MICROBIAL COMMUNITY ASSEMBLY PROCESSES IN AGRICULTURAL AND NATURAL ECOSYSTEMS

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ABSTRACT

Microbial communities can support plant and soil health through disease suppression, growth promotion, improving stress tolerance and increasing nutrient supply and cycling. The microbiome of crops has been suggested as a target to improve agricultural sustainability and crop performance. Targeted breeding efforts may allow for the selection of more robust crop microbiomes, lessening the need for additional agricultural inputs. However, how crop associated microbiomes assemble is still poorly understood. To more effectively engineer the microbiome, a more complete knowledge of the selection pressures that shape these communities over time and space is needed. Within this, an understanding of what is acting as biotic or abiotic filters and imposing these selection pressures is also needed. Once these aspects of microbial community assembly in crops are known, more effective selection of microbiomes can occur.

In addition to agriculture, a more complete understanding of soil microbial community assembly processes in natural ecosystems is needed. Invasive species are expected to expend in both range and abundance with climate change. Invasive species cause decreases in local plant diversity and alter local soil microbial communities and ecosystem services. The invasive plant will also disturb native microbial community assembly processes. Understanding how invasive species impact soil microbial community assembly processes and the associated ecosystem services will provide vital information to aid in restoration efforts. Furthermore, when restoration might not be feasible, understanding these processes will allow for more accurate predictions of how ecosystem services will change and the proper remediation planning can then occur. However, the knowledge of how invasive species affect assembly processes is still unclear.

This dissertation found that the primary factor affecting microbial community assembly processes in both agricultural and natural ecosystems was time. In the agricultural system, time was the growth stage of *Brassica napus*. In the native grassland, seasonal changes impacted bacterial and fungal assembly processes more than invasive biomass. Ecosystem services were also affected more by seasonal changes than invasive biomass. Given the annual cyclical nature of both plant growth stage and seasonal changes in a grassland, the patterns observed will likely endure from year to year.

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DEDICATION

This dissertation to dedicated to Marie Ruth Gregory Jordan. Love you always.

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ACE	Abundance-based Coverage Estimate
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ASV	Amplicon sequence variants
AAFC	Agriculture and Agri-Food Canada
βMNTD	Beta mean nearest taxon distance
βNTI	Beta Nearest Taxon Index
dbRDA	Distance-based redundancy analysis
DNA	Deoxyribonucleic acid
MNTD	mean nearest taxon distance
NCBI	National Center for Biotechnology Information
NAM	Nested Association Mapping
NRI	Net Relatedness Index
NTI	Nearest Taxon Index
PERMANOVA	Permutational multivariate analysis of variance
PCoA	principle-coordinates analysis
PNA	peptide nucleic acid
pPNA	plastid peptide nucleic acid
mPNA	mitochondrial peptide nucleic acid
RC_{bray}	Bray-Curtis-based Raup-Crick
rRNA	ribosomal ribonucleic acid
SAD	species abundance distribution
SAR	species-area relationships
VPA	Variance partitioning analysis
WAP	Weeks after planting

1. INTRODUCTION

Microbial community assembly processes can be broadly defined as how microbial communities grow and change over time and space. More specifically community assembly processes are what ecological processes shape the abundance and distribution of species within a community (Kraft and Ackerly 2014). We will need to have a more complete understanding of what shapes microbial abundances and distributions if we want to harness the microbiome more effectively for improved crop performance (Chaparro et al. 2012. Busby et al. 2017). Once we understand the shaping forces, we can manipulate them to select for more robust microbial communities. Additionally, a better understanding of how microbial communities respond to disturbance and alter their assembly processes in natural ecosystems could help improve remediation efforts (Van der Putten et al. 2013, Graham et al. 2016). Understanding how natural ecosystems compare to disturbed systems not only provides a baseline for remediation efforts, it will also provide a more complete understanding of how microbial communities operate in general.

One of the core concepts of community assembly is the regional species pool and the area for colonization (Kraft and Ackerly 2014). The regional species pool for the purpose of this dissertation is the source of microbes that colonize the plant, or the local soil microbial diversity. The area to be colonized is the *B. napus* plant, or the soil surrounding native grassland roots that is undergoing the disturbance of invasion. Once the colonists have arrived, various filters or processes act upon the colonists to select which species survive and thrive (Kraft and Ackerly 2014).

The filters or processes shaping the microbial communities can broadly be defined following Vellend's conceptual synthesis of community ecology (Vellend 2010). The first process is selection, which are deterministic processes what will change the composition from the regional species pool into the local species pool (Vellend 2010). Examples of well-studied selection pressures for microbial communities are habitat (soil vs human gut, Caporaso et al. 2011) and within those habitats, other factors such pH (Fierer and Jackson 2006) or soil characteristics such as C:N ratio (Kuramae et al. 2010). The second process is dispersal, or the movement of microbes across space (Vellend 2010). In microbial communities this process varies greatly from

macrobiotic communities as the primary dispersal mechanism for microbes is passive dispersal (Nemergut et al. 2013). The third process is drift, or random division, death or other stochastic changes in the relative abundance of community members (Vellend 2010). Microbial communities may be more subject to drift as most of the species in a community are present in low abundances leading to higher chances of local extinction through drift (Nemergut et al. 2013). The final process shaping microbial communities is diversification which is the generation of new genetic material within a community (Vellend 2010). In microbial communities the source of the new genetic information can occur through random mutation or horizontal gene transfer (Nemergut et al. 2013). Rates of diversification will be faster in microbial communities than macrobiotic communities due to the short generation time of most microbial species.

In this dissertation microbial community assembly processes will be examined at three levels of increasing complexity. The first and simplest system examined in the phyllosphere of eight lines of *B. napus* over ten weeks. The phyllosphere is the surface and interior of the leaf surface (Vorholt 2012). The phyllosphere is a harsh environment with large fluctuations in environmental conditions, high amounts of disturbance and low carbon availability (Vacher et al. 2016) Due to the harsh conditions, phyllosphere microbial communities are generally less diverse than soil communities and the assembly processes are likely to be less complex (Vorholt 2012, Vacher et al. 2016).

The second level of complexity is the roots and rhizosphere of eight lines *B. napus* over ten weeks. The rhizosphere is a more complex environment than the phyllosphere. Microbial communities in the rhizosphere are much more diverse than the communities found in the phyllosphere (Bulgarelli et al. 2013). More diversity will lead to more complex assembly processes due to increased inter- and intraspecific competition. In addition to increased diversity, there are many more carbon sources available for the microbial community in the rhizosphere, including soil organic matter and root exudates (Jones et al. 2009). The increased availability of carbon means that there are more potential niches than in the carbon limited phyllosphere. The increase in potential niches will also increase the complexity of the assembly processes. However, *B. napus* was planted in a monoculture, which is not reflective of natural ecosystems making this system less complex than a natural ecosystem.

The third and final level of complexity is a natural grassland undergoing invasion, primarily by the invasive grass *Bromus inermis*. The plant diversity in this study is much higher than the monoculture of *B. napus*. The quantity and quality of root exudates is highly plant species specific (Jones et al. 2009). The complexity of root exudate chemistry would increase with increasing plant diversity (Jones et al. 2009). Much like the increase in niches between the *B. napus* leaf and rhizosphere, we can expect a higher level of available niches in this system and thus more complex assembly processes. Smooth brome invasion provides a natural soil microbial community assembly experiment as it has shown to reduce plant diversity (Stotz et al. 2019). This reduction in plant diversity, as well as the introduction of smooth brome biomass, causes a disturbance between the invaded and native plots that helps elucidate the assembly processes. Not only can we examine the assembly processes in the native grassland, but we can also examine them in the disturbed plots, giving a better overall understanding of how assembly processes in natural ecosystems function.

1.1 Dissertation Organization

This dissertation is comprised of six chapters, including three research chapters that have been written for submission to peer-reviewed journals. Chapter 1 introduces the scientific questions addressed by this dissertation and the objectives of the dissertation. Chapter 2 covers the key literature and concepted for the following research chapters, namely what is microbial community assembly and how it is measured. Chapters 3 to 5 are the research chapters. Each chapter consists of preface abstract, material and methods, results and discussion section.

Chapter 3 focuses on the bacterial community composition in the phyllosphere of eight lines of *Brassica napus* over ten weeks and what influences the observed changes in composition. Chapter 4 examines the bacterial community assembly processes in the rhizosphere, roots and leaves of the same eight lines of *Brassica napus* over ten weeks. Chapter 5 looks at the soil bacterial and fungal assembly process in a native grassland undergoing invasion by *Bromus inermis*. This chapter also incorporates five ecosystem services and examines how they are impacted by both invasion and assembly process. Chapter 6 is a synthesis of the findings and future research directions.

2. Literature Review

2.1 Determinants of Microbial Diversity

Microbial ecology is a relatively young field that has recently boomed due to the advent of next generation sequencing (Widder et al. 2016). To date, most research into microbial ecology has focused on microbial community structure and function, or in other words, what microbes are there and what they are doing there. For example, we know that every surface of the plant is colonized by microorganisms and that these microbial communities can provide many benefits to the plant (Bulgarelli et al. 2013). Microbial communities can protect the host plant from disease (Newton et al. 2010), increase nutrient supply (Jones et al. 2009), improve stress tolerance (Piccoli and Bottini 2013) and improve overall plant productivity (Gopalakrishnan et al. 2015). However, despite this close relationship with plant, microbial communities are subject to their own evolutionary processes and life histories, but little is known about how or why plant associated microbial communities change throughout the growth of the plant and with differing space (Coyte et al. 2021).

