomic enrichment between rhizosphere communities at the seedling and flower stages ($p < 0.05$; Fig. 3B). The bacterial rhizosphere communities at the seedling and flower stages also had widespread enrichment of taxa compared to the bulk soil and root communities (Fig. S9A & D). The rhizosphere communities were also


A
Bacterial Bulk Soil and Rhizosphere Communities by Soil History
$R^2 = 0.0569$ p < 0.001
Soil History
Monocrop
Wheat-Canola
PBC
Wheat–Canola
Monocrop Canola
Constrained axis 2 (34 %)
Constrained axis 1 (66 %)
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.2
−0.1
0.0
0.1
0.2

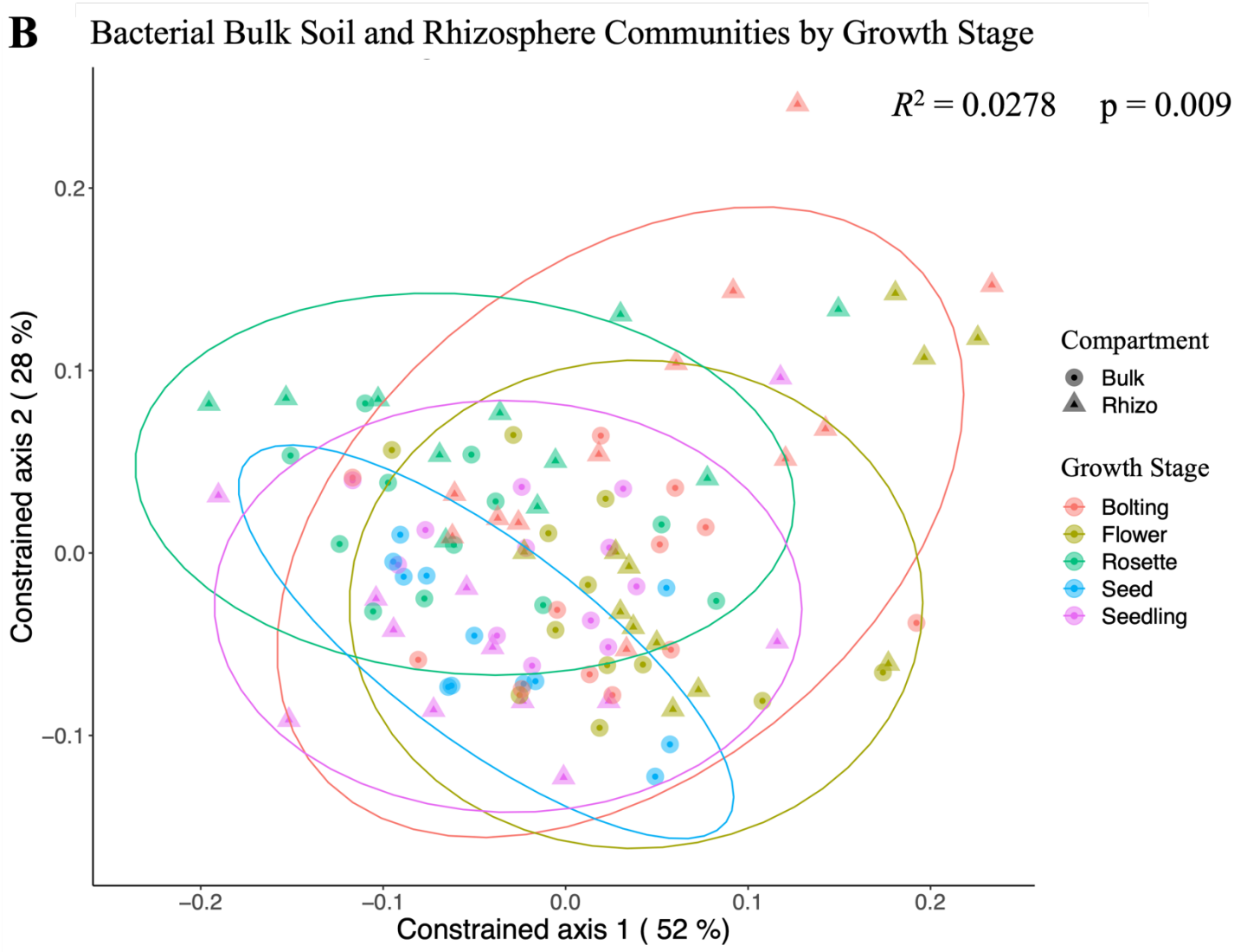

B
Bacterial Bulk Soil and Rhizosphere Communities by Growth Stage
$R^2 = 0.0278$ p = 0.009
Constrained axis 2 (28 %)
Constrained axis 1 (52 %)
0.2
0.1
0.0
−0.1
−0.2
−0.1
0.0
0.1
0.2
Compartment
Bulk
Rhizo
Growth Stage
Bolting
Flower
Rosette
Seed
Seedling

Figure 2. Bacterial communities identified from the bulk soil and rhizosphere of *Brassica napus* were more phylogenetically similar in: (A) plots with pea-barley-canola soil history (black, PBC) versus other soil histories regardless of growth stage, and (B) early growth stages (seed and seedling) versus later growth stages (rosette, bolting, and flower). Samples were harvested throughout the 2019 growing season during the Test Phase of a multi-year crop rotation in Lacombe, Alberta. Distance-based redundancy analysis, using UniFrac distances weighted by absolute abundance, quantified how the experimental factors (A, constrained by soil history) and (B, constrained by growth stages) structured the bacterial communities, where those with similar phylogenetic composition appear closer together.

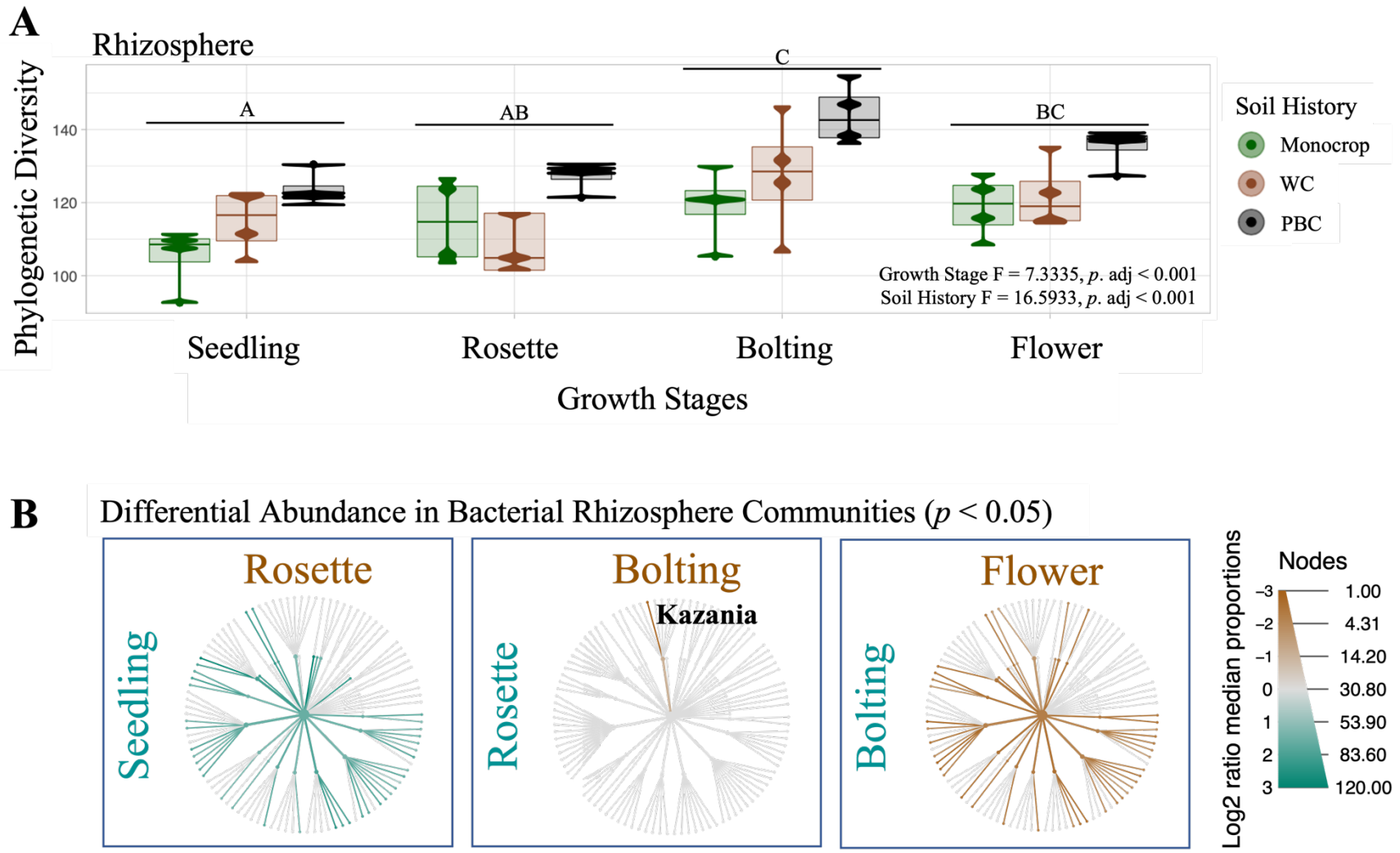

A
Rhizosphere
Phylogenetic Diversity
140
120
100
A
AB
C
BC
Growth Stage F = 7.3335, p. adj < 0.001
Soil History F = 16.5933, p. adj < 0.001
Seedling
Rosette
Bolting
Flower
Growth Stages
Soil History
Monocrop
WC
PBC
B
Differential Abundance in Bacterial Rhizosphere Communities (p < 0.05)
Rosette
Seedling
Bolting
Rosette
Kazania
Flower
Bolting
Nodes
Log2 ratio median proportions
−3
−2
−1
0
1
2
3
1.00
4.31
14.20
30.80
53.90
83.60
120.00
Number of Taxa

Figure 3. Bacterial rhizosphere communities varied significantly between growth stages of their *Brassica napus* plant host. Samples were harvested throughout the 2019 *Brassica napus* growing season during the Test Phase of a multi-year crop rotation in Lacombe, Alberta. (A) Phylogenetic diversity was significantly higher in the bolting and flower growth stages than the seedling and rosette stages ($p < 0.001$). Bacterial rhizosphere communities with the pea-barley-canola (PBC) soil history were also significantly more diverse at each growth stage than the other soil histories ($p < 0.001$). Diversity across growth stages and soil histories was tested with a Multi-Factor ANOVA, which confirmed the previously established soil histories and the Test Phase *B. napus* growth stages did not interact. Statistically significant groups were identified using Tukey's *post hoc* test. (B) The seedling-rosette transition and bolting-flower transition were significantly different ($p < 0.05$) in bacterial taxa. Significantly enriched taxa, labelled in bold, were tested between each pair of growth stages and soil history. Taxa that were significantly more abundant are highlighted brown or green, following the labels for each compared host. These differential taxa clusters identified significantly enriched (ie, abundant), using the non-parametric Kruskal test, followed by the *post hoc* pairwise Wilcox test, with an FDR correction.

enriched in a number of taxa compared to the roots at the rosette and bolting stages, though noticeably less widespread than at the seedling and flower stages (Fig. S9B & C). Finally, the bacterial rhizosphere communities were largely stable in terms of different taxa abundance between the rosette and bolting stages, except for an enrichment in *Kazania* bacteria (Fig. 3B, phylum *Desulfobacterota*).

The β-diversity of the rhizosphere communities remained phylogenetically similar between the bacterial bulk soil and rhizosphere communities across the growing season; the bacterial communities appeared most similar at the seedling stage, as well as to the initial seed stage in the bulk soil (Fig. 2B). We also observed more variation in β-diversity among the rhizosphere communities at the rosette, bolting and flower stages compared to communities in the bulk soil (Fig. 2B). Like the bulk soil communities, the β-diversity illustrated that the PBC communities were more phylogenetically consistent to each other, regardless of GS, than to either the monocrop, or WC (Fig. 2A).

Root communities were only impacted by growth stages, and not soil history

Finally, we analyzed how soil history and GS impacted the bacterial root communities. Only GS was significant in structuring root communities (PERM $R^2 = 0.16454$, $p < 0.006$, Table 2), unlike the bulk soil and rhizosphere communities where both GS and soil history were significant. β-diversity further illustrated this, as there was no impact of soil history on the root communities. We also observed that the bacterial root communities were more phylogenetically consistent at the seedling stage and became more variable at each subsequent GS (Fig. 5).

In the root communities, PD was low compared to the bulk soil and rhizosphere communities (Fig. S7). Nonetheless, these communities were very stable across GS, except at the

bolting stage, where PD was significantly lower compared to the other GS (Fig. 4A). We did not detect any impact of the different soil histories on the PD, or differential abundances of bacterial taxa, in the root communities at any GS. The root communities were significantly depleted in a wide variety of bacterial taxa at each GS when compared to the rhizosphere ($p < 0.05$; Fig. S9C). Nevertheless, the root communities were also significantly enriched in specific taxa when compared to the rhizosphere. First, at the rosette stage the most prominently enriched taxa in the roots were in the *Verrucomicrobiae*, *Actinobacteria*, *Proteobacteria*, and *Bacterodia* ($p < 0.05$; Fig. S9B9). Second, at the bolting stage the root communities were enriched in the *Gammaproteobacteria* & *Bacterodia* when compared to the rhizosphere communities ($p < 0.05$; Fig. S9C). We also detected two enriched taxa in the root communities when compared among themselves at different GS; *Bacterodia* were enriched at the rosette stage, compared to the bolting stage, while *Gammaproteobacteria* were enriched at the bolting stage, compared to the flower stage ($p < 0.05$; Fig. 4B).

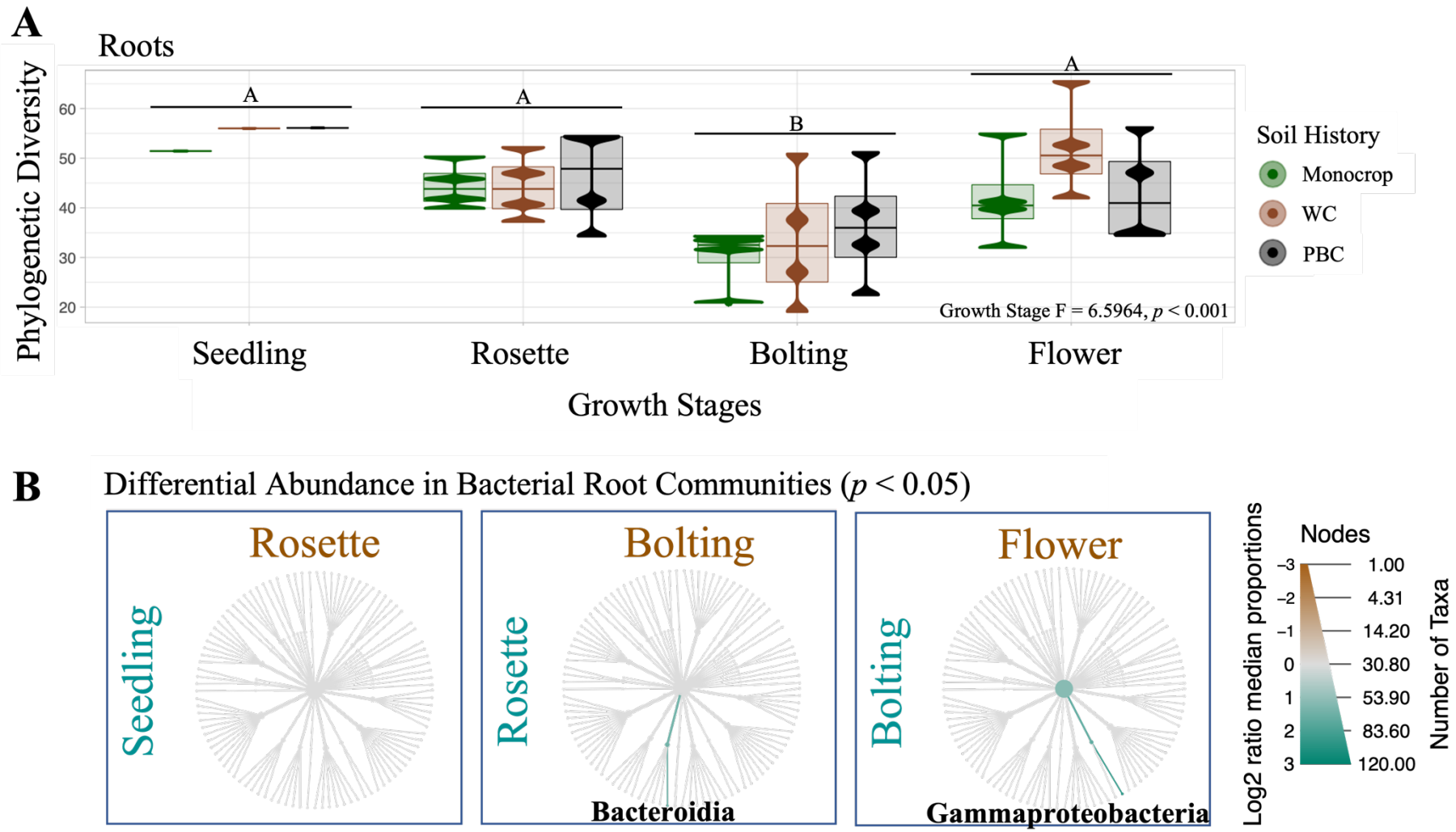

A
Roots
Phylogenetic Diversity
60
50
40
30
20
A
A
B
A
Growth Stage F = 6.5964, p < 0.001
Seedling
Rosette
Bolting
Flower
Growth Stages
Soil History
Monocrop
WC
PBC
B
Differential Abundance in Bacterial Root Communities (p < 0.05)
Rosette
Seedling
Bolting
Rosette
Bacteroidia
Flower
Bolting
Gammaproteobacteria
Nodes
−3
−2
−1
0
1
2
3
1.00
4.31
14.20
30.80
53.90
83.60
120.00
Log2 ratio median proportions
Number of Taxa

Figure 4. Bacterial root communities were stable across different growth stages and soil histories. Samples were harvested throughout the 2019 *Brassica napus* growing season during the Test Phase of a multi-year crop rotation in Lacombe, Alberta. (A) Phylogenetic diversity was significantly lower among the root communities at the bolting stage compared to other growth stages ($p < 0.001$). Diversity across growth stages was tested with a Multi-Factor ANOVA, which confirmed that the previously established soil histories were not significant and did not interact with the Test Phase *B*. *napus* growth stages. Statistically significant groups were identified using Tukey's *post hoc* test. (B) *Bacteroidia* were significantly enriched ($p < 0.05$) in the bacterial root communities at the rosette stage compared to the subsequent bolting stage, while *Gammaproteobacteria* were enriched in root communities at the bolting stage compared to communities at the flower stage. Significantly enriched taxa, labelled in bold, were tested between each pair of growth stages and soil history. Taxa that were significantly more abundant are highlighted brown or green, following the labels for each compared host. These differential taxa clusters identified significantly enriched (ie, abundant), using the non-parametric Kruskal test, followed by the *post hoc* pairwise Wilcox test, with an FDR correction.

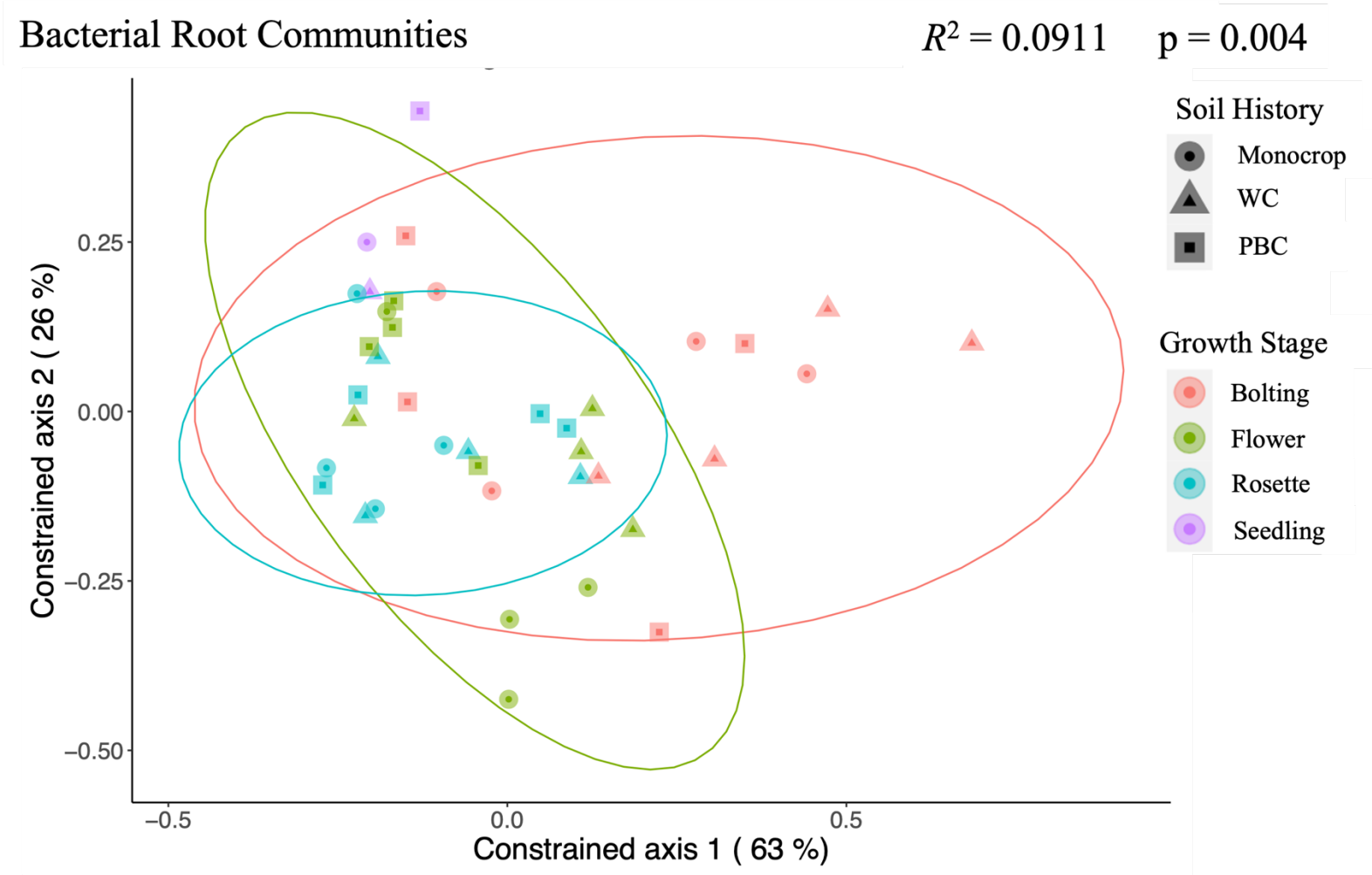

Bacterial Root Communities
$R^2 = 0.0911$
p = 0.004
Soil History
Monocrop
WC
PBC
Growth Stage
Bolting
Flower
Rosette
Seedling
Constrained axis 2 (26 %)
0.25
0.00
−0.25
−0.50
−0.5
0.0
0.5
Constrained axis 1 (63 %)

Figure 5. Bacterial root communities from the seedling (purple) and rosette stage (blue) were more phylogenetically similar to each other, regardless of different soil histories (shapes; not significant), than to the bolting (red) or flower (green) growth stages. Samples were harvested throughout the 2019 growing season during the Test Phase of a multi-year crop rotation in Lacombe, Alberta. Distance-based redundancy analysis (constrained by growth stages), using UniFrac distances weighted by absolute abundance, quantified how the experimental factors structured the bacterial communities, where those with similar phylogenetic composition appear closer together.

Discussion

Soil history implies that information is transmitted through time to condition the assembly of future plant-soil microbial communities (Kaisermann *et al*., 2017; Bakker *et al*., 2021; Hannula *et al*., 2021). Although our previous work has shown this can be true (Blakney *et al*., 2022; Blakney *et al*., 2023), the influence or longevity of soil history on bacterial community structure across growth stages is not clear. In this study, we tested how previously established soil histories endured across growth stages throughout a growing season. We took advantage of an agricultural field experiment to bridge the gap between controlled greenhouse conditions and experiments in "natural" environments, as such studies are currently lacking to understand bacterial temporal dynamics (Revillini *et al*. 2016; Martinović *et al*., 2021; Gu *et al*., 2022). We hypothesised that previously established soil histories would decrease in influencing the structure of *Brassica napus* bacterial communities over the growing season. We sampled bulk soil, rhizosphere and roots successively throughout the growing season from different soil histories and used 16S rRNA metabarcoding to estimate the bacterial communities. Contrary to our hypothesis, we actually found a strong impact of soil history on the bacterial bulk soil and rhizosphere communities throughout the growing season.

Bacterial community diversity changed across growth stages

We found that the time points of each GS were significant in structuring bacterial communities from all three compartments, the bulk soil, rhizosphere and roots (Table 2). However, the data illustrated that the rhizosphere communities were exceptionally dynamic across GS, while the bacterial bulk soil and root communities tended to remain more stable. The most striking feature we observed among the bacterial rhizosphere communities were the large-scale taxonomic

enrichments between GS (Fig. 3B & S9), which were entirely absent in the bulk soil communities (Fig. 1B & S9) and quite limited in the roots (Fig. 4B & S9). The increased dynamism of the rhizosphere in particular seems reasonable since these communities are at the confluence of the bulk soil and the plant. Therefore, the rhizosphere ought to experience the changing influences of the *B*. *napus* plant host in addition to the local edaphic factors and climate variables. Furthermore, the compositional enrichments in the bacterial rhizosphere communities may illustrate the plant hosts capacity to increase recruitment from the surrounding bulk soil, particularly at the seedling and flower GS.

In contrast, bacterial root communities should be primarily shaped by the plant host, as we observed. Moreover, the bulk soil communities should largely experience similar abiotic conditions as the rhizosphere, but with minimal influence from the plant host (Vieira *et al*., 2020; Blakney *et al*., 2022; Blakney *et al*., 2023). That there were no compositional changes among the bulk soil communities between GS may be an encouraging indication that the bulk soil samples were appropriately harvested as they appear free from the influence of the host plant.

A second key feature we detected in the rhizosphere communities was an increase in phylogenetic diversity over GS (Fig. 2B & 3A), while a similar trend was confined to only the flower stage time point in the bulk soil communities (Fig. 1A). Conversely, the roots remained relatively phylogenetically stable throughout the experiment (Fig. 4A). The increase in late-stage phylogenetic diversity also appears in changes in β-diversity between rhizosphere and bulk soil communities (Fig. 2). In both cases we observed increased phylogenetic diversity over time, as older soils can slowly accumulate new community members due to different dispersal, drift, selection or speciation/diversification events (Nemergut *et al*., 2013).

A key difference between the bulk soil and rhizosphere communities, however, is that communities in the rhizosphere are directly influenced by the plant host, while those in the bulk soil are not since they are non-planted controls. Therefore, since we observed similar increases in diversity late in the growing season among the rhizosphere communities and their cognate bulk soil controls, it is perhaps obvious to suggest a common cause. However, it is important to be mindful that the trend among the rhizosphere communities may actually be due to the plant host, unlike in the bulk soil. Similar increases in phylogenetic diversity across GS have also been observed in the rhizosphere of other plants, such as rice (Edwards *et al*., 2018). Unlike previous findings in the perennial *Brassicaceae Arabidopsis alpina* that showed quite static bacterial communities (Dombrowski *et al*., 2016), our data aligned similarly to other annual crops that exhibit quite dynamic bacterial rhizosphere communities across GS (Edwards *et al*., 2018).