Much like macrobiotic communities, we know that microbial communities are shaped by their environment, but unlike many larger organisms, microbial communities are also shaped by their host (Trivdei et al. 2020). Host genetics, or plant variety can influence the microbial community associated with that plant. For example, crop variety has been shown repeatedly to impact rhizosphere communities (Edwards et al. 2015, Colemam-Derr et al. 2016, Dombrowski et al. 2017). However, this is not consistent throughout the plant, as Bell et al. (2020) found that B. napus line had little to no effect on the bacterial community present in the phyllosphere. The effect of host genotype is not limited to just crop variety. Wagner et al. (2016) found that the host genotype of *Boechera stricta*, a wild perennial mustard, shaped both the leaf and root microbiome.

Host genotype will also affect host root morphology and root exudation patterns, both of which will shape the microbial community throughout the plant. Differences in root morphology can cause differences in water and nutrient uptake, both of which will affect the structure and function of microbial communities in the rhizosphere. These difference in water and nutrient uptake will also inevitably affect aboveground plant tissues and thus the associated microbial communities associated with these plant tissues (Toju et al. 2019).

Differences in plant variety and root structure will also alter root exudation patterns. Root exudates are the primary driver of microbial recruitment and retention and will vary between plant species or varities (Haichar et al. 2014). Additionally, we know that root exudate patterns can vary with plant development stage (Haichar et al. 2014), which is the likely cause of the variation in rhizosphere communities with plant development stage that has been well documented (Smalla et al. 2001, Wagner et al. 2014, Wagner et al. 2016, Copeland et al. 2015, Hilton et al. 2017).

Land use as well as abiotic stress are additional factors shaping microbial community composition. Microbial community structure varies with organic and conventional agriculture (Hartman et al. 2015, Jangid et al. 2008), fertilizer inputs both in agricultural ecosystems and grassland ecosystems (Leff et al. 2015, Ramirez et al. 2012) and different tillage regimes (Mbuthia et al. 2015). In natural systems, soil microbial communities vary with grazing (Yang et al. 2012), and forest sites have demonstrated lower diversity than pasture or agricultural sites (Mendes et al. 2015). Drought or other alterations change soil microbial communities and the resulting local biogeochemistry (Nielsen and Ball 2015). In addition to drought, plants can alter their microbial community when experiencing nutrient deficiencies (Fabiańska 2019). Finally, wildfires alter both the structure and function of soil microbial communities (Ferrenberg et al. 2013).

While we know some of the influences on the structure and function of microbial communities, we still lack an understanding of the ecological processes and selection pressures that cause the observed variations. With the increasing intensity of global climate change it is becoming increasingly pressing to understand how microbial communities assemble. In order to understand how natural soil microbial communities will change due to the disturbances associated with climate change, we must elucidate the microbial community assembly processes in systems undergoing invasion now. Finally, with the population expected to expand greatly while the climate rapidly changes, we will need to make agriculture both more productive and sustainable (Gilbert et al. 2010, Ryan et al. 2009). Targeting the microbiome of crops can help provide more productive and sustainable agriculture (Coleman-Derr and Tringe 2014). However, we must first gain a better understanding of the microbial community assembly processes occurring in crops in order to target the microbiomes more efficiently.

2.2 Microbial Community Assembly Processes

Microbial communities can, and should, be thought of as exactly that, a community. In any community, the passing of time and differences in space will affect how the community grows and changes. The factors that affect these differences are called microbial community assembly processes. Historically, much of the work on community assembly processes has taken place in plant and animal communities (Martiny et al. 2006, Vellend 2010). However, in the last few years there has been a push to apply these same principles to microbial communities because just like macrobiotic communities, microbial communities interact with the biotic and abiotic components of their environment (Trivedi et al. 2020), undergo selection processes (Evans et al. 2017) and experience both intra- and interspecific competition (Freilich et al. 2011). Microbial communities follow many of the same biogeographic patterns as macrobiotic communities (Martiny et al. 2006), however the ecological rules determining these patterns remain understudied and are likely to differ from macrobiotic communities in several important ways (Nemergut 2013).

It is unlikely that microbial communities assemble in the same ways that plant and animal communities do because microbes are subject to different selection pressures, ecological processes and have a much larger distribution of life histories (Nemergut 2013). Microbes are phenotypically plastic and experience horizontal gene transfer, both of which allow for quick responses to a changing environment. This, coupled with short generation times, allows for rapid evolution of microbial communities, which macrobiotic communities do not experience. Furthermore, while microbes do not actively disperse, they still experience near limitless dispersion due to their small size and ubiquity in all systems, suggesting that differences in microbial community composition are likely due to environmental selection (Martiny 2006). An example of this was found by Fierer and Jackson (2006) where the largest determinant of microbial community richness in soils was pH, not latitude which is the primary driver of macrobiotic community richness. A better understanding of how microbial communities assemble and what environmental factors drive this assembly process is needed.

Assembly processes in microbial communities can be split between two board ecological selection processes: deterministic and stochastic. Deterministic selection can be thought of as niche-based processes (Chase and Myers 2011, Dumbrell et al. 2010, Vellend 2010). These are the non-random selection pressures, such as niche availability, environmental variables or

inter/intra-specific competition that shape the structure of a community (Chase and Myers 2011). Within deterministic processes where are several sub-categories, namely homogeneous selection and heterogenous selection. Homogeneous selection leads to communities that are more similar compositions whereas heterogenous selection will lead to more distinct communities (Stegen et al. 2012, 2015). Stochastic processes are processes that are more random than factors influencing deterministic processes. These random events can be disturbance related, dispersal events, both homogenous dispersal (no limitation in dispersal) and dispersal limitation, as well as genetic drift (Chase and Myers 2011). Genetic drift is the random death, division or mutation of individual community members. Together, both deterministic and stochastic processes will shape the composition of microbial communities.

2.3 Metrics of Microbial Community Assembly Processes

Traditionally microbial community assembly processes have been examined using a variety of statistical techniques, primarily multivariate statistic. The most straightforward of these methods are comparing microbial community structure between various treatments using such methods like permutational multivariate analysis of variance (PERMANOVAs) or ordination methods like principle-coordinates analysis (PCoA) (Zhou and Ning 2017). Significant differences in treatments can be interpreted as differences in selection pressures, however this method only indirectly measures selection and assembly processes. Neutral models like species-area relationships (SARs) or species abundance distribution (SADs) are other methods that have been employed (Matthews and Whittaker 2014). However, these models have unrealistic assumptions that assume no deterministic selection is occurring and community composition is determined by stochastic processes alone. Variance partitioning analysis (VPA) is another popular approach to look at assembly processes (Smith and Lundholm 2010). This analysis determines that all accounted for variance arises due to deterministic processes and all unaccounted-for variance arise from stochastic processes. However, it not possible to measure all environmental variables, so it is highly likely that at least some of this undetermined variance is actually deterministic selection.

A useful tool in untangling assembly processes that is more comprehensive than the previously mentioned methods is to use a null-model framework (Kembel et al. 2009, Stegen et al. 2012, 2013, 2015). These frameworks use observed microbial phylogenies that are repeatedly randomized to give a theoretical phylogeny that would occur in the absence of any selection

pressures (Webb 2000). Ecological selection is occurring if the observe phylogeny falls more than two standard deviations outside of the theoretical phylogeny (Stegen et al. 2015). Not only can this process allow for the estimation of the selection processes occurring within a community (Stegen et al. 2012, Dini-Andreote et al. 2015) it can also give an indication of the strength of the selection pressures as the farther the community falls outside of theoretical phylogeny, the stronger the selection pressures. There are two different ways to calculate community relatedness based on phylogenies. The Nearest Taxon Index (NTI) is the mean nearest nodal distance for a given phylogeny whereas the Net Relatedness Index (NRI) is the mean pairwise distance for the same phylogeny (Webb 2000). While these two metrics are similar, NRI is more sensitive to fundamental shifts in community structure and NTI is more sensitive to changes near the edges of the phylogeny, or in other words, individual species abundances (Webb 2000). An NTI/NRI value of -2 indicates that the community is phylogenetically more clustered than expected whereas a value of +2 indicates that the community more phylogenetically dispersed the expected.

The NTI metric can also be used to look at beta diversity. βNTI is the phylogenetic turnover in a microbial community. Similar to NRI/NTI, βNTI utilizes multiple iterations and a null distribution of the local phylogeny. $|\beta NTI| > 2$ indicate that the community is undergoing deterministic selection processes. βNTI values that are greater than two indicate that the deterministic process is heterogenous selection, or that a more diverse community is being selected for (Stegen et al. 2013). βNTI values that are less than negative two indicate that the community is undergoing homogeneous selection, or that the community will be more similar than expected by chance. Stochastic processes are indicated by $|\beta NTI| < 2$. In these instances, it is useful to use the Bray-Curtis-based Raup-Crick (RC_{bray}) index. This index is the probability that any given sample has the same composition as other samples. Much like βNTI, it uses successive iterations to create a null distribution and compares the actual abundances of AVSs to the null hypothesis. This metric can be used to estimate the type of dispersal occurring in that community (Ning et al. 2020). Observations with values $|\beta NTI| < 2$ and $RC_{bray} > +0.95$ were classified as dispersal limitation and $|\beta NTI| < 2$ and $RC_{bray} < -0.95$ classified as homogenizing dispersal (Ning et al. 2020, Lin et al. 2012, Vellend 2010). Pairwise observations not falling within the constraints of $|\beta NTI| < 2$ or $|RC_{bray}| < 0.95$ were categorized as drift. Drift in the

context of microbial communities is the random division, death or mutation of individual members of the community.