Nonetheless, we also observed that the variation within the rhizosphere and root communities increased through time; communities at the bolting and flower GS were more variable than at earlier GS (Fig. 2B & 5). It is possible that this variation could be due to the inherent stochasticity of priority effects, where community composition at later GS is constrained by earlier GS (Meisner *et al*., 2021; Chase *et al*., 2021; Debray *et al*., 2022). This could be better tested in the future as high-frequency sampling from multiple hosts of the same genotype may be experimentally valid to detect priority effects (Debray *et al*., 2022). However, given the dramatic morphological changes that occur at different GS, as has been noted in *B*. *napus*, attention must be paid to ensure that the same physical thing is repeatedly sampled (Mamet *et al*., 2022). Failure to do so could mistake a lack of priority effects for high community turnover.

Bacterial diversity was highest in the most diverse soil history

Our results also illustrated a clear trend that bulk soil and rhizosphere communities with the PBC soil history consistently had the highest phylogenetic diversity, compared to the monocrop and WC soil histories across all GS (Fig. 1A, 2 & 3A). We can be confident that the increased phylogenetic diversity among the PBC soil communities was due to the different soil history for two reasons. First, we observed an increase in PD among PBC plots in the bacterial bulk soil communities beginning from the first GS, before any host plants was even present. Second, the increase in phylogenetic diversity in the bulk soil and rhizosphere communities from the PBC plots remained throughout the growing season. Thus, even with the addition of a host plant the common PBC soil history still impacted the bacterial communities at each GS, or concordant sampling time in the case of the bulk soil.

Conversely, we found no difference between the three soil histories in the root communities (Fig. 4A & Fig. 5) where soil history was not significant to those communities (Table 2). Thus, from our experiment we can only observe a change in the root communities according to GS (Fig. 5). This is consistent with our previous work that also demonstrated bacterial root communities tend to be strongly influenced by the host plant, and not to impacted by soil history, unless the host plant host is stressed (Blakney *et al*., 2022).

Although we found a clear impact of different soil histories on phylogenetic diversity in the bacterial bulk soil and rhizosphere communities, it is interesting to note that we only identified a slight compositional change in the bulk soil due to soil history (Fig. 1B). Moreover, we did not detect different, or specific ASVs within the rhizosphere communities according to their different soil histories (Fig. 3B). First, this lack of compositional difference between soil histories might suggest that the different agricultural treatments involved in establishing the previous soil history

were not sufficiently diverse. However, given our previous results using crop rotations this seems unlikely (Blakney *et al*., 2022; Blakney *et al*., 2023). Alternatively, the lack of compositional differences between rhizosphere communities despite coming from different soil histories, could be evidence for the common host plant structuring similar rhizosphere communities. This would be consistent with other studies that found declining site-specific bacteria over time, and increasing plant-specific bacteria (Edwards *et al*., 2018). Including other diverse host plants, similar to our previous experimental design (Blakney *et al*., 2022; Blakney *et al*., 2023), would allow us to better support this conclusion.

Conclusion

In this experiment we tested the hypothesis that previously established soil histories would decrease in influencing the structure of *Brassica napus* bacterial communities over the growing season. We sampled the bulk soil, rhizosphere and roots successively throughout the growing season from plots with different soil histories and used 16S rRNA metabarcoding to estimate the bacterial communities. We largely confirmed our first prediction that the bacterial bulk soil communities would remain stable throughout the experiment and continue to be structured by their soil history. Our second prediction, that the bacterial bulk soil and rhizosphere communities would remain similar at the initial seed and seedling GS, according to their different soil history, was also well supported. In fact, we found that the bacterial bulk soil, rhizosphere, and root communities all diverged more as they aged. However, this divergence tended to refute our final prediction, as the rhizosphere communities did not converge in similarity over the growing season, regardless of their soil history. Yet, this was largely true among the root communities, which only divered according to growth stage. Moreover, we did not identify specific community changes in either the

rhizosphere or the roots due to different soil histories. Nevertheless, soil history continued to be more influential than GS among the rhizosphere communities and was not significant for root communities. Therefore, based on our data, our initial hypothesis that posited that the influence of soil history on structuring the bacterial community would decline across GS was not well supported. Instead, we found a strong impact of soil history on the bacterial bulk soil and rhizosphere communities throughout the growing season, but not in the root communities.

Our results highlight the importance of studying microbial communities through time, which has largely been ignored to date. Studying how communities arrive at a given composition is more instructive than just a static snapshot. Here we found that different soil histories persisted and impacted bacterial diversity throughout the growing season. This suggests that the host plant's capacity to "re-write" different soil histories may be quite limited as key components that constitute the soil history's identity remained present and continued to impact the bacterial communities. From the agricultural perspective, persisting soil histories may have important long-term consequences. This presents exciting future experiments to uncover the transmission components, or memory, of soil history among soil bacterial communities. Given the significant and myriad human-induced changes throughout the biosphere (IPCC 2021), there is a clear need to better account for how historical events may structure plant-soil microbial communities going forward through time, and more broadly influence the mechanisms of community ecology.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada. We thank Chantal Hamel for her support and assistance in establishing the field experiment with Agriculture and Agri-foodCanada. We also thank Jennifer Zuidof for setting up, managing, and harvesting the field experiment at Lacombe, Albert. We thank Stéphane Daigle for assistance in statistical analysis, Jacynthe Masse, Simon Morvan, Morgan Botrel, and Stephanie Shosha, for their helpful comments and discussions.

Author Contributions

AJCB designed the experiment, prepared the samples for sequencing, performed the qPCR experiment, analyzed the data, and wrote the manuscript with input from all co-authors. MSA & MH designed the experiment, supervised the work, contributed reagents, analytical tools, and revised the manuscript.

Supplementary Materials

Table S1. Bacterial strains included in the mock community (BEI Resources, USA) of known composition, was included on each plate (Fig. S2). The mock community contains DNA of 20 bacterial species in equimolar counts (10^6 copies/μL) of 16S rRNA genes. Taxa have been provided to illustrate the level of comparison.

Bacteria	Taxonomy		
	Phyla	Class	Order/Family
Actinomyces odontolyticus	Actinobacteria (P)	Actinomycetales (C)	
Propionibacterium acnes	Actinobacteria (P)	Actinomycetales (C)	
Bacteroides vulgatus	Bacteroidetes (P)		
Deinococcus radiodurans	Deinococcus (P)		
Bacillus cereus	Firmicutes (P)	Bacilli (C)	Bacillales (O)/ Bacillaceae (F)
Listeria monocytogenes	Firmicutes (P)	Bacilli (C)	Bacillales (O)/ Listeriaceae (F)
Staphylococcus aureus	Firmicutes (P)	Bacilli (C)	Staphylococcaceae (F)
Staphylococcus epidermidis	Firmicutes (P)	Bacilli (C)	Staphylococcaceae (F)
Enterococcus faecalis	Firmicutes (P)	Bacilli (C)	Lactobacillales (O)/ Enterococcaceae (F)
Lactobacillus gasseri	Firmicutes (P)	Bacilli (C)	Lactobacilliaceae (F)
Streptococcus pneumoniae	Firmicutes (P)	Bacilli (C)	Streptococcaceae (F)
Streptococcus agalactiae	Firmicutes (P)	Bacilli (C)	Streptococcaceae (F)
Streptococcus mutans	Firmicutes (P)	Bacilli (C)	Streptococcaceae (F)

Clostridium beijerinckii	Firmicutes (P)	Clostridia (C)	Clostridiale (O)
Rhodobacter sphaeroides	Proteobacteria (P)	Alphaproteobacteria (C)	
Neisseria meningitides	Proteobacteria (P)	Betaproteobacteria (C)	
Helicobacter pylori	Proteobacteria (P)	Epsilonproteobacteria (C)	
Escherichia coli K12	Proteobacteria (P)	Gammaproteobacteria (C)	Enterobacteriales (O)
Acinetobacter baumannii	Proteobacteria (P)	Gammaproteobacteria (C)	Pseudomonadales (O)/ Moraxellaceae (F)
Pseudomonas aeruginosa PAO1-LAC	Proteobacteria (P)	Gammaproteobacteria (C)	Pseudomonadaceae (F)

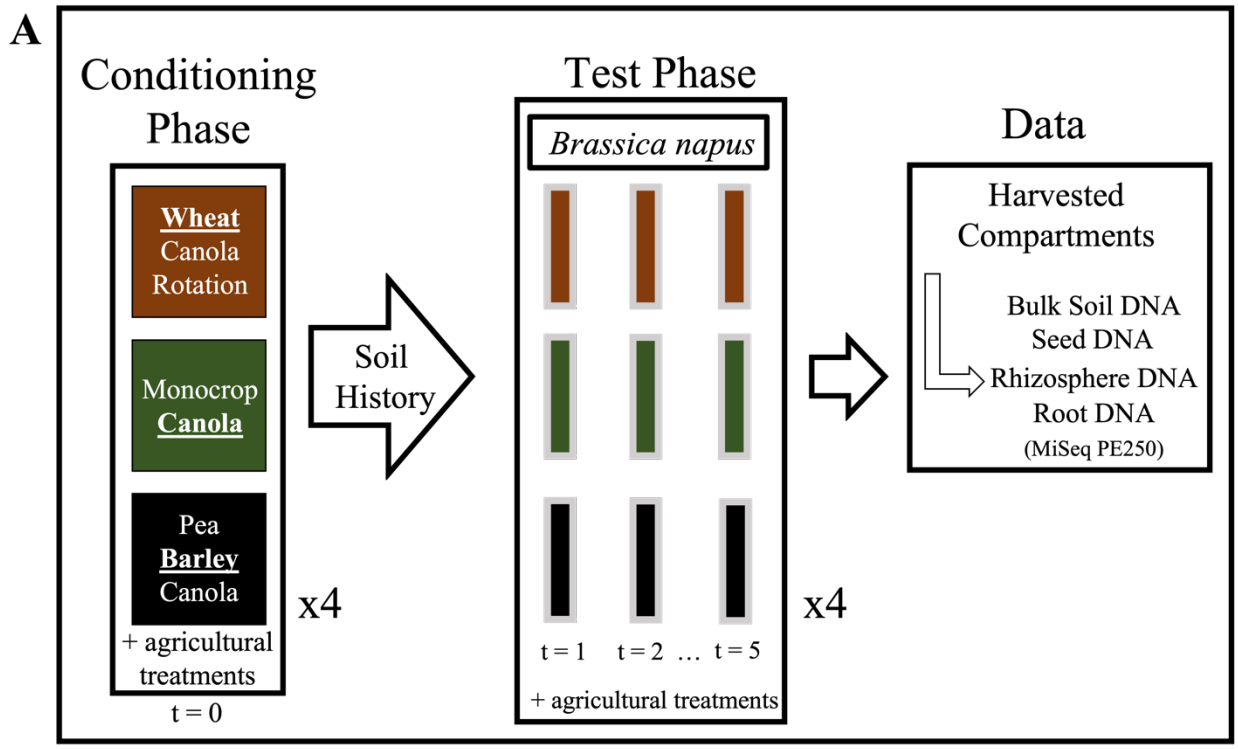
Table S2. Primers used in this study.

Name	Sequence (5'-3')	Reference
S-D-Bact-0341-b-S-17	CCTACGGGNGGCWGCAG	Klindworth *et al*., 2012
S-D-Bact-0785-a-A-21	GACTACHVGGGTATCTAATCC	Klindworth *et al*., 2012
CS1 Adapters	ACACTGACGACATGGTTCTACA	Illumina, 2013
CS2 Adapters	TACGGTAGCAGAGACTTGGTCT	Illumina, 2013
16S PA-27F-YM	AGAGTTTGATCCTGGCTCAG	Bruce *et al*., 1992
16S PH-R	AAGGAGGTGATCCAGCCGCA	Bruce *et al*., 1992
Eub338	ACTCCTACGGGAGGCAGCAG	Fierer *et al*., 2005
Eub518	ATTACCGCGGCTGCTGG	Fierer *et al*., 2005

Table S3. PERMANOVA for all the sampled Test Phase communities identified compartment (bulk soil, rhizosphere, or root), growth stage, and soil history established in the Conditioning Phase, as significant experimental factors. PERMANOVA was calculated using a Bray-Curtis and Weighted Unifrac distance matrix, with 9999 permutations.

Experimental Factors	Bray-Curtis[a]			Weighted Unifrac		
	F Model	R^2	Pr (> F)	F Model	R^2	Pr (> F)
Compartment[b]	2.82430	0.05461	**0.001**	81.305	0.60135	**0.001**
Growth Stage[c]	1.12762	0.02907	**0.006**	1.655	0.01632	0.090
Soil History[d]	1.14988	0.01482	**0.016**	2.924	0.01442	**0.025**
Compartment ~ Growth Stage	1.29513	0.05008	**0.001**	2.576	0.03811	**0.005**
Compartment ~ Soil History	1.03763	0.02675	0.154	1.458	0.01438	0.156
Soil History ~ Growth Stage	0.97479	0.05026	0.762	0.767	0.01514	0.724
Compartment ~ Soil History ~ Growth Stage	1.01258	0.07832	0.268	1.150	0.03402	0.296

[a], Values in bold indicate significant factors
[b], Rhizosphere or roots
[c], Test Phase growth stages: seed, seedling, rosette, bolting, or flower
[d], Soil history established the previous year: monocrop canola, wheat-canola rotation, or pea-barely-canola rotation


A
Conditioning Phase
Wheat
Canola
Rotation
Monocrop
Canola
Pea
Barley
Canola
+ agricultural treatments
t = 0
x4
Soil History
Test Phase
Brassica napus
t = 1
t = 2
…
t = 5
+ agricultural treatments
x4
Data
Harvested Compartments
Bulk Soil DNA
Seed DNA
Rhizosphere DNA
Root DNA
(MiSeq PE250)

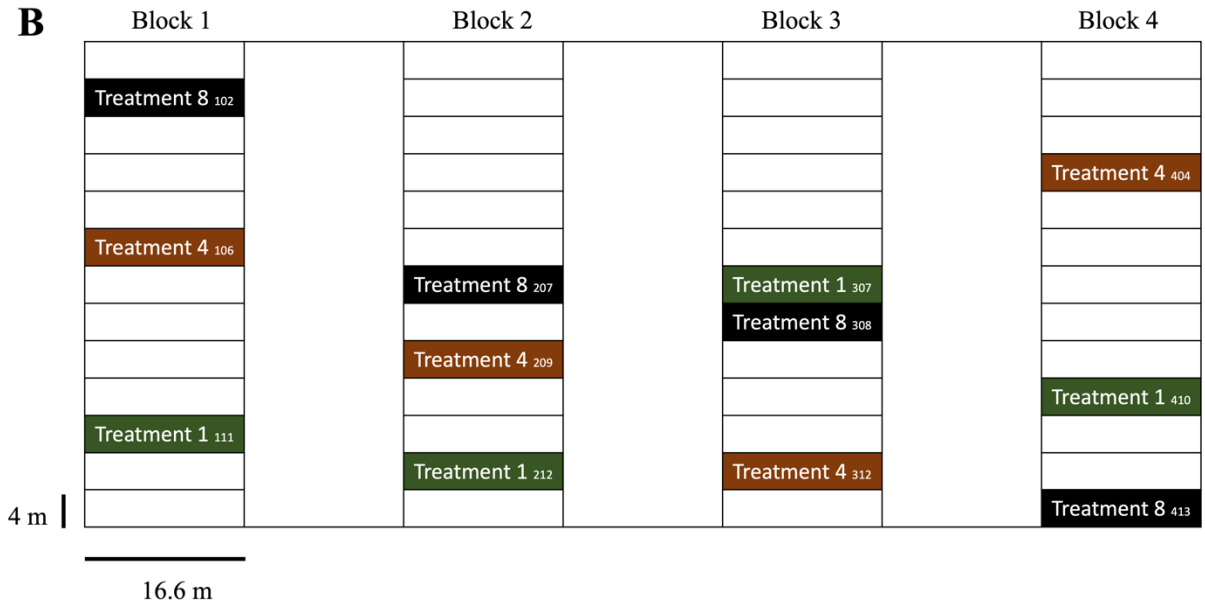

B
Block 1
Block 2
Block 3
Block 4
Treatment 8 102
Treatment 4 106
Treatment 1 111
Treatment 8 207
Treatment 4 209
Treatment 1 212
Treatment 1 307
Treatment 8 308
Treatment 4 312
Treatment 4 404
Treatment 1 410
Treatment 8 413
4 m
16.6 m

Figure S1. Conceptual design of the experiment. (A) Soil history was established during the growing season of 2018 (Conditioning Phase, t = 0), while the effect of soil history on *Brassica napus* bacterial rhizosphere and root communities, or associated bulk soil, was observed the following season, in 2019 (Test Phase, t = 1, 2, 3 … 5). Samples were harvested throughout the growing season at five growth stages; seed, seedling, rosette, bolting, and flower. (B) Field plan for the experiment. The experimental design was a split-plot replicated in four complete blocks. In the 'Conditioning Phase', three soil history treatments were randomly assigned: treatment 1 (green) another generation of canola (*B*. *napus* L., cv. L252LL) as a monocrop, treatment 4 (brown) spring wheat (*Triticum aestivum* cv. AAC Brandon), as the wheat phase of a two-year crop rotation with *B*. *napus*, and treatment 8 (black) barley (Hordeum vulgare cv. Canmore), as a three-year crop rotation with *B*. *napus* and pea (*Pisum sativum* L. cv AAC Lacombe). Plot numbers appear in subscript.

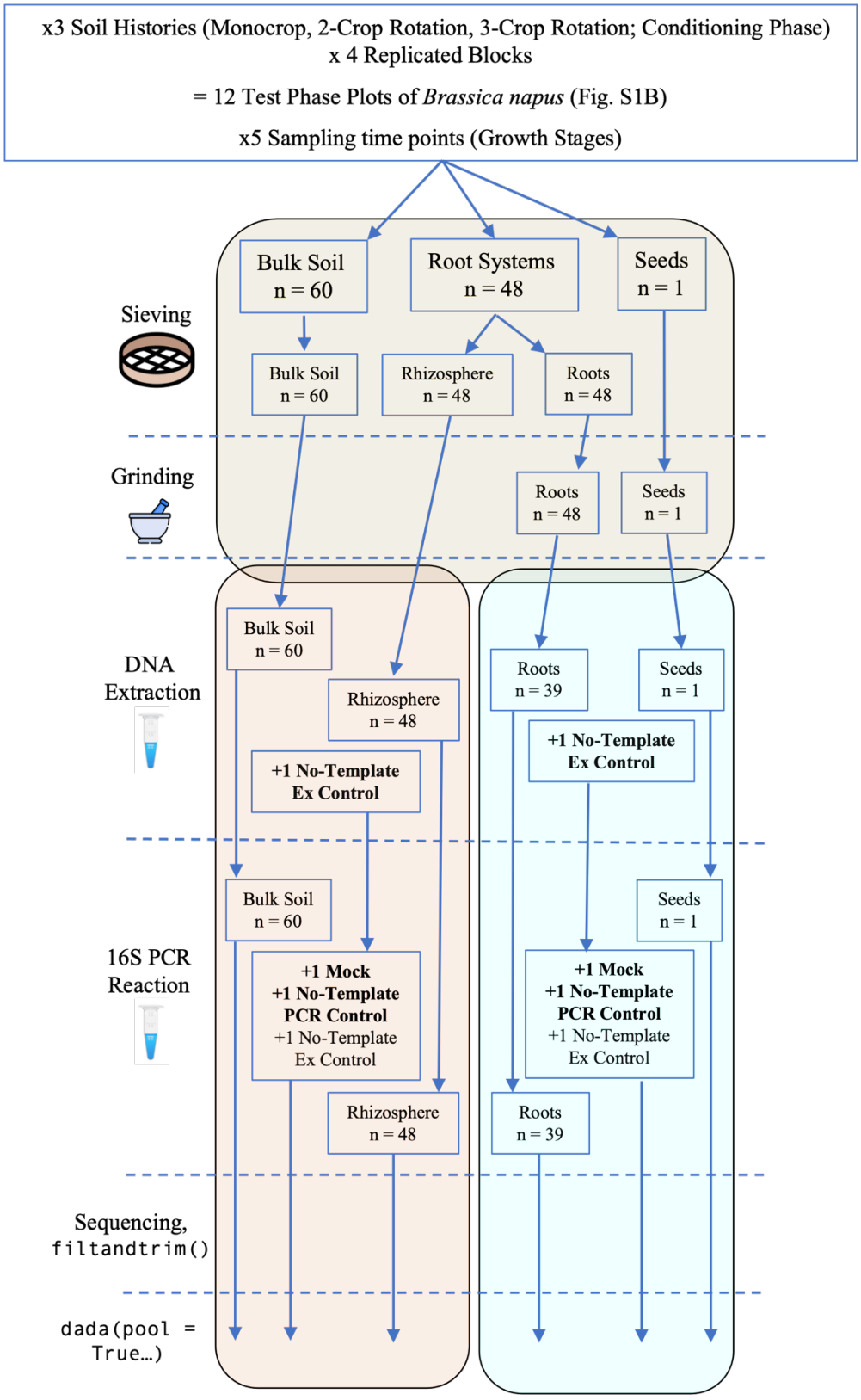

x3 Soil Histories (Monocrop, 2-Crop Rotation, 3-Crop Rotation; Conditioning Phase)
x 4 Replicated Blocks
= 12 Test Phase Plots of *Brassica napus* (Fig. S1B)
x5 Sampling time points (Growth Stages)
Bulk Soil n = 60
Root Systems n = 48
Seeds n = 1
Sieving
Bulk Soil n = 60
Rhizosphere n = 48
Roots n = 48
Grinding
Roots n = 48
Seeds n = 1
DNA Extraction
Bulk Soil n = 60
Rhizosphere n = 48
+1 No-Template Ex Control
Roots n = 39
Seeds n = 1
+1 No-Template Ex Control
16S PCR Reaction
Bulk Soil n = 60
+1 Mock +1 No-Template PCR Control +1 No-Template Ex Control
Rhizosphere n = 48
Seeds n = 1
+1 Mock +1 No-Template PCR Control +1 No-Template Ex Control
Roots n = 39
Sequencing, filtandtrim()
dada(pool = True…)

Figure S2. Organization of our lab workflow for the Test Phase *Brassicaceae* samples from harvest to generating amplicon sequence variants (ASVs). The Test Phase *Brassicaceae* samples were harvested in from 12 plots organized as split plots that were randomly assigned three soil history treatments (Fig S1B). Each plot was harvest at five growth stages (seed, seedling, rosette, bolting and flower); note the seed was all the same for each plot (n = 1). Bulk soil samples were taken from each plot at each growth stage (n = 60). In the field, each plant had its root systems and associated immediately flash-frozen in liquid nitrogen and kept on ice. In the lab, samples were sieved to remove debris (rocks, undecomposed straw, twigs etc…), and root systems were divided into rhizosphere and root samples (n = 48 each). Roots and seeds were then ground in liquid nitrogen, and DNA was extracted from all the Test Phase samples. No-template extraction controls were included to assess what contaminates, or biases, the extraction kits (Machery-Nagel Nucleospin Soil gDNA kit, at left in brown, and Qiagen Plant DNEasy kit in green) might impart.

We also included no-template PCR negative controls, and confirmed by gel electrophoresis that none of the no-template extraction controls, nor the no-template PCR controls, contained DNA. To help identify sequencing biases, or bath effects, a replicate of the bacterial mock community (BEI Resources, USA) was included on each plate submitted for sequencing. All DNA samples were submitted to Génome Québec for 16S rRNA PCR amplification, library preparation, and paired-end 250 bp Illumina MiSeq sequencing. All reads were subsequently trimmed and processed through the DADA2 pipeline for ASV inference.

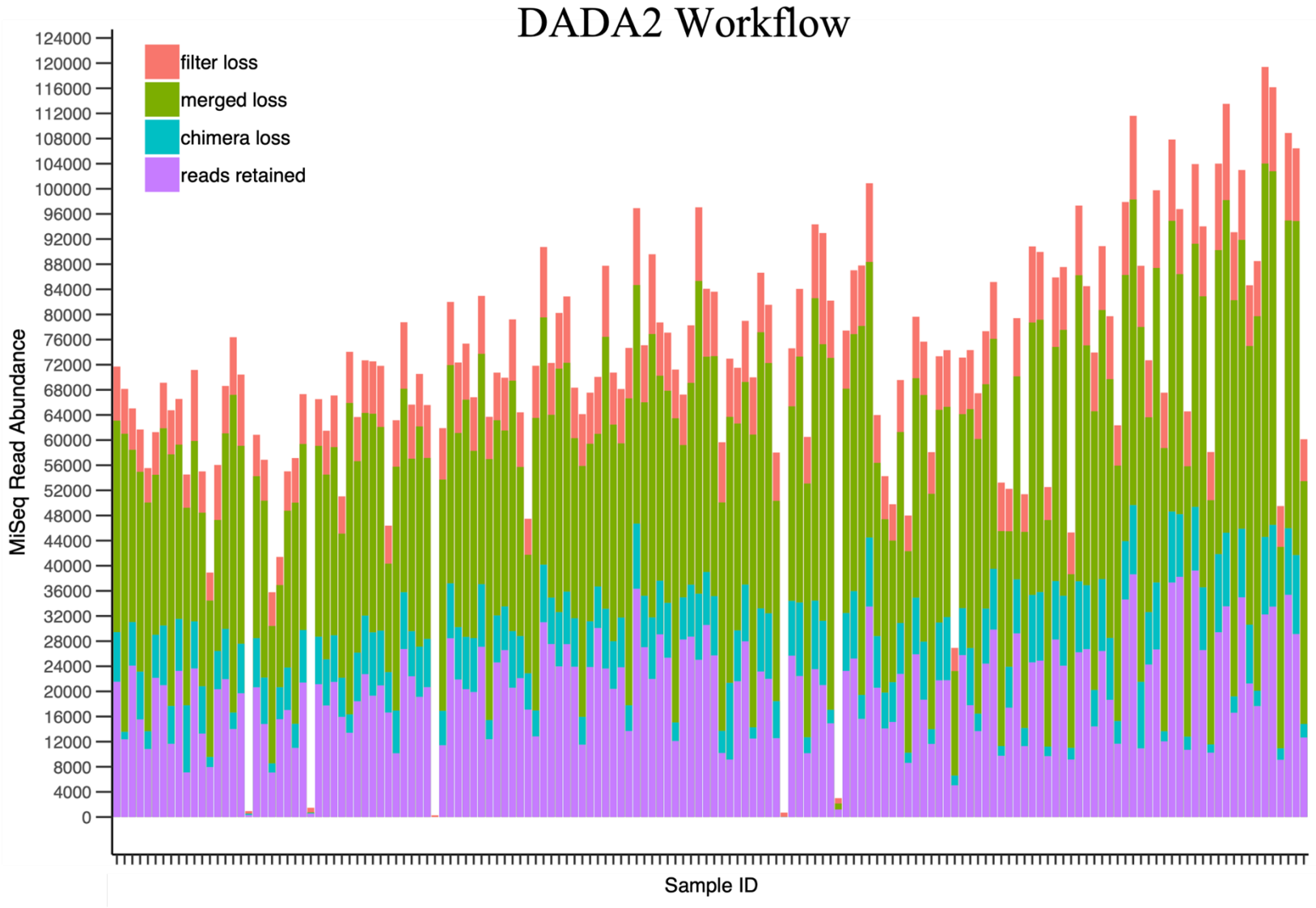

DADA2 Workflow
filter loss
merged loss
chimera loss
reads retained
MiSeq Read Abundance
124000
120000
116000
112000
108000
104000
100000
96000
92000
88000
84000
80000
76000
72000
68000
64000
60000
56000
52000
48000
44000
40000
36000
32000
28000
24000
20000
16000
12000
8000
4000
0
Sample ID

Figure S3. The DADA2 workflow processed 11 010 728 raw reads, produced from one lane of sequencing via Illumina's MiSeq at Génome Québec, and retained 2 770 390 reads which were used to infer amplicon sequence variants (ASVs).