The use of microbial phylogenies to determined microbial community assembly processes is a fairly recent innovation (Stegen et al. 2012, Zhou and Ning 2017). Like previously used methods, phylogeny based null-model frameworks are not without drawbacks. The largest of these drawbacks is related to sampling effort. The null-model created relies on the regional species pool (Webb 2000) which is directly related to sampling effort. This also means that these metrics are not directly comparable to other studies as the sampling effort and regional species pool will not be the same. The second drawback to phylogeny based null-model frameworks is that it relies on the assumption that short sequencing reads can accurately depict microbial traits related to fitness based on the constructed phylogeny. Horizontal gene transfer can homogenize the traits in a given community, however, many bacterial traits that confer specific advantages such as methanogenesis or other unusual metabolism strategies are deeply conserved (Martiny et al. 2015). Finally, given the difficulty of sequencing and constructing fungal phylogenies, the same assumptions of trait conservation are not as robust nor are most surveys of fungal diversity complete (Blackwell et al. 2011). However, despite its limitation, the null-model framework remains the best current measure of microbial community assembly processes.

The coupling of the βNTI and the RC_{bray} metrics allows for the estimation of the types of ecological assembly processes that are occurring in a given community. Previous methods only allowed for a very course estimation of these assembly processes. Additionally, these metrics can be combined with traditional statistical methods for analyzing microbial communities which can give an approximation of the influences on these assembly processes (Stegen et al. 2012, 2013, 2015, Bao et al. 2020, Ning et al. 2020). The knowledge of what type of assembly processes are occurring could allow for the better approximation of how future microbial communities will assemble. If it is known what selection pressures and the external factors that influence those pressures a future community will face, this may allow for more targeted microbiome manipulation through plant breeding or restoration efforts.

2.4 Assembly Processes in Agriculture

Most agricultural ecosystems are planted in monocultures, making them a simpler system in which to study microbial community assembly processes than natural ecosystems. The

domestication of plants had led to an overall reduction in soil microbial diversity with mycorrhizal symbiosis being particularly effected (Pérez-Jaramillo et al. 2016, Mariotte et al. 2018). However, this decreased soil microbial diversity and monoculture structure of agriculture has proved to be an effective system in which to study microbial community assembly processes. For example, as previously mentioned, we know that plant genotype can influence community structure and therefore assembly processes (Edwards et al. 2015, Colemam-Derr et al. 2016, Dombrowski et al. 2017). Along with plant genotype, manipulating plant function traits to examine their effect on assembly processes is much more straightforward in agriculture (Wood et al. 2009). Given that plant species richness is much higher in natural ecosystem it is often difficult to differentiate the rhizosphere of one plant species from another. Agriculture has also allowed for the close study of how plants recruit microbes in the field (Pérez-Jaramillo et al. 2016) which is an important aspect in microbial community assembly processes. Nutrient input studies are a common theme in agricultural experiments and have played an important role in analyzing how microbial communities assemble under different soil nutrient conditions (Mariotte et al. 2018, Hartman et al. 2015, Jangid et al. 2008). Due to the reduced complexity and easier manipulation of the system, agricultural experiments provide excellent opportunities to study microbial community assembly processes.

Assembly processes in agriculture can vary significantly, much like community composition. Jiao et al. (2020) found that community assembly was dominated by species sorting and dispersal in both maize and rice fields in Eastern China at high altitudes, but the effect weakened at lower altitudes. Lurie et al. (2015) found assembly processes differed on the root interior, root surface and the rhizosphere of rice within the same plant and also that assembly differed by rice genotype as well as soil source. In another study with rice, Edwards et al. (2015) found that assembly varied by field location and agricultural practice (conventional or organic). Carvalho et al. (2020) found that phyllosphere assembly processes in citrus trees also varied between organic and conventional practices. The study of microbial community assembly processes in agricultural systems is relatively new but is needed as we continue to unravel the microbiomes of crops and their potential manipulation.

2.5 The Canola Microbiome

Work on the canola microbiome has focused primarily on the roots and rhizosphere (Smalla et al. 2001, Copeland et al. 2015, Cordero et al. 2020, Gopalakrishnan et al. 2015, Schlatter et al. 2019, Glaeser et al. 2020, Taye et al. 2020), or on cultured microbial isolates (Ramero et al. 2019) or pathogens from *Brassica* species, and not on the microbiome as a whole (Wassermann et al. 2017). Copeland et al. (2015) is the only study examining the phyllosphere microbiome in addition to the rhizosphere microbiome. They found that the canola microbiome diversity varied seasonally and with rainfall. They also found that the canola phyllosphere was distinct from the rhizosphere. The canola microbiome varies with soil type or sampling location (Codero et al. 2020, Schlatter et al. 2019). Sampling time, growth stage and seasonality are important factors in shaping the canola microbiome as well (Smalla et al. 2001, Schlatter et al. 2019, Copeland et al. 2015). Canola variety inconsistent influences bacterial community composition (Copeland et al. 2015, Taye et al. 2020, Bell et al. 2021).

Given previous findings, sampling site as well as growth stage (or seasonality) are the most plausible influences on microbial community assembly processes in canola. However, is it improbable that all of the bacterial communities associated with canola assemble in the same manner. The phyllosphere environment is a harsh environment with large daily fluctuations in temperature, moisture and UV radiation (Vorholt 2012, Vacher et al. 2016). These factors will cause very different selection pressures than those occurring in the root and rhizosphere which is a comparatively protected environment. In order to work with breeders to create canola with a more robust microbiome, we need to first have a better understanding of the assembly processes occurring in the canola microbiome.

2.6 The Soil Microbiome and Invasion

Invasive species cause over $130 billion in economic damage and loss annually (Pejchar and Mooney 2009) and this impact is predicted to increase with global climate change (Vilá et al. 2011). For example, the United States government spent an estimated $3 billion dollars on the removal, remediation and control of invasive species (Crafton and Angadjivand 2018). Additionally, invasive species drastically alter the ecosystem services which are broadly defined as the benefits human derive from natural ecosystems (Daily et al. 1997). An example of an

invasive species that has had far reaching effects of ecosystem services is the invasion of cheatgrass (*Bromus tectorum*) in the semi-arid grasslands of North America. Like many invasive plants, cheatgrass decreases native plant diversity and abundance, disrupts grazing, increases fire frequency and intensity, reduces soil microbial community diversity and abundance and makes the soil more susceptible to erosion (Knapp 1996, Lekberg et al.2013).

Changes in plant species composition will change the quantity and quality of root exudates to the soil (Ehrenfeld 2003, Van der Putten et al. 2007, Knapp and Kovács 2012), and alter rates of nutrient cycling (Mack and D'Antonio 1998, Mack et al. 2002, Fraterrigo et al. 2010) as well as change inputs of organic matter to the soil (Ehrenfeld 2003, Suding et al. 2013). Despite an increasing knowledge of how invasive plants change plant-soil feedbacks less is known about how these changes impact local assembly processes (van der Putten et al. 2007, Suding et al. 2013). However, changes in the chemical and physical properties of soil, as well as in litter and exudate inputs, are almost certain to impact microbial community assembly processes and community composition, which in turn, could give the invading plant species a competitive advantage over native plants, perpetuating the invasion and hindering restoration efforts.

Smooth brome is commonly planted as a forage grass despite being an invasive species that has been shown to drastically reduce local plant diversity and alter soil properties (Slopek and Lamb 2017, Bahm et al. 2011, Salesman and Thomsen 2011, Stoz et al. 2019, Otfinowski et al. 2007, Chagnon et al. 2018) Likely due to these changes, smooth brome alters soil microbial communities (Piper et al. 2015, Piper et al. 2015, Mamet et al. 2017). In the same studies, it was found that smooth brome increased total soil nitrogen and gross nitrogen mineralization and the associated ammonia oxidizing archaea. Smooth brome also increased overall root and shoot biomass which will increase both root exudation and litter inputs. Finally, levels of diversity were altered in all three studies as well. These results suggest that smooth brome can change soil microbial community composition and consequently nutrient cycling for its own betterment. Given these alterations to the soil microbial community structure and function, it would follow that smooth brome invasion alters microbial community assembly processes. In order to remediate soil from smooth brome invasion, a better understanding of how these processes are changed relative to native communities is needed.