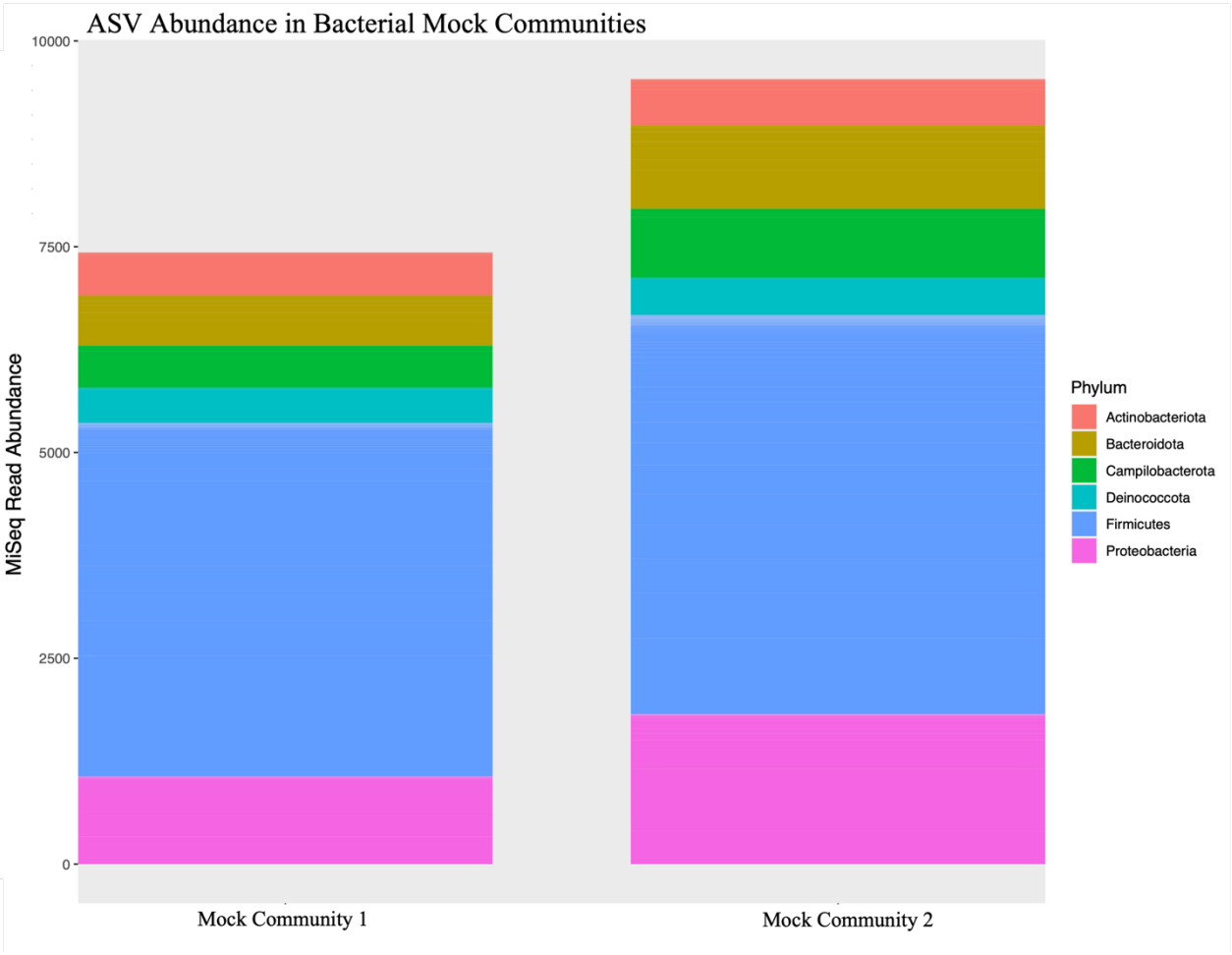


Figure S4. High-quality MiSeq reads retained through the DADA2 pipeline from among the mock community replicates, were inferred as inferred amplicon sequence variants (ASVs), and assigned taxonomy using the Silva database, represented here as phlya. Every bacterial group included in the mock community was detected in both replicates, and no others. The *Actinobacteria*, and *Deinococcus*, were the most accurate between what was included in the community, and retrieved in our pipeline. Community 2 showed an expansion among the ASVs identified as *Bacteriodetes*, *Campilobacterota*, *Firmicutes*, and *Proteobacteria*.

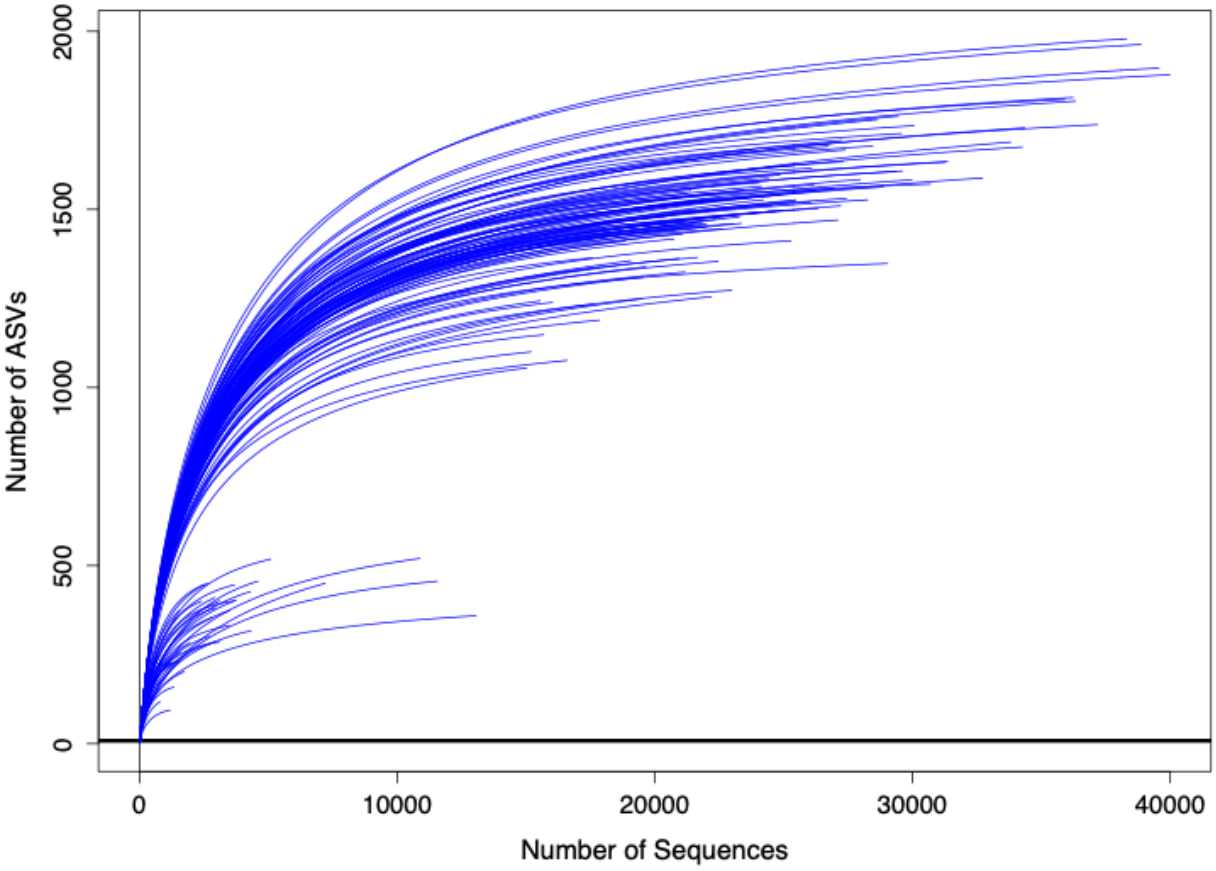


Figure S5. Rarefaction curves illustrated that the majority of the bacterial communities were identified in the samples harvested in 2019 from the bulk soil, rhizosphere, and roots, from five growth stages during the Test Phase of a multi-year crop rotation, in Lacombe, Alberta.

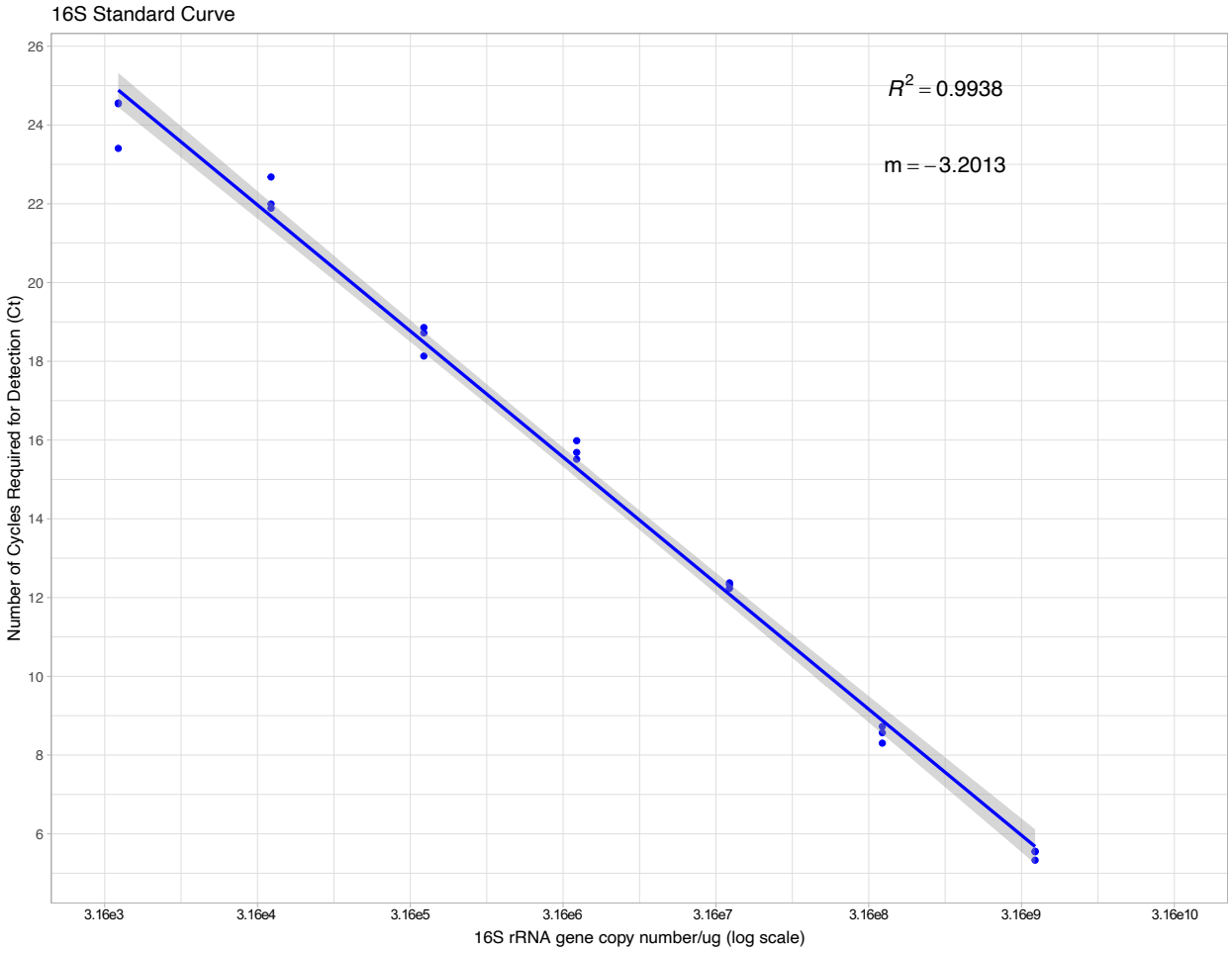


Figure S6. A standard curve of the 16S rRNA gene copy numbers (X-axis) versus the number of cycles required for detection (cycle threshold, Ct, Y-axis), as determined from the serial dilution of a quantified 16S rRNA gene.

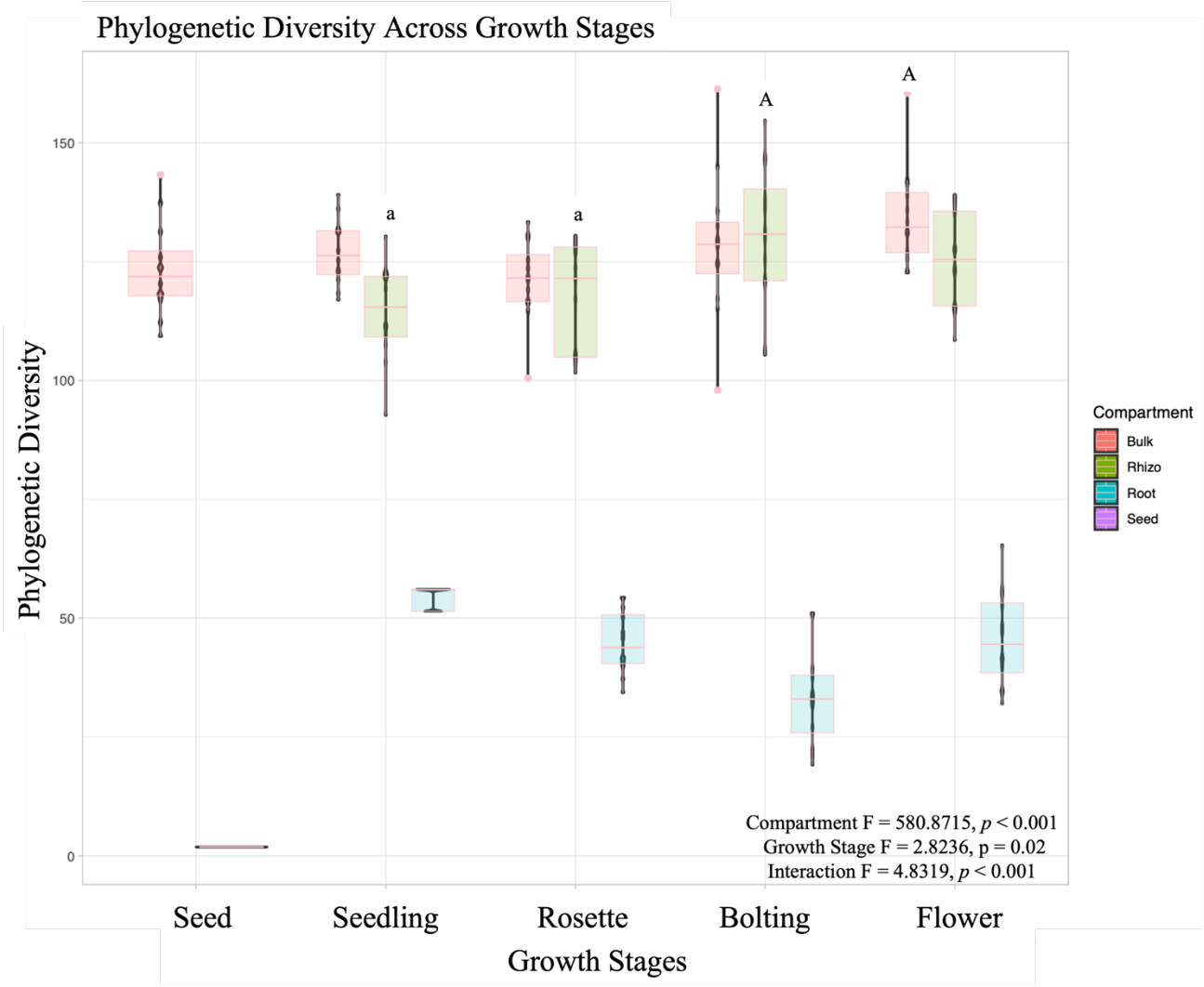
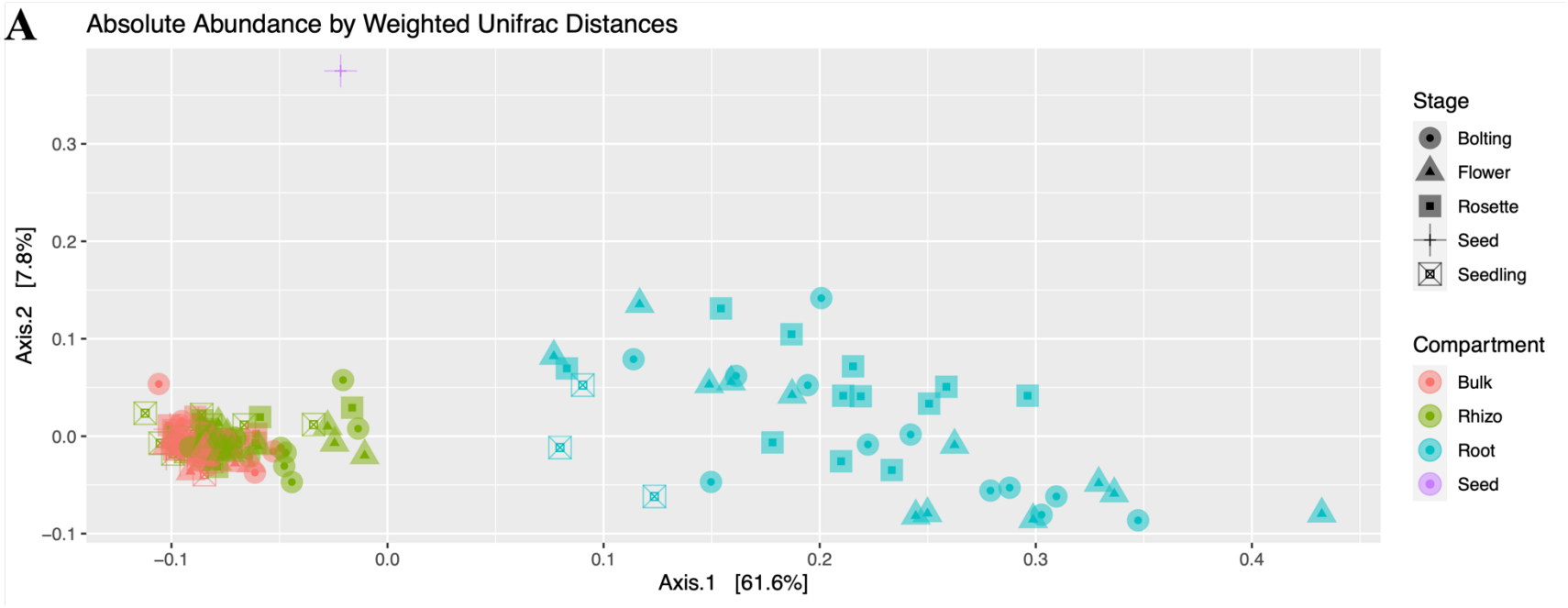

Phylogenetic Diversity Across Growth Stages
Phylogenetic Diversity
150
100
50
0
a
a
A
A
Compartment
Bulk
Rhizo
Root
Seed
Compartment F = 580.8715, p < 0.001
Growth Stage F = 2.8236, p = 0.02
Interaction F = 4.8319, p < 0.001
Seed
Seedling
Rosette
Bolting
Flower
Growth Stages

Figure S7. Phylogenetic diversity was significantly different between compartments ($p < 0.001$) in bacterial communities throughout the *Brassica napus* growing season. Root and seed communities were significantly different from each compartment across growth stages. Bulk soil and rhizosphere communities were strikingly similar across growth stages, except for the flower and bolting stage, respectively, which were both more diverse than the rhizosphere communities at the seedling and rosette stages. Diversity across growth stages was tested with a Multi-Factor ANOVA, which confirmed that the compartments and the Test Phase *B*. *napus* growth stages were significant and did interact. Statistically significant groups were identified using Tukey's *post hoc* test.


A
Absolute Abundance by Weighted Unifrac Distances
Axis.2 [7.8%]
0.3
0.2
0.1
0.0
−0.1
−0.1
0.0
0.1
0.2
0.3
0.4
Axis.1 [61.6%]
Stage
Bolting
Flower
Rosette
Seed
Seedling
Compartment
Bulk
Rhizo
Root
Seed

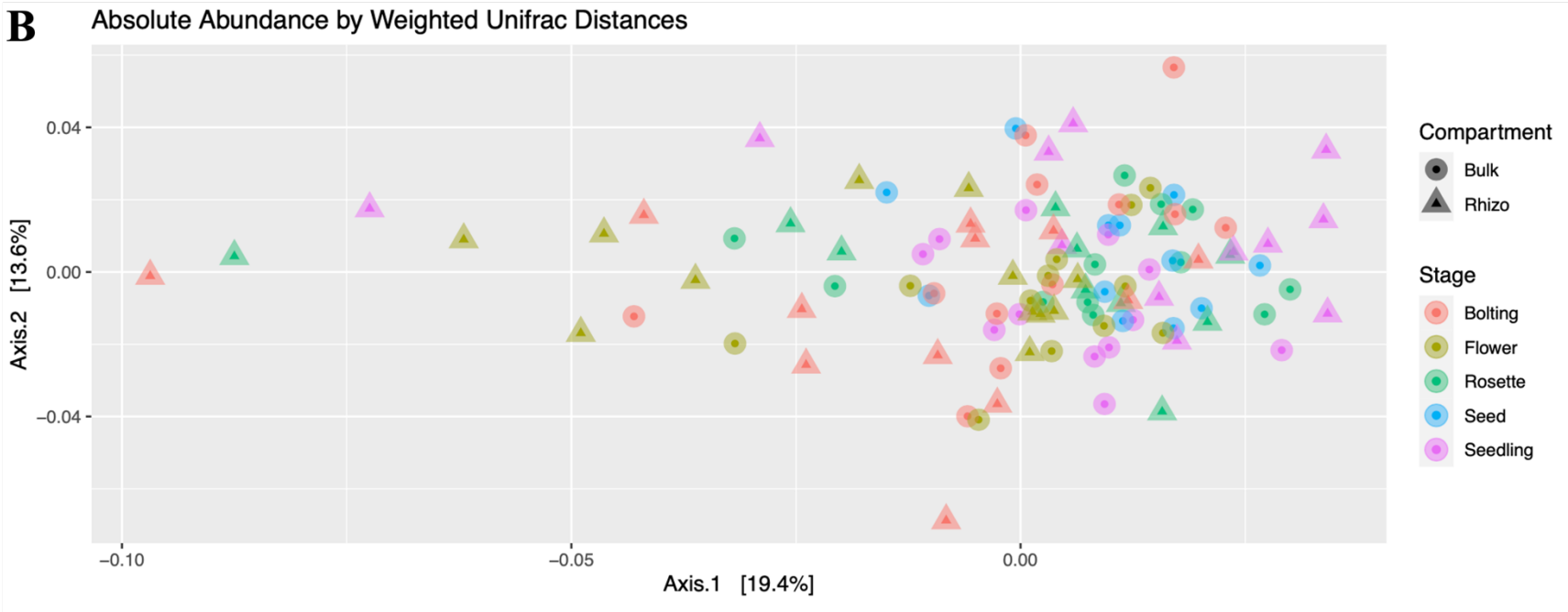
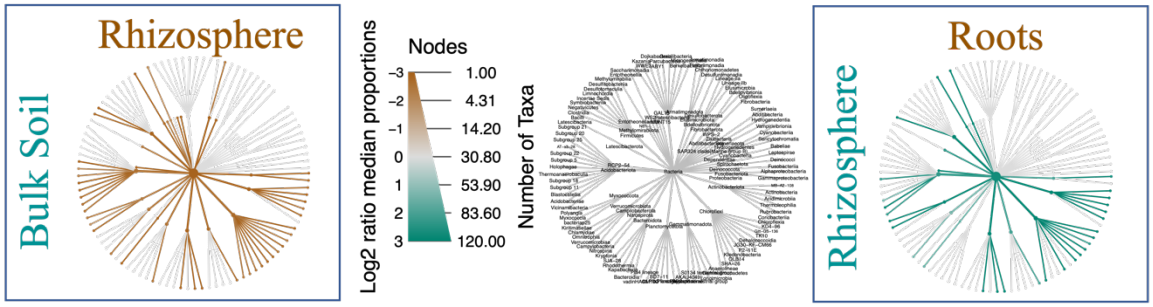
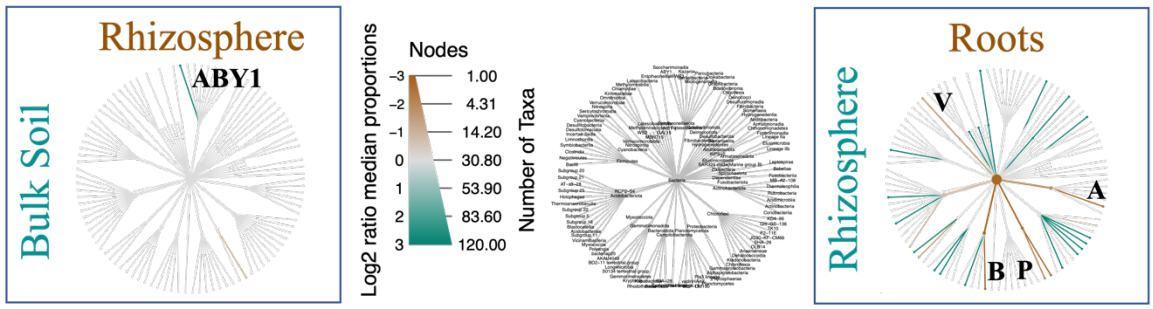

B
Absolute Abundance by Weighted Unifrac Distances
0.04
0.00
−0.04
Axis.2 [13.6%]
−0.10
−0.05
0.00
Axis.1 [19.4%]
Compartment
Bulk
Rhizo
Stage
Bolting
Flower
Rosette
Seed
Seedling

Figure S8. Bulk soil and rhizosphere bacterial communities were more phylogenetically similar than to root or seed communities. (A) Principal co-ordinate analysis illustrated that seed and root bacterial communities were phylogenetically distinct from bulk soil and rhizosphere communities. Bacterial root communities were more similar at the seedling and rosette stages, and appeared more diverse over time. Axis 1 and 2 captured 69.4% of the variability among the bacterial communities. (B) Bacterial communities from the bulk soil and rhizosphere were more phylogenetically similar at the seed and seedling stages, and increasingly diverse at following growth stages. Axis 1 and 2 captured 35% of the variability among the bacterial bulk soil and rhizosphere communities. Principal co-ordinate analysis were plotted using UniFrac distances weighted by absolute abundance, where phylogenetically similar communities were plotted closer together.