2.7 Natural Assembly Processes

Much like agricultural ecosystems, natural ecosystems are subject to a wide variety of influences. For example, grassland assembly processes are influenced by warming with warming increasing the proportion of drift over time (Ning et al. 2020). Homogenous selection in warmed plots was also more correlated with drought and plant productivity than control plots. In other study, soil nutrients were a key driver of bacterial assembly processes in soil vadose zone but depth was the largest determinant of assembly processes in the groundwater saturated zone (Sheng et al. 2021). Tripathi et al. (2018) found the soil pH rather than soil successional age, was the largest determinant of soil bacterial assembly processes. Zhou et al. (2019) found that deterministic processes dominated assembly in phyllosphere and rhizosphere of *Phragmite australis* in a wetland system. Climate change will cause the range of many plant species to shift as well as increase the incidence of many disturbances and the spread of invasive species (Nicotra et al. 2010). In order to understand how nature ecosystems will respond to these changes we need a better understanding of the soil microbial community assembly processes associated with them.

2.8 Invasion and Ecosystem Services

Much like range shift and disturbance regimes, the abundance and distribution of invasive species is expected to expand with climate change (Tylianakis et al. 2008). The effect of invasive species on ecosystem services has been well documented, such as the aforementioned cheatgrass. Moreover, the economic costs of invasive species control, removal and remediation, invasive species can severely impact ecosystem services and human health. Invasive plants can greatly affect provisioning services, which are defined as the production of food, fiber, or fuel (Pejchar and Mooney 2009). For example, there are more than 300 invasive species in rangelands in the United States that cause an estimated $2 billion in economic losses annually (Ditomaso 2017). In addition to rangeland losses, invasive species also cause major agricultural losses each year. One way that invasive species can cause agricultural losses is through the disruption of pollination services provided by native bees which are reliant on native plant species (Potts et al. 2016).

Ecosystem services are any benefits that human beings and society receive from an ecosystem (Daily 1997). The term became more broadly used after the United Nations released the Millennium Ecosystem Assessment in 2005. In the document, ecosystem services were split into four categories: provisioning, regulating, cultural and supporting services. Provisioning services are any production of food, fiber, timber or water for human consumption. Regulating services include the maintenance of the climate including such things are greenhouse gas emissions and flooding, disease regulation, waste and water regulation. Cultural services are the recreational and spiritual aspects that human societies gain from ecosystems. Finally, supporting services are soil formation, photosynthesis and nutrient cycling. Given these broad definitions, assessing the monetary value of ecosystem services is difficult. The earliest estimation of these services was that ecosystem services added $16-54 trillion USD to the global economy annually which is a conservative estimation (Costanza et al. 1997). More recently, the World Wildlife Foundation estimated global ecosystem services to be worth an estimated $125 trillion (WWF 2018). Regardless of the monetary value, all ecosystem services continue to be threatened by global climate change (Costanza et al. 2017).

Soil specific ecosystem services that are reliant in some way on soil microbial communities are water cycling, climate regulation, nutrient cycling, and soil conservation (Daily et al. 1997) Invasive species will often increase the rates of both nitrogen and carbon cycling within a system as well as increasing water demands (Ehrenfeld 2003). Hall and Asner (2007) found elevated rates of nitrous oxide emissions with increased invasive biomass demonstrating that invasive species can alter climate regulation as well. The invasive shrub *Tamarix* alters both local water cycling and soil conditions (Shafroth et al. 2005). The deep tap root of the shrub brings up salts from lower soil horizons salinizing both the surrounding soil and water. The soil salinization will impact microbial communities as well as impacting soil conservation efforts.

3. BRASSICA NAPUS PHYLLOSPHERE BACTERIAL COMPOSITION CHANGES WITH GROWTH STAGE

3.1 **Preface**

In this chapter I analyze the composition of the bacterial community on the leaves of eight lines of *Brassica napus*. I discuss how line and growth stage impact this community as well as the core community present in all lines. This chapter has been published Bell, J.K., B. Helgason, and S.D. Siciliano. 2021. Brassica napus phyllosphere bacterial composition changes with growth stage. Plant Soil: 501–516. Dr. Helgason designed the experiment, secured funding and provided critical feedback for the manuscript. Dr. Sicilinao designed the experiment, secured funding and supervised the laboratory work, statistical analysis and writing.

3.2 Abstract

Aims

Phyllosphere bacteria play critical roles in plant growth promotion, disease suppression and global nutrient cycling but remain understudied.

Methods

In this project, we examined the bacterial community on the phyllosphere of eight diverse lines of *Brassica napus* for ten weeks in Saskatoon, Saskatchewan Canada.

Results

The bacterial community was shaped largely by plant growth stage with distinct communities present before and after flowering. Bacterial diversity before flowering had 111 core members with high functional potential, with the peak of diversity being reached during flowering. After flowering, bacterial diversity dropped quickly and sharply to 16 members of the core community, suggesting that the plant did not support the same functional potential anymore. *B. napus* line had little effect on the larger community but appeared to have more of an effect on the rare bacteria.

Conclusions

Our work suggests that the dominant bacterial community is driven by plant growth stage, whereas differences in plant line seemed to affect rare bacteria. The role of these rare bacteria in plant health remains unresolved.

3.3 Introduction

The phyllosphere is one of the largest microbial habitats globally yet remains understudied (Vorholt 2012). The phyllosphere can encompass all the aerial parts of the plant (Vorholt 2012), but most commonly is referred to as the surface and interior of the leaves (Redford et al. 2010, Vacher et al. 2016). Phyllosphere microorganisms provide benefits to the plant, such as increased nutrient cycling, disease suppression, and growth promotion among many others. Fürnkranz et al. (2008) found that some species of tropical plants had higher rates of beneficial nitrogen fixation by diazotrophic phyllosphere bacteria. Vogel et al. (2016) and Jarvis et al. (2015) both found that the pre-existing phyllosphere bacterial communities combined with plant genetics, impacted the reaction to and the extent of disease on plants that had been inoculated with known pathogens. Phyllosphere microbial communities also play important roles in promoting plant growth (Batool et al. 2016), and the diversity and abundance of these communities can impact the levels of insect herbivory (Humphrey et al. 2014). Finally, phyllosphere bacteria are likely an important driver in the global methane cycle and other global nutrient cycling processes (Iguchi et al. 2015).

Despite the importance of phyllosphere microbial communities for overall plant and ecosystem functioning, they remain poorly described, particularly in agricultural systems, which account for 37% (1385 mil. ha) of the global land use in 2017 (FAOSTAT 2017). With the global population predicted to reach 9 billion by 2050, agricultural intensification will increase (Glibert et al.2010). Producing more food on decreasing arable land and in more erratic climate conditions poses a unique and difficult problem for crop breeders and farmers. Breeding crops to have a more robust microbiome may be a sustainable way to improve crop yield without additional inputs or an increase in cultivated land (Ryan et al. 2009). However, before crops can be successfully bred to optimize microbiome contributions, we must first understand the diversity and dynamics of the microbiome of crop plants.

Canola (*Brassica napus*) is produced for its high-quality oil, and increasingly, to be used as high-quality animal feed and for the production of biofuels. Canada is the leading producer of canola, with an estimated 3 million tons produced in 2018 (AFFC 2019). The importance and

large-scale production of canola makes it a good target for microbiome studies. Canola also has large nitrogen requirements. Using microbiome manipulation to increase nitrogen use efficiency could help make canola a more sustainable crop. Additionally, the use of genetically modified crops, including canola, is widely banned in Europe. Breeding canola with improved microbiomes could help lessen the disease pressure placed on canola or rapeseed crops that cannot be modified genetically to ameliorate some of this pressure. Work on the microbiome of canola and oilseed crops has focused primarily on the roots and rhizosphere (Copeland et al. 2015, Cordero et al. 2020, Gopalakrishnan et al. 2015, Glaeser et al. 2020), or on cultured microbial isolates (Ramero et al. 2019) or pathogens from *Brassica* species, and not on the microbiome as a whole (Wassermann et al. 2017). In some cases, the interaction of canola root- and rhizosphere-associated microbiomes with other crops was considered (Hilton et al. 2018, Schlatter et al. 2019). To date, only one study has examined the canola phyllosphere microbiome (Copeland et al. 2015) observing differences compared to common bean and soybean. They further observed that the canola phyllosphere microbiome was affected by rainfall and became increasingly different from the rhizosphere community as the growing season progressed.
Not only does the composition of the phyllosphere remain understudied but what impacts the assembly of these communities has also been largely ignored. Many of the studies on the canola microbiome have both limited sampling dates and canola lines. In order to successfully breed *B. napus* to have a more robust microbiome we must first gain an understanding of how the microbiome develops and changes throughout the growing season for the whole plant, not just in the rhizosphere. In this study, we examined the bacterial phyllosphere microbiome of eight different lines of *B. napus* in Saskatchewan over a ten-week period to determine the composition of the phyllosphere and what factors are important for the structuring of bacterial communities on leaf surfaces. We hypothesized that the phyllosphere bacterial community composition would vary with developmental stage and *B. napus* line.

3.4 Methods

Eight lines of *B. napus* (Mason et al. 2017, Clarke et al. 2016) were seeded on May 28 or 29, 2017 in the field at the Agriculture and Agri-Food Canada (AAFC) research farm outside of Saskatoon, Saskatchewan, Canada (52.1718° N, 106.5052° W) which lies in the Dark Brown soil zone (Chernozem). The plots were seeded using a 6 row, double disc drill with 1284 *B. napus*

seeds applied per plot (6.1m long by 1.8m wide). A granular blend of 20-8-0-20 fertilizer was applied on May 23 at the rate of 139 kg ha^{-1}. These lines of *Brassica napus* are part of the AAFC canola breeding program and thus were not commercial canola varieties, but rather different lines created by nested associating mapping, referred to as NAM lines (Clarke et al. 2016). They ranged in seed origin and color, fiber content, erucic acid content and seed glucosinolate levels (SI table 1) meaning that some of them were not technically canola which requires a low erucic acid content, but all lines were varying varieties of *B. napus.* Differences in these traits will result in differences in oil quality and are related to phenological development. Bazghaleh et al. (2020) have described the experimental design extensively, but briefly, the site consisted of three replicate blocks with each *B. napus* line seeded randomly within each block, but not repeated within a block. All lines were planted on May 28, 2017. The site received 127.9 mm of precipitation throughout the growing season with a mean air temperature of 16.4 °C. Several leaf samples were collected from each of the eight lines in each block every week for ten weeks beginning on June 20, 2017 until August 22, 2017. Plants on the edges of the block were avoided as these leaves were visibly dusty and thus would likely have a different microbiome than most leaves on the interior of the rows. Leaves that were clearly diseased, had extensive insect damage or were senescing as the plant matured were also avoided, as these leaves did not represent the majority of the leaves present and would again bias the microbiome results. During flowering, uppermost leaves were avoided as these leaves had a thick layer of petals on them and differentiating the leaf microbiome from the flower microbiome would have been impossible. However, some petal contamination was impossible to avoid, but was bypassed when feasible. Finally, during the later stages of seed development and ripening, leaves that were mostly senesced were avoided as dead tissue would cause large changes in the microbiome. Leaf samples, consisting of only one or two leaves were placed into sterile whirl-pak bags (Nasco, Wisconsin, USA) and placed onto ice. Leaf samples from the same NAM line, but from different blocks were not combined and plants were not destructively sampled as only a single leaf or two were sampled. One sample from each NAM line in each block was collected (24 samples) as well as one randomly selected duplicate sample within block (line was not considered here), leading to the collection of 27 samples collected weekly, or 270 samples over the entire growing season. Samples were then returned to the lab and stored at – 80 °C until further processing.

Frozen, brittle leaves were crumbled manually in the whirl-pak and a 0.05 g subsample was taken and extracted using Qiagen PowerPlant extraction kit (Hilden, Germany) following manufacturer instructions. After extraction, DNA was tested for quantity and quality following the standard Qubit protocol (Thermo Fisher Scientific, Waltham Massachusetts). Template DNA was standardized to 4 ng ml^{-1} prior to amplification. Bacterial diversity was assessed by amplifying the V4 region of the bacterial 16S rRNA using the primer set 515F with Illumina adapters (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA 3') and the 806R (5' - GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGA CTA CCG GGG TAT CT - 3') (Walters et al. 2015). The PCR reaction mix consisted of 7 ml Invitrogen Platinum SuperFi PCR master mix (Thermo Fisher Scientific, Waltham, Massachusetts), 0.1 ml of each primer (10 mM stock), 3 ml (5 mM stock) plastid peptide nucleic acid blocker (pPNA) and 2 ul (5 mM stock) mitochondrial peptide nucleic acid blocker (mPNA) (PNA Bio, California, USA) and 10.3 ml nuclease free water, and 2 ml of the standardized template DNA. PNAs were included to block the amplification of host DNA, plant mitochondria and chloroplasts, which are a common contaminant from plant tissues (Ray et al. 2000, Von Wintzingerode et al. 2000). The PCR conditions were 95 °C for 5 min as an initial denaturization, followed by 95 °C for 30 sec., 78 °C for 10 sec., 54 °C for 45 sec., 72 °C for 60 sec. for 35 cycles, and a final elongation of 72 °C for 7 min. Negative controls and PCR duplicates were included. PCR product was purified to eliminate primers and impurities using 1:1 ratio of Nucleomag NGS clean-up and size select (D-mark Biosciences, Scarborough, Ontario). Randomly selected duplicates were included during DNA extraction, amplification, and sequencing stages adding in 56 duplicates, bringing the total sample size to 326. After purification, samples were indexed following the Illumina protocol (Illumina, San Diego, California), purified again to remove excess index primers, quantified and standardized to 4 nM, and pooled. Pooled libraries were then sequenced using the Illumina MiSeq platform using V3 chemistry.

A total of 326 samples were sequenced resulting in 10,839,325 reads with an average of 18,186 reads per sample. Sequences were imported into QIIME2 v 2019.1 (Bolyen et al. 2018) and primers were removed using cutadapt (Martin 2011). Reads were then processed into amplicon sequence variants (ASVs) and chimeras were removed using Deblur (Amir et al. 2017) resulting in 1,968 ASVs. ASVs were then classified using a 515/806 trained Greengenes

database classifier (Desantis et al. 2006). Host mitochondria and chloroplasts were removed after classification. Host DNA ranged from 6% to 100% of the read in each sample with an average of 32% across samples. The abundance and taxonomy artifacts produced in QIIME2 were exported to BIOM format (McDonald et al. 2012) for processing in R v. 3.5.3 (R Core Team 2018). Where available, species level classification was used although the authors recognize that there are issues (Johnson et al. 2019), this is standard practice at the time of writing. If a species level classification was not available, the ASV was marked as Unclassified. This was also done for higher levels of taxonomy.

To select the core microbiome (Shade and Handelsman 2012, Lundberg et al. 2013, Vandenkoornhuyse et al. 2015, Shade and Stopnisek 2019), each ASV was converted from number of reads to presence or absence. No singletons or doubletons formed part of the core, with all members being both prevalent (present every week during sampling) and abundant (> 0.05% of all reads). The ASVs that were present across all lines and weeks were selected as the Core and represents 15 ASVs, which fall into four different classifications (Table 1). Upon this conversion, it was noted that there appeared to be a shift in community composition after flowering. The alpha diversity indices for the weeks before and after flowering were similar despite there being a shift in community composition. To investigate if there was a shift in composition, that was not being reflected accurately in the diveristy indices, the community was subset into (i) ASVs present in all weeks up to and including flowering (Flowering) and (ii) those present in all weeks after flowering (Pod), using the same prescence and absence matrix that was used to select the core that was present across all ten weeks. In order for an ASV to be selected for either the Flowering or Pod communities, the ASV had to be present consistently every week either before or after flowering. There were 111 ASVs present before and during flowering (Flowering) and 16 present post flowering (Pod), a small porportion of the original 1,968 ASVs. This separation into Flowering and Pod allowed for the conservation of the shift in community composition that would not have been captured by examining the entirety of the sampling period together, nor by looking at diversity metrics alone. The Flowering community has distinct ASVs from that of the Pod community as many of the ASVs present during and before the flowering stage disappeared once the plants matured into seed and pod development. However, for analysis both of these communites do inlcude the original 15 Core ASVs, as this small subset of ASVs were present all ten weeks. This resulted in Flowering consisting of 126 ASVs and Pod having

31 ASVs. Unweighted UniFrac distance matrices were performed on the entire data set of 1,968 ASVs (Total) and well as the combined Core, Flowering and Pod communities (Expanded Core).

Alpha diversity was calculated using the package vegan in R (Oksanen et al. 2019). All diversity measures calculate the variety of organisms in a community based on combinations of species richness and evenness (Kim et al. 2017). The Shannon-Weaver index, Abundance-based Coverage Estimate (ACE) and the Simpson Index were chosen because they represent a mix of approaches to diversity. The Shannon-Weaver index places more emphasis on species richness whereas the Simpson index places more emphasis on species evenness (Kim et al. 2017). Unlike the previous two indices, the ACE index takes into consideration the abundance of the species present and for this study most closely reflected the observed diversity. Shannon-Weaver index, ACE (Chao and Lee 1992), Simpson index and species richness (Lou 2007) estimates were calculated and showed similar trends, with peak diversity occurring across all lines during week 4 of sampling and declining steadily with plant maturity (Table 2). However, both the Shannon-Weaver and the Simpson indices showed an initially very high level of diversity, but this is likely an over estimation due to a high abundance of rare ASVs present in the first few weeks.

The BBCH-scale is a scale used to uniformly identify and quantify the phenological stages of plant development, with scales developed for species specific development (Lancashire 1991). Despite differences in crop species as well as varietal differences, all plants have the same growth stages of leaf development, formation of side shoots, stem elongation, harvestable vegetative plant part development, inflorescence emergence, flowering, seed development, ripening and senescence. All *B. napus* lines were assigned BBCH weekly using the Canola Council of Canada BBCH guide (Canola Council of Canada, 2020) and were averaged. This was done because despite identical planting times, the eight *B. napus* lines did exhibit some differences in plant development. Sampling week 1 and 2 took place during the leaf development stage for most *B. napus* lines sampled, with bolting happening during week 3 (Figure 1). Peak flowering was reached for most lines during sampling week 4 (Flowering) with seed development occurring in the following two weeks (Pod). The last four weeks of sampling were characterized by ripening of the *B. napus* seed pods (Pod).