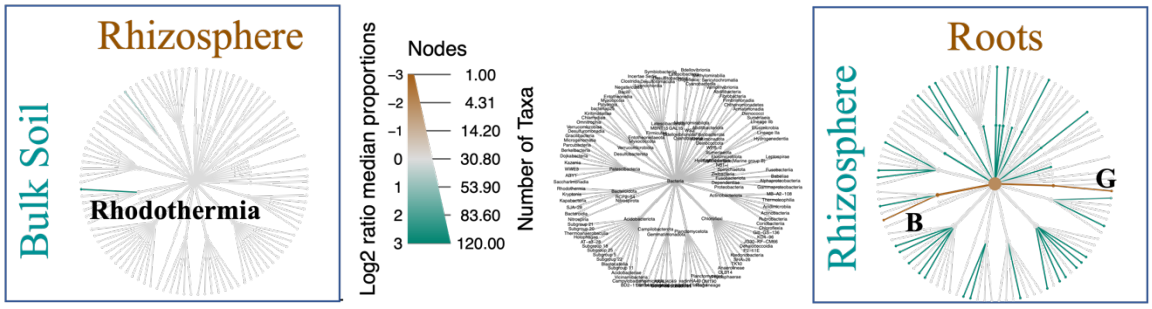
A Differential Abundance in Bacterial Seedling Communities ($p < 0.05$)



B Differential Abundance in Bacterial Rosette Communities ($p < 0.05$)



C Differential Abundance in Bacterial Bolting Communities ($p < 0.05$)



D Differential Abundance in Bacterial Flower Communities ($p < 0.05$)

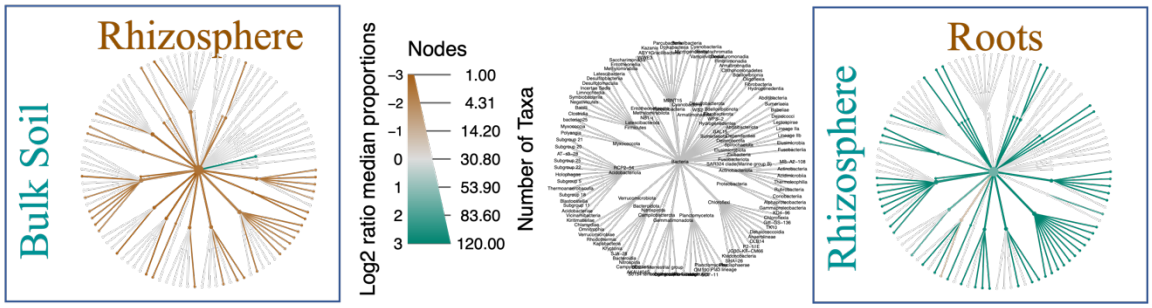


Figure S9. Specific bacterial taxa were significantly different between adjacent compartments at specific *Brassica napus* growth stages. (A) Bacterial rhizosphere communities at the seedling stage were enriched in taxa compared to the bulk soil and root communities. (B) At the rosette stage, bulk soil and rhizosphere communities were relatively similar, while both the rhizosphere and root communities were enriched in different taxa. (C) The bacterial communities in the bulk soil and rhizosphere were also similar at the bolting stage, though the the rhizosphere and root communities both exhibited specific enrichments in different taxa. (D) Bacterial rhizosphere communities were enriched in taxa compared to both the bulk soil and root communities. The abundance of each taxonomic group was compared between compartments, using the using the non-parametric Kruskal test and the *post hoc* pairwise Wilcox test, with the FDR correction. Taxa that were significantly (p. adj < 0.05) more abundant were highlighted.

General Conclusions

How current microbial communities impact future ones, and more broadly, how microbial communities vary through time, is a key knowledge gap in understanding how microbial communities assemble. Accounting for legacy, or historical, effects will be fundamental in developing accurate models of microbial community assembly and evolution, accounting for functional roles of communities, and for exploiting microbial technologies for use throughout society (Rittmann, 2006; De Vrieze *et al*., 2020; Gundersen *et al*., 2021), including medicine (Shafquat *et al*., 2014), waste management (Graham *et al*., 2004), and agriculture (Quiza *et al*., 2015). As such, the goal of my thesis was to investigate how soil history impacts future microbial communities in agroecosystems. By modelling soil history in agricultural rotations, and using metabarcoding to identify the microbial communities, I was able to test different hypotheses concerning the duration and impact of soil history in uncontrolled field conditions on soil bacterial and oomycetes.

In **Chapter 1**, I showed that bacterial communities with different soil histories converged toward similar phylogenetic compositions via the plant-soil microbial community feedback of their new *Brassicaceae* plant hosts. This was an interesting result, as it illustrated that three different soil histories could all be equally modified by the new plant hosts, and that despite variation in their agricultural treatments, the *Brassicaceae* hosts assembled phylogenetically similar bacterial communities from diverse soil histories. That the *Brassicaceae* host with the most distinct bacterial communities was also the least phylogenetically related among the five hosts is also fascinating, as it hints at the shared evolutionary history between host and communities. Moreover, the unplanned water-stressed conditions proved serendipitous, as it provided supporting evidence that active plant-soil community feedback processes drive the bacterial community changes between

host plants. This helped clarify that the bacterial communities were not just a result of variation between agricultural treatments, or the mere presence of the plant. The absence of an active feedback process also illustrated that soil history continued to shape the bacterial communities for up to a year after its establishment.

I also documented that soil history could continue to shape oomycetes communities for up to a year in **Chapter 2**. In this study, I found that the different soil histories had more significant influence on structuring the oomycete communities than the *Brassicaceae* plant hosts. Furthermore, soil chemistry actually appeared to be the primary factor in structuring the oomycete communities. This was particularly interesting since the oomycetes, and the bacterial communities reported in **Chapter 1,** were inferred from the same samples, and had to contend with similar edaphic factors. Yet, I found that these different microbial communities were influenced to varying degrees by soil chemistry, soil history, or their *Brassicaceae* hosts.

In **Chapter 3** I then investigated how previously established soil histories influenced the bacterial communities of *Brassica napus* throughout a growing season. Building on the data from the first experiment, I found that the different soil histories did continue to structure the bacterial rhizosphere communities across different growth stages, but had no influence on the root communities. This indicates that there are long-term components to soil history that are not degraded, or "re-written" by the host plant. Rather, the host plant masks the existing soil history, and derives a host-specific community from it. Furthermore, this data also suggested that the host plant assembles similar communities from diverse soil histories.

Synthesis

For Agricultural

Taken together, my thesis highlights a number of practical considerations for agricultural applications. First, as discussed, to date there has been little work on how previous crops and their agricultural inputs effect subsequent soil microbial communities (Lay *et al*., 2018). My data directly addresses this knowledge gap and suggests that different crops, and their established soil histories, have little impact on the subsequent *Brassicaceae* oilseed crops in establishing similar bacterial communities. This is a positive result for agriculture on several fronts including for crop breeding programs that disrupt soil microbial communities (Pérez-Jaramillo *et al*., 2018; Bailey-Serres *et al*., 2019; Taye *et al*., 2020), as well as issues regarding intensive practises degrading "soil health" (Yang *et al.*, 2020; Fierer *et al*., 2021).

In parallel with the concerns over how productive rotations and intensive agricultural practices have degraded local environments stems the idea that agriculture has also decreased the quality of the soil, or its health. Therefore, with little data available concerning the impact of Big Agriculture, and its overuse of fertilizers and pesticides, it has been assumed that the soil microbial communities have also been damaged along with the soil (Yang *et al.*, 2020; Moore *et al*., 2023). This inadvertent harm to the resident microbial communities may have negatively impacted their functionality in agricultural soils and the ecosystem services they provide (Yang *et al.*, 2020; Fierer *et al*., 2021). My data illustrates that, despite diverse agricultural treatments and sites, soil histories established by lentil, wheat, barley, canola, or fallow conditions, have little impact on diverse *Brassicaceae* oilseed crops establishing their own bacterial communities. That the diverse *Brassicaceae* hosts still established phylogenetically similar bacterial communities despite various

soil histories suggests that at least this fraction of the resident microbial community has not been irreparably harmed in these soils in terms of composition.

Another result of not sufficiently studying the soil microbial communities associated with plants has been the concern that crop domestication and on-going selection for yields may have inadvertently harmed the capacities of crop plants to form healthy microbial communities (Pérez-Jaramillo *et al*., 2018; Bailey-Serres *et al*., 2019; Taye *et al*., 2020). However, my data demonstrates that the different *Brassicaceae* hosts continue to assemble similar bacterial communities from a range of soil histories, while complementary agricultural studies from the same crop rotation experiment in Swift Current, Saskatchewan, has illustrated that these diverse *Brassicaceae* oilseed crops are also productive (Hossain *et al*., 2019; Liu *et al*., 2019). This suggests that the breeding of these oilseed crops has not come at the expense of degraded microbial communities.

Furthermore, there has been interest in diversifying *Brassicaceae* oilseed crops to expand breeding options for improving resistance to phytopathogens, as well as heat and drought-stress (Bailey-Serres *et al*., 2019; Hossain *et al*., 2019; Liu *et al*., 2019). My data highlights that during the drought event, the diverse *Brassicaceae* hosts shifted their root bacterial communities to include more *Actinobacteria*, as has been reported elsewhere for plants experiencing drought (Naylor *et al*., 2017; Santos-Medellín *et al*., 2017; Fitzpatrick *et al*., 2018). This suggests that the on-going breeding of these various *Brassicaceae* oilseed crops has not impaired this generic plant response to drought. In fact, this result provides an interesting foundation for further research into the drought response of the *Brassicaceae* oilseed holobiont and future avenues for breeding programs.

My investigation into the oomycete communities also has insights for breeding *Brassicaceae* oilseed crops with improved resistance to phytopathogens. My data suggests that *B*.

carinata and *S. alba* may be less able to prevent oomycete colonization compared to the other *Brassicaceae* crops. This should be further investigated and considered in future breeding programs. Concurrently, none of the different *Brassicaceae* crops appeared to restructure the oomycete communities, which indicates that these plant hosts had weaker selection on the rhizosphere, be it positive or negative, compared to the soil history. Therefore, this may indicate that producers ought to be cautious with these particular rotations, as they may establish optimal conditions for oomycete phytopathogens. On-going biomonitoring and quantification should be mandated given that oomycetes have been identified throughout the Canadian Prairies (Taheri *et al*., 2017a; Karppinen *et al*., 2020; Taheri *et al*., 2021).

However, I also identified a strategy to engineer greater resilience into bacterial communities, which could improve resistance to pathogens, or other biotic or abiotic perturbations (Loreau *et al*., 2021; Hong *et al*., 2022; Li *et al*., 2022). My data indicates that bacterial communities from soil histories with more diverse crop rotations had significantly increased phylogenetic diversity. From greater diversity, bacterial and other microbial communities tend to stock more genetic and functional variation in order to better adapt to changing conditions, as per the insurance theory (Loreau *et al*., 2021). Thus, agricultural producers should be shifting to greater crop diversity in an effort to engineer greater resilience into the soil microbial communities (Yang *et al.*, 2020; Moore *et al*., 2023).

For Plant-Soil Microbial Community Feedback

In my thesis, I demonstrated the use of crop rotation experiments to model how PSF established a soil history that would structure the subsequent plant-soil microbial community. This is an important step forward in studying PSF, as these studies have traditionally not been conducted in the field (van der Putten *et al*., 2013; Revillini *et al*., 2016; De Long *et al*., 2019; Chung, 2023). A strength of this approach is that it incorporates a full range of biotic and abiotic factors into the PSF, both above and below-ground (De Long *et al*., 2019). Moreover, through the use of different crop rotations, and their assortment of varying agricultural management practices, multiple soil histories were established and tested. Demonstrating that large-scale field experiments involve PSF and establish soil history is a key proof-of-concept, as exposing theories and ideas about PSF and soil history to "the real world" is an important step in building-up the model (Revillini *et al*., 2016; De Long *et al*., 2019).

Further to this, PSF is often characterised as unpredictable to detect, partially due to the high degree of variability among initial microbial communities (De Long *et al*., 2019; Van Nuland *et al*., 2019; De Long *et al*., 2023). However, my thesis tested multiple soil histories in two different field experiments, 600 km apart, over different years, which repeatedly demonstrated that soil history structured the subsequent bacterial and oomycete communities of *Brassicaceae* hosts. This is a robust, replicated test of various PSF (lentil, wheat, or barley) that each established a clear, detectable common soil history that structured the subsequent bacterial and oomycete communities. This occurred despite the complex biotic and abiotic interactions, including the multifaceted connections between different resident microbial communities (Van Nuland *et al*., 2019). Thus, my data suggests that PSF and the established soil history can be readily and consistently detected in field trials.

Not only do these replicated studies illustrate PSF can establish detectable soil histories in a field experiment, but they also demonstrate the primacy of the resident plant host in the hierarchy of forces involved in PSF (De Long *et al*., 2023). The resident *Brassicaceae* hosts incorporated numerous biotic and abiotic factors—variable initial microbial communities, different soil histories, and various management practices—into a coherent environmental filter and established phylogenetically consistent microbial communities in the rhizosphere. Compared to the previously established soil histories, or the different agricultural practices that were employed, the *Brassicaceae* plant hosts melded them together and established, or "re-wrote" a consistent filter for bacterial communities in the rhizosphere.

Conversely, my data illustrated that a strong abiotic factor, water stress, could nullify the resident plant's PSF, and reveal the previously established soil history. I also found that the resident *Brassicaceae* PSF does not apply equally to all microbes; the oomycete communities inferred from the same rhizosphere samples as their bacterial cognate communities were virtually unstructured by plant hosts. These observations both point to subtleties in the hierarchy of forces involved in PSF, as the resident plant host is not always preeminent according to, at least certain, biotic (i.e. different groups of microbes), and abiotic (i.e. water stress) factors (De Long *et al*., 2023). This is particularly useful to note, as these parameters can be tested in future experiments.