Permutational analysis of variance (PERMANOVA) was performed using the adonis function in the vegan package in R (Oksanen et al. 2019). The weighted and unweighted UniFrac (Lozupone and Knight 2005) distances were calculated using the phyloseq package (McMurdie

and Holmes 2013), for the entire community (Total), the Expanded Core (Core, Flower and Pod communities) as well as the communities present before and during flowering (Flower) and the

Table 0.1 The core microbiome present across all *B. napus* lines throughout the ten-week sampling period. The relative abundance is expressed as a percentage of the total bacterial abundance, the core relative abundance, for all lines for the entire sampling period. Plant pathogenesis, pigment production, and biofilm formation ability are all adaptations giving these core bacteria advantages to colonizing the leaf surface. Expression of these traits are denoted with a plus sign if the bacteria has the capability and a negative sign if the bacteria do not express this capabilities.

Order	Genus	Species	Relative Abundance	Relative Abundance Core	Pathogenic	Pigmented	Biofilm Forming
Pseudomonadales	Pseudomonas	Viridiflava	12.9	31.9	+ (Hu et al. 1998)	-	+ (Bartoli et al. 2014)
Enterobacteriaceae	Pantoea	Unclassified	10.6	12.5	+/- (Walterson and Stavrinides 2015)	+ (Walterson and Stavrinides 2015)	+ (Walterson and Stavrinides 2015)
Oxalobacteraceae	Massilia	Unclassified	5	13.1	-	-	-
Xanthomonadales	Stenotrophomonas	Retroflexus	2.9	5.6	-	-	+ (Ren et al. 2014).

communities present after flowering (Pod). UniFrac distances were used instead of Bray-Curtis or other similar methods, to maintain the phylogenetic signals as there was a shift in community composition from the community present pre-flowering and post-flowering. Other distance matrices that do not contain phylogenetic information would not accurately capture this shift.

Distance-based redundancy analysis (dbRDA) (Legendre and Andersson 1999) was performed using the capscale function in the vegan package in R (Oksanen et al. 2019). The weighted and unweighted UniFrac matrices for both the Total and the Expanded Core communities were constrained by NAM line and BBCH growth stage, as well as the interaction, and these constraints were then analyzed using the anova function (base R). Given that block was found to not be significant for any of the communities in the PERMANOVA results it was not included in the dbRDAs.

To isolate the effect of *B. napus* NAM line more thoroughly, a dbRDA was performed constraining each UniFrac matrix by growth stage only. This was done to investigate whether NAM line influenced the community when it was not outweighed by the importance of growth stage. The residuals of this model were extracted, and an additional dbRDAs were performed constraining by NAM line. By doing this we accounted for the effect of growth stage without the influence of NAM line, then after this variation was accounted for, we could isolate the additional variation caused by NAM line alone without the large signal of growth stage overshadowing the effect of NAM line.

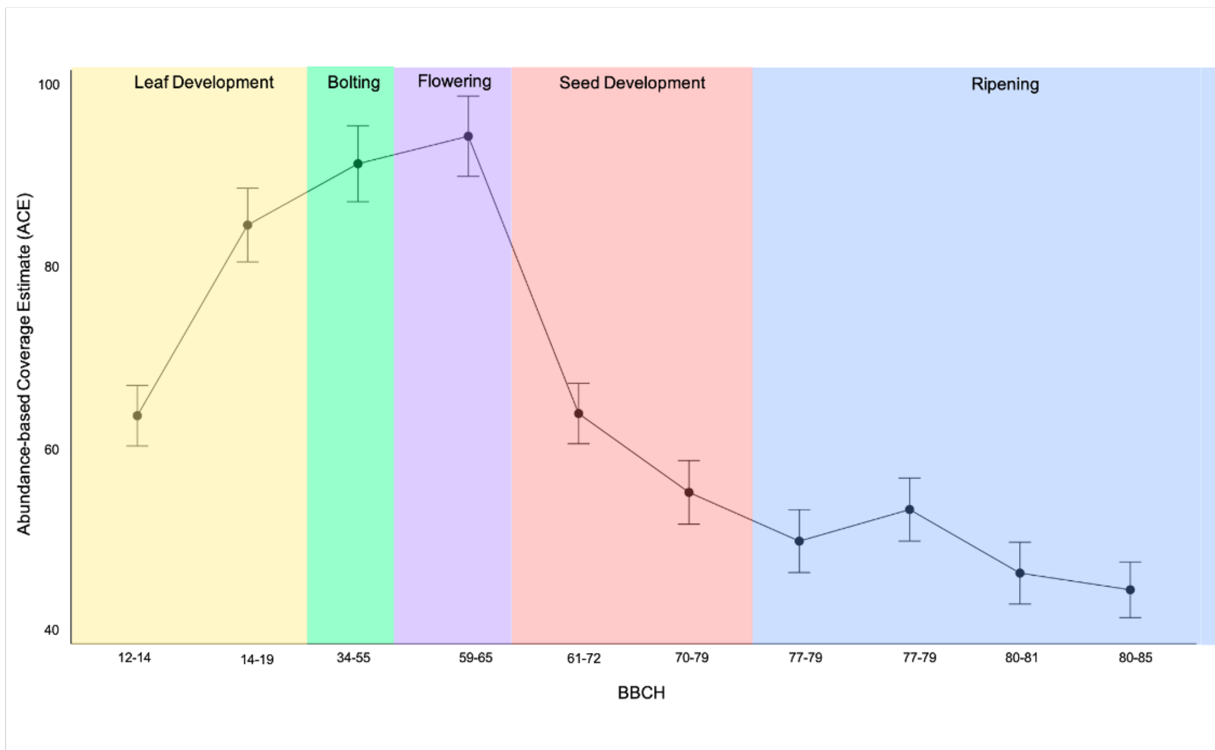


Figure 0.1 Abundance-based Coverage Estimate (ACE) average of the bacterial community on the leaf surface against the average BBCH of all 8 lines of *B. napus* across the ten sampling weeks. Each point is the average ACE for that week for the community microbiome (1,968 ASVs (amplicon sequence variants) with error bars representing the standard error of the estimate. The BBCH range represents the range of BBCH observed across the eight different lines for that sampling week. Change in BBCH stage is indicated by changes in background color with the name of the stage indicated at the top of the colored bar.

3.5 Results

The leaf bacterial community was dominated by a small subset of the total ASVs present. The leaf microbial community (Figure 2) of 1,968 ASVs was primarily Proteobacteria (79%), followed by Bacteroidetes (7%), Acidobacteria (6%) and Firmicutes (6%). The Proteobacteria were primarily Gammaproteobacteria (74%), followed by Betaproteobacteria (20%) and Alphaproteobacteria (4.7%). However, despite 1,968 ASVs being present, the majority of the community was dominated by a few species. The Expanded Core microbiome of 136 ASVs were primarily *Pseudomonas virdiflava* (Table 1) (12.9% of the Total, 31.9% Core), followed by *Pantoea sp.* (10.6% Total, 12.5% Core), *Massilia sp.* (5% Total, 13.1% Core) and *Stenotrophomonas retroflexus* (2.9% Total, 5.6% Core) (Figure 2). However, it should be noted

that these identifications are tentative due to the difficulty of assigning taxonomy using only one region of the bacterial genome.

Bacterial diversity was highest during week four, or the flowering period for both the observed diversity (the number of ASVs present) and abundance-based coverage estimator (ACE) (Table 2, Figure 1). The highest diversity for the Shannon index and inverse Simpson was observed during the first sampling week (Table 2), however there was a sharp decline in both of these indices during the second sampling week with both reaching a peak during the flowering period. The initially high diversity found during week one was likely due to the plants being recently emerged from the soil and thus still being colonized by primarily by soil bacteria. After flowering, all the diversity indices declined steadily to levels similar to, or lower than those observed during the leaf development stages. There was a sharp decrease in diversity between weeks six and seven (Fig. 2, Table 2) which could have been caused by low precipitation. Diversity increased again during weeks seven to nine, only to decrease again at week ten. The overall decline in diversity after flowering could have been caused by the gradual leaf senescence that occurs with *B. napus* during seed production.

PERMANOVA results indicated that stage was the primary factor shaping the community structure. The PERMANOVA of the Total weighted UniFrac distances (Table 3) showed that plant development stage (BBCH) significantly affected the community composition ($p=0.026$, $R^2 = 0.029$) and that NAM line was nearly significant ($p=0.06$, $R^2 = 0.021$) (Table 3). There were no significant terms for the PERMANOVA of the Total Unweighted UniFrac Distances (Table 3). For the Expanded Core, stage was significant in the Weighted Unifrac distances ($p=0.018$, $R^2 = 0.026$). In the unweighted Flower community, there was only a marginally significant interaction between NAM line, stage and block (Table 3). Stage was significant for both the Weighted ($p=0.001$, $R^2 = 0.039$) and Unweighted ($p = 0.001$, $R^2 = 0.052$) UniFrac distances of the Pod community. Additionally, NAM line was significant ($p = 0.045$, $R^2 = 0.048$) for the weighted UniFrac distances in the Pod community (Table 3).

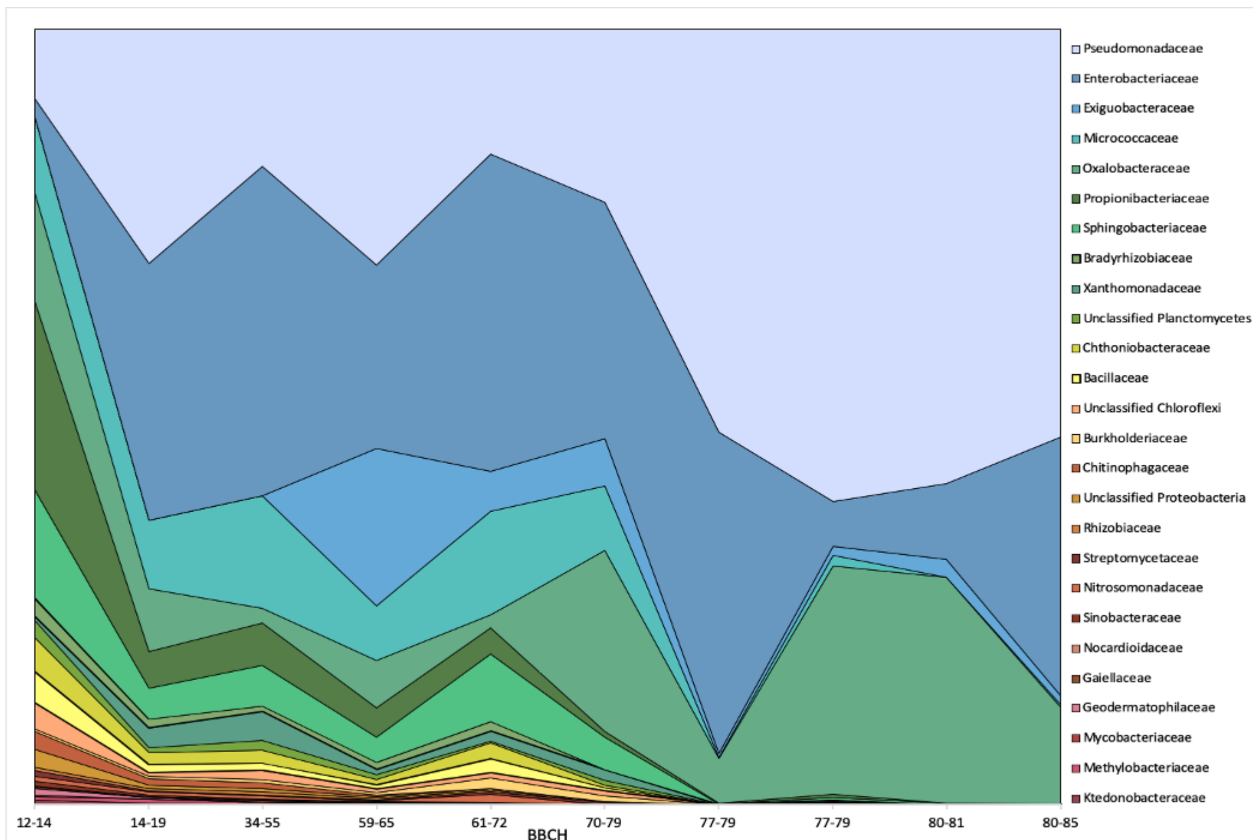


Figure 0.2 Relative abundance of genera that composed the Core as well as the communities present during the Flowering and Pod stages (Expanded Core). Many of the ASVs (amplicon sequence variants) in the Expanded Core were classified into the same genus or even species, but due to the nature of ASVs, they remained separate. For this graphic, the ASVs present in the Expanded Core have been grouped into genus instead of showing all 136 individual ASVs in the Expanded Core. Colors are grouped as genus and the weekly range of BBCH observed is present on the x axis.

Given the significant, but low, explanatory values given by the PERMANOVAs, dbRDAs were done to further isolate the effects driving community composition while maintaining the phylogenetic relationships provided by the UniFrac distance matrices. Similar to the PERMANOVAs, the db-RDAs indicated that growth stage was the dominate factor shaping the bacterial community. BBCH stage significantly ($p < 0.001$) affected Total bacterial community structure for both weighted and unweighted UniFrac dbRDA analyses (Fig 3). NAM line was significant only for the unweighted UniFrac analysis of the Total community ($p = 0.048$; Table 3, Fig 3B). There was a marginally significant NAM line by growth stage interaction for the unweighted Total community ($p=0.058$; Table 3) and for the weighted, Expanded Core community ($p = 0.030$; Table 3), likely caused by slight differences in plant development times

between lines. In the dbRDA models of the Total community, BBCH stage and NAM line explained 28% of the variance in the weighted Unifrac analysis (Fig 3A) and 24% of the variance in the unweighted UniFrac analysis (Fig 3B). The slight decrease in explanatory power between the weighted and unweighted analysis could have been caused by the bacterial community being largely dominated by a small number of ASVs, which would not have been reflected in the unweighted distance matrix. When only the Expanded Core was analysed using dbRDA, this increased to 41% (SI Fig 1A) and 32% (SI Fig 1B) for the weighted and unweighted community analyses (Table 3). This increase in explanatory power is likely caused by the reduction in the number of ASVs included in the analysis (1,968 ASVs in the Total to 136 ASVs in the Expanded Core). Despite there being a large number of rare ASVs, they were not abundant thus reducing the noise for the analysis. For the Flowering community, stage was significant for both the weighted ($p=0.002$) and unweighted ($p=0.001$) UniFrac matrices, but neither NAM line nor the interaction of NAM line and stage were significant for either (Table 3). For the Pod Development community, stage was significant for both the weighted ($p=0.003$) and the unweighted ($p=0.001$) UniFrac matrices, but again, neither NAM line nor the interaction was significant (Table 3).

For the Total community dbRDA, BBCH stage alone explained 19% of the variation observed for the weighted UniFrac matrix (Fig 3A) and 12% of the variation for the unweighted UniFrac matrix (Fig 3B). NAM line was observed to have slight differences in development time and visual appearance of the leaves (observations from the field) however these differences had little effect on the bacterial communities present on the leaves. After isolating the residuals of each of the above models, NAM line explained only 2% of the variation observed and was not significant for the weighted UniFrac distance residuals. Similarly, for the unweighted UniFrac distance residuals, NAM line explained 3% ($p = 0.1$) of the variation. For the Expanded Core community, growth stage alone explained 28% of the variation observed for the weighted UniFrac matrix (Fig 3A) and 19% for the unweighted matrix (Fig 3B). NAM line explained 3% for the weighted and unweighted Expanded Core residuals and was not significant. The leaf is an inhospitable environment which can change drastically with plant growth stage, thus the small differences in NAM line were likely insignificant to shaping the bacterial community when compared to the much larger force of plant growth stage.

Table 0.2 Average diversity metrics of all eight lines of *B. napus* for each week. Observed diversity is simply the average number of ASVs (amplicon sequence variant) per sample present and ACE is the Abundance-based Coverage Estimate. Larger numbers represent higher diversity.

Stage	Week/BBCH	Observed	ACE	Shannon	Inverse Simpson
Leaf Development	1/12-14	52.7	36.6	3.4	34.2
Leaf Development	2/14-19	68.5	84.7	2.5	7.7
Bolting	3/34-55	76.1	91.4	2.5	7.5
Flowering	4/59-65	77.4	94.4	2.7	11.1
Seed Development	5/61-72	48.6	63.9	2.2	7.2
Seed Development	6/70-79	45.1	55.2	2.2	6.4
Ripening	7/77-79	41.3	49.8	2.2	6.6
Ripening	8/77-79	44.4	53.3	2.5	8.9
Ripening	9/80-81	39.2	46.3	2.1	5.1
Ripening	10/80-85	37.5	44.4	2.3	7.4

3.6 Discussion

B. napus growth stage is the dominant factor shaping the bacterial community present on the leaves, throughout the growing season with a distinct community present before and after flowering. The distinct community is evidenced by the Flowering community with 111 ASVs present every week before flowering, with most of these disappearing after flowering, leaving only 16 ASVs present in the Pod community. Additionally, significant differences in both PERMANOVAs and dbRDAs were found when comparing the weighted UniFrac to the unweighted UniFrac distances, with BBCH stage being significant for both the total community and the Expanded Core. Differences between weighted UniFrac, which uses phylogenetic relationships as well as abundance, and unweighted UniFrac, which does not use abundance, indicate that both community structure, and evenness change throughout the sampling period (Lozupone et al. 2007). If both community structure and evenness were not significantly impacted by BBCH growth stage, there would be no significant differences between the weighted and unweighted UniFrac distances.

The dominance of growth stage appears to outweigh the importance of different *B. napus* lines on shaping the bacterial community despite differences in NAM line as the Expanded Core remained abundant and constant and only the composition of the rare ASVs seemed to vary. Furthermore, NAM line significantly impacted the community structure only a limited number of times (Table 3). It is unclear if this is a *B. napus* specific finding or perhaps a phyllosphere specific finding. Crop variety has been shown to impact rhizosphere communities (Edwards et al. 2015, Colemam-Derr et al. 2016, Dombrowski et al. 2017). Additionally, Bokulich et al. (2014) found that microbial communities in grape must varied with grape cultivar. However, crop line has variable impacts on phyllosphere communities. Singh et al. (2019) found that grape cultivar impacted the microbial community present in the phyllosphere. Conversely, Johnston-Monhe et al. (2016) found that maize genotype had no effect on microbial communities in the phyllosphere. Most importantly, Copeland et al. (2015) did not find that *B. napus* line impacted the microbial communities in the phyllosphere. The lack of impact of line on bacterial community structure could be due to thick, waxy cuticle

present on *B. napus* leaves. The thick cuticle likely makes the *B. napus* leaf an inhospitable environment for bacteria with low availability of plant derived carbon (Vorholt 2012, Vacher et al. 2016). More work is needed to characterize the phyllosphere and impact of line on *B. napus* as well as other crops.

Table 0.3 Permutational analysis of variance (PERMANOVA) tables. *B. napus* (NAM) line, growth stage, block and all the interactions were used as covariates. Both weighted UniFrac distances and unweighted UniFrac distances were analyzed for the Total community, the Expanded Core community and the communities present before and after flowering.

Data	Variable	Component	D.F.	Sum of Squares	Mean Squares	F. Model	R2	p-value
Total								
Weighted Unifrac	Stage	Fixed	4	0.500	0.125	2.333	0.029	0.026*
	NAM	Fixed	7	0.353	0.044	0.824	0.021	0.0667
	Block	Random	2	0.150	0.075	1.403	0.009	0.227
	Stage x NAM		29	1.706	0.059	1.098	0.100	0.295
	Stage x Block		8	0.270	0.034	0.631	0.016	0.889
	NAM x Block		15	0.934	0.062	1.162	0.055	0.25
	NAM x Stage x Block		56	2.207	0.039	0.736	0.129	0.968
Unweighted Unifrac	Stage	Fixed	4	1.132	0.283	1.133	0.014	0.237
	NAM	Fixed	7	1.584	0.198	0.792	0.020	0.938
	Block	Random	2	0.462	0.231	0.924	0.006	0.524
	Stage x NAM		29	6.725	0.232	0.928	0.083	0.797
	Stage x Block		8	2.105	0.263	1.054	0.026	0.327

	NAM x Block		15	3.504	0.234	0.935	0.043	0.685
	NAM x Stage x Block		56	13.966	0.249	0.998	0.173	0.505
Expanded Core								
Weighted	Stage	Fixed	4	1.294	0.324	1.980	0.026	0.018*
	NAM	Fixed	7	1.201	0.172	1.050	0.025	0.362
	Block	Random	2	0.367	0.183	1.121	0.008	0.335
	Stage x NAM		29	4.162	0.149	0.910	0.085	0.738
	Stage x Block		8	1.377	0.172	1.053	0.028	0.36
	NAM x Block		15	2.544	0.182	1.112	0.052	0.274
	NAM x Stage x Block		56	8.992	0.167	1.019	0.184	0.442
Unweighted	Stage	Fixed	4	1.172	0.293	1.298	0.018	0.125
	NAM	Fixed	7	1.467	0.210	0.929	0.022	0.595
	Block	Random	2	0.402	0.201	0.891	0.006	0.558
	Stage x NAM		29	6.405	0.229	1.014	0.096	0.408
	Stage x Block		8	1.400	0.175	0.776	0.021	0.877

	NAM x Block		15	3.204	0.229	1.014	0.048	0.455
	NAM x Stage x Block		56	12.902	0.239	1.059	0.193	0.229
Flower								
Weighted	Stage	Fixed	2	0.107	0.053	0.647	0.011	0.647
	NAM	Fixed	7	0.719	0.103	1.246	0.073	0.253
	Block	Random	2	0.101	0.053	0.610	0.010	0.649
	Stage x NAM		14	0.931	0.665	0.806	0.094	0.764
	Stage x Block		4	0.423	0.106	1.283	0.043	0.247
	NAM x Block		14	0.824	0.059	0.714	0.083	0.873
	NAM x Stage x Block		28	2.178	0.778	0.944	0.220	0.587
Unweighted	Stage	Fixed	2	0.558	0.279	0.996	0.015	0.446
	NAM	Fixed	7	1.944	0.278	0.992	0.053	0.487
	Block	Random	2	0.558	0.279	0.996	0.015	0.435
	Stage x NAM		14	3.905	0.279	0.996	0.107	0.526
	Stage x Block		4	1.288	0.322	1.150	0.035	0.160
	NAM x Block		14	3.678	0.263	0.939	0.101	0.726

	NAM x Stage x Block		28	8.794	0.314	1.122	0.242	0.06
Pod								
Weighted	Stage	Fixed	1	0.211	0.211	7.654	0.039	0.001*
	NAM	Fixed	7	0.188	0.027	0.973	0.034	0.477
	Block	Random	2	0.107	0.054	1.947	0.020	0.084
	Stage x NAM		7	0.224	0.032	1.164	0.041	0.282
	Stage x Block		2	0.055	0.275	0.999	0.010	0.376
	NAM x Block		14	0.358	0.026	0.930	0.066	0.556
	NAM x Stage x Block		12	0.345	0.029	1.044	0.063	0.388
Unweighted	Stage	Fixed	1	1.870	1.875	10.660	0.052	0.001*
	NAM	Fixed	7	1.724	0.246	1.401	0.048	0.045*
	Block	Random	2	0.366	0.183	1.041	0.101	0.369
	Stage x NAM		7	1.477	0.211	1.199	0.041	0.165
	Stage x Block		2	0.411	0.205	1.168	0.011	0.284

NAM x Block	14	2.370	0.169	0.963	0.066	0.572
NAM x Stage x Block	12	2.604	0.217	1.234	0.072	0.099

Copeland et al. (2015) found that the canola phyllosphere was dominated by a few high abundant genera with many rare ASVs, with decreasing diversity as the growing season progressed. However, they examined only sampling date, but not plant growth stage, so while the trends are similar, it is difficult to extrapolate further. The dbRDAs, as well as the PERMANOVA results suggest that perhaps *B. napus* line plays a larger role in structuring the rare ASVs present on the leaves, but not the Expanded Core microbiome which was comprised of relatively abundant bacteria. This is especially true for the Pod community which showed a significant effect of NAM line on the unweighted UniFrac distances, but not in the weighted UniFrac distances. Since the unweighted UniFrac distances do not incorporate abundance, simply presence or absence of an ASV, this indicates that there might be a larger influence of line on the rarer ASVs, which are not given as much weight when using the abundance weighted UniFrac distance matrix. However, there appears to be no consistent patterns in what rare ASVs are present throughout the sampling season, unlike the Expanded Core community, that was generally highly abundant, relative to other taxa. This lack of both consistency and abundance suggests that perhaps these rare ASVs are transitory inhabitants of the leaves that may be selected for by more nuanced biochemical or morphological characteristics that are line specific, such as cuticle thickness or unmeasured secondary metabolites. In other words, the composition of the rare ASVs is likely determined by the stochastic effects of random replication, death and immigration, whereas the Expanded Core bacterial community is likely selected primarily by plant growth stage, making it a more deterministic process.

Changes in the plant bacterial microbiome with plant growth stage are well documented for rhizosphere soils (Smalla et al. 2001, Wagner et al. 2014, Wagner et al. 2016, Copeland et al. 2015, Hilton et al. 2017) so it is not surprising that growth stage changes were found in *B. napus* leaves. Additionally, it has been well documented that plants undergo large physiological and biochemical changes during flowering and fruit production (Nitsch 1965, Mohan and Rao 1984, Shu et al. 2010). *B. napus* leaves have a thick, waxy cuticle which thickens with age. This thickening of the cuticle would make access to plant derived carbon more limited as the plant ages, which could be the cause of the shifts observed during the growing season. Furthermore, during flowering, the petals are quickly shed, many of which land on the leaf. This additional and large source of labile carbon could be one of the factors accounting for the large increase in bacterial diversity and abundance seen around the time of flowering. After flowering, as the

seeds begin to develop and ripen, many of the different lines started to show signs of leaf senescence and death, in some cases shedding their leaves entirely. This slow leaf senescence coupled with the thickened waxy cuticle likely drives the rapid decrease in bacterial diversity seen after flowering. While both the PERMANOVA results and the db-RDAs have relatively small R^2, their cumulative effect explains quite a bit of the variation. It is well established that environmental variables or unmeasured variables explain most of the variation seen in microbial communities and it is not uncommon to have low R^2 (Redford et al. 2010, Wagner et al. 2014, Coleman-Derr et al. 2016, Leff et al. 2017,), therefore these results are valid despite the relatively low explanatory power of each variable.

The most prevalent non-pathogenic ASVs in the phyllosphere appear to be well suited for life in this difficult environment. *Stenotrophomonas retroflexus* forms biofilms, which could aid in the colonization of the leaf surface and protect the bacteria from desiccation and UV stress (Ren et al. 2014, Lui et al. 2014). *Oxalobacteraceae massilia* exists in the rhizosphere, root surface and seed coat of cucumber plants, so it's not unexpected to find it on the leaf surface as well (Ofek et al. 2012). Additionally, this species varies with plant development stage in cucumber plants so given that we see large growth stage patterns in *B. napus* leaves, we might expect to see a similar pattern here (Ofek et al. 2012). *Enterobacteriaceae Pantoea* is a functionally diverse genera with plant growth promoting abilities, nitrogen fixing capabilities, can produce antibiotics and might be an opportunistic pathogen (Walterson and Stavrinides 2015, Coutinho and Venter 2009). Many species of *Pantoea* are also pigmented, which also likely increases their ability to withstand UV stress, making them well suited