For example, if I had included metabarcodes of additional microbial groups, algae and fungi, or functional sub-group, such as mycorrhiza and phytopathogens, we could expect these groups to fall along a continuum of influence between the resident plant and the established soil history. Functionality of fungi, such as mutualists versus parasites, has been shown to be important in PSF, particularly whether the outcome for the plant host is beneficial or not (Geisen *et al*., 2022; Semchenko *et al*., 2022). Therefore, tracking how different microbial groups are influenced by the

hierarchy of forces involved in PSF could provide a better understanding of the spatiotemporal reach, or strength of these different parameters (De Long *et al*., 2023).

To date PSF and the soil history that is established is largely assumed to occur instantly, and little work has attempted to dissect how these processes occur through time (Chung, 2023). My Lacombe experiment starts to quantify PSF working through time from the "belowground-up", where the changes in bacterial communities can be indictors of the plant host's status, requirements, and potential influence (Bennett & Klironomos, 2018; Li *et al*., 2019). From the dynamics of various microbial communities across different developmental stages, we can develop a more nuanced and predictable vision of PSF, and derive clearer concepts of microbial community assembly (Chung, 2023; Martiny *et al*., 2023).

On Community Assembly

Returning to first principles, what do these data tell us about how previously established soil history impacts microbial community assembly? First, our data illustrates that different soil histories endure; they last for up to a year, are maintained through seasonal extremes, and are not degraded by the presence of a new host plant. As the Trial 2 data from **Chapter 1** and the results from **Chapter 3** show that the soil history remains intact and continues to influence the bacterial rhizosphere communities, even in the presence of a new plant host. This is more obvious in **Chapter 2** where the oomycete communities are continuously structured by the previously established soil histories, despite the presence of new host plants.

Furthermore, the elements responsible for soil history continually assembled consistent microbial communities, as we observed for the bacterial communities in the results from Trial 2 in **Chapter 1** and again in **Chapter 3**, as well as for the oomycete communities in **Chapter 2**.

Therefore, this suggests that the foundational components of soil history should be resilient against variation in the microbial community and should dominate any influence from dispersal, drift, or speciation. This suggests that the defining components of soil history should not be physiochemical in nature (e.g. pH, moisture, salinity, cation exchange capacity, total carbon, etc…) as this would be expected to change over the course of a year.

This points to some component of soil history that continually, and strongly, selects for similar microbial communities, or functions, through time. Broadly, this might be accomplished by decaying plant material; as it takes time to breakdown, this could provide a continued source of selection. More specifically, particular residual secondary metabolites from the plant-soil microbial community feedback process could play long-term roles in framing soil history (Zhang *et al*., 2023). In sufficient quantities, or stocked in decaying plant material, secondary metabolites could continually select for a common microbial community through time.

Furthermore, during the Test Phase of each experiment, the data demonstrated a number of fates for the previously established soil histories. In the presence of an active feedback process, the selective force of soil history was overtaken by selection from the host plant for bacterial rhizosphere communities. This could be tested to determine if the previously established soil history reasserts itself at the end of a growing season after the plant is removed. Conversely, in the absence of an active feedback process, soil history continued to dominate as a selective process in the rhizosphere (**Chapters 1** and **3**) and the bulk soil (**Chapter 3**). Nonetheless, the bacterial bulk soil communities also continued to change over the growing season similar to the rhizosphere communities. This might suggest that secondary non-selective forces, such as dispersal events through the air or water, are also active.

From this work it appears that soil history limits the influence of drift and speciation on microbial community assembly. In well provisioned agroecosystems, such as the research farms where these experiments were conducted, it seems less likely that microbial communities would experience more stochastic events where drift might play a critical role (Gundersen *et al*., 2021). Equally, given the relative stability of the different soil histories, it seems unlikely for speciation events to be influential. Though, it is possible that the introduction of a new host plant could be sufficient to drive speciation in the rhizosphere. Future metagenomic experiments may be better adapted to detect speciation events as genome-level changes in different samples, or populations, throughout a growing season.

Perspective

There are also outstanding questions, or limits, to the analysis of microbial communities in soils or other environments. First, it has been well documented that there are biases throughout metabarcoding pipelines, from soil to sequencing, and the necessity of striving to follow the best practices (Klindworth *et al*., 2013; Alteio *et al*., 2021; Lloréns-Rico *et al*., 2021; Tedersoo *et al*., 2022). Adapting new technologies is always accompanied by growing pains to establish appropriate protocols and new norms withing the scientific community vis à vis reporting data, including proper sampling, use of controls, ASV inference, taxonomic identification, and the use of functional assignments (Meyer *et al*., 2018; Fleishman *et al*., 2022; Roche & Mukherjee, 2022). As such, the onus is on the community to push for more rigorous standards at each step of the research process. Preregistered reports, peer community journals, and preprints may all help the research community avoid stagnating by setting rigorous practices.

Beyond these technically oriented limits, there are also several biological assumptions baked into metabarcoding, including questions about the value of which organisms are identified without contextual details, such as if they are still alive, dormant, their numbers or what functions they have. For example, nucleic acid isolation protocols do not discriminate between biologically packaged molecules, i.e. those inside cells and viral particles, versus "relic" nucleic acids, or those present in the environment, including the microbial necromass of lysed cells and particles (Sokol *et al*., 2022). This relic DNA has been reported to account for 50-70% of the obtained reads and artifactually increase the α and β diversity of studies (Carini *et al*., 2016; Bowsher *et al*., 2019).

However, as Lennon *et al*., (2017) point out, relic DNA bias is not universal, even when it is in high concentrations. Highly active and dynamic environments, such as agricultural soils, are thought to be less biased versus more stagnant samples, such as in biofilms or sediments (Carini *et al*., 2016; Lennon *et al*., 2017; Bowsher *et al*., 2019). Furthermore, since my data illustrated significant plant host-induced changes to the bacterial communities within a growing season in **Chapters 1** and **3**, it seems unlikely that relic DNA caused a significant bias. This is more difficult to assess for the oomycete communities, however, since there were fewer dynamic changes. Therefore, it could be possible that the strong soil history effect among the oomycete rhizosphere communities was due to relic DNA bias. Given this, future studies should make use of treatment options to limit the impact of relic DNA. Propidium monoazide, for example, can be used on environmental samples, where upon exposure to light it irreversibly intercalates into unprotected DNA strands preventing this pool of unprotected DNA from being PCR amplified (Carini *et al*., 2016; Emerson *et al*., 2017; Carini *et al*., 2020; Ouyang *et al*., 2021). Through these types of innovation, metabarcoding can avoid some the assumptions associated with it and increase its use and accuracy.

Additionally, there are a few key concepts that more microbial ecologists are calling for to strengthen the discipline and move it in from a descriptive "pre-science" toward a productive period of "normal science" (Kuhn, 1962). This includes, incorporating quantification, investigating functionality, and proposing testable and falsifiable hypotheses. For example, at its base, ecology asks what organisms are present and how many? Metabarcoding has been very useful for the former; at this point, the literature is replete with what organisms are present in the soil (Thompson *et al*., 2017), yet we still have a poor grasp of how many individuals there are (Roche & Mukherjee, 2022). Hence, the literature is increasingly articulating the need to quantify community sizes, or absolute abundances (Gu *et al*., 2022; Roche & Mukherjee, 2022).

Recall, however, that reporting the number of sequencing reads, or the percentage which were assigned to a particular ASV is not quantifying the size of the community (Smets *et al*., 2016; Gloor *et al*., 2017). Rather, the number of NGS reads obtained are a fixed value that has no biological relationship to the absolute abundance of a community (Alteio *et al*., 2021; Lloréns-Rico et al., 2021) Consequently, additional tools must be employed to estimate the total size of the community. Conveniently, however, there are already a number of strategies that exist to quantify microbes, such as qPCR, or flow cytometry (Alteio *et al*., 2021).

Therefore, I made it a point of my thesis to attempt to quantify the microbial communities studied. This was achieved for all the bacterial communities, and the oomycete mock community, but not for the oomycete experimental communities in **Chapter 2**, though the technical limitation of using oomycete-specific ITS primers on environmental samples has since been overcome (Foster *et al*., 2022). The lack of regular reporting of estimated community sizes in the literature had a clear impact on my interpretation of the data as there was simply less context with which to evaluate it. Therefore, although incorporating the qPCR data into my work was not as productive as it might

have been, doing so did help reveal gaps in our understanding which would have otherwise gone unnoticed (see **Chapter 2**). Moreover, the qPCR estimates did nothing to hinder my results, while publishing my protocol and data will help future experiments establish quantification as a norm.

A drawback, however, of whole sample quantification methods, like qPCR, is the lack of distinction between which organisms are active in the community, i.e. growing, versus those that are dormant. This is an important distinction since many soil microbes are actually dormant, such that the dominant portion of ASVs identified via metabarcoding may actually be alive, but inactive (Lennon & Jones, 2011; Blagodatskaya & Kuzyakov, 2013). Given that dormancy is a common and phylogenetically pervasive trait (Lennon & Jones, 2011; Blagodatskaya & Kuzyakov, 2013), it may be more relevant to pair metabarcode biodiversity surveys with estimates of the actively growing portion of the community and the total size of the community (Bowsher *et al*., 2019). This can be achieved through biochemical assays, by measuring the 16S rRNA transcription rate (Bowsher *et al*., 2019), or through the incorporation rate of isotopes (Orsi, 2022).

In the case of my data, quantifying the active portion of the bacterial and oomycete communities would have given a more detailed understanding of their dynamics. However, since there was only one time point sampled for the Swift Current experiment (**Chapters 1 & 2**), it is unclear if identifying the active portion of those communities would have changed the overall interpretation. More likely, additional fine-scale data would simply have further refined or focused the data for those chapters. Conversely, estimating the total size and the active portion of the bacterial communities from the Lacombe experiment (**Chapter 3**) would have been very enriching since there were multiple time points. This would have yielded a much more detailed understanding of how these communities changed across different growth stages.

This also highlights the importance of studying communities through time, and not just in snapshots (Chung, 2023). Through routine estimates of community size at different proximal time points we can begin to catalogue these growth dynamics. This missing data was particularly relevant for my oomycete data since the presence of potential phytopathogens is an insufficient diagnostic indicator. Given that density-dependence impacts so many biological processes, including infection, and plant-soil microbial community feedback, quantifying soil microbes will be fundamental to understanding community assembly and functionality (van der Putten *et al*., 2013; Chung, 2023).

Furthermore, in the descriptive phase of soil microbial ecology, it has been important to determine which taxa are present. In-line with taxa-centric thinking comes the view that knowing the taxa will elucidate knowing the functions; an assumption often seen with metabarcoding (Gundersen *et al*., 2021; Wicaksono *et al*., 2022). However, this is also a macro-ecological bias; microbiologists do not have agreed upon biological species concepts, which limits the use of taxa as units of functionality (Escalas *et al*., 2019; Martiny *et al*., 2023). Moreover, many microbes, bacteria in particular, can be phylogenetically identical, and yet possess different functions (Blakney & Patten, 2011; Gundersen *et al*., 2021). Thus, it has been proposed that a "microbial ecology without species" would be more useful, along with a shift to understanding and quantifying functionality (Escalas *et al*., 2019).

There are several methods available to investigate the functions of microbial communities and overcome the limits imposed by metabarcoding taxonomy surveys. First, potential functions of a community can be determined via a metagenomics approach, which can catalogue the known coding and non-coding genetic elements and their copy numbers (Hug & Co, 2018). This is also useful to observe phylogenetically divergent microbes that might be missed using metabarcoding

(Eloe-Fadrosh *et al*., 2016; Hug *et al*., 2016). For more specific questions and tighter budgets, targeted amplicon sequencing can determine if known functions are present in a microbial community (Hink *et al*., 2016). There are some disadvantages with these metagenomic strategies, however, including that they could be prone to relic DNA bias, as well as not accounting for active versus dormant microbes.

An alternative approach to estimate the functions of microbial communities would be to measure gene expression of the community with a metatranscriptomics strategy. By capturing what genes are being actively transcribed, or translated via ribosome profiling, these strategies have the advantage of detailing what the microbial community was actually doing near the moment of sampling (Grenga *et al*., 2020). Furthermore, they tend to limit the disadvantages of relic DNA and dormant microbes, unlike metagenomic strategies. Although metatranscriptomic approaches may be more technically challenging than comparing barcodes to a functional database, they can produce far more detailed results (Alteio *et al*., 2020).

These functional approaches would have no doubt produced very different datasets than what I have reported for the Swift Current and Lacombe experiments using metabarcoding. It would have been interesting to see if the community structure due to the plant hosts or water stress reflected different functions in the bacterial communities of **Chapter 1**. Also, knowing the potential or active functions of the oomycete communities may have helped interpret why they were so strongly determined by soil history and chemistry, but not the plant hosts. However, as discussed, since there was only one time point in the Swift Current experiment, having functional profiles would still only have given a singular snapshot, which could make interpreting why those functions were present, or active, difficult. Conversely, coupled with the multiple time points and compartments in the Lacombe experiment (**Chapter 3**), assessing the functionality of the different

bacterial communities would have been very productive. For example, that data showed that increased diversity in the soil history increased the phylogenetic diversity of the bacterial bulk soil and rhizosphere communities; therefore, observing more diverse bacterial functions would have considerably supported this point.

Moreover, focusing on the ecological patterns of functions, whether as gene content, or biochemistry, will deepen our understanding of holobiont theory and help re-invigorate uncovering the responsible mechanisms (Doolittle & Booth, 2017). Although given the ease of using metabarcoding, perhaps it should be unsurprising that incorporating biological mechanisms into microbial ecology is currently a challenge. In a meta-analysis of the five leading microbial ecology journals (Applied and Environmental Microbiology, Environmental Microbiology, FEMS Microbiology Ecology, ISME Journal and Microbial Ecology), approximately only 10% of the published articles in 2020 included an appropriate hypothesis i.e., testable, falsifiable, mechanistic (Prosser 2020; Prosser 2022). Since there is certainly no lack of microbial phenomena, proposing the how and why for a mechanistic explanation should not be so rare, or so difficult. As has been said “an accumulation of facts is no more a science than a heap of bricks is a house” (Poincaré, 1902).

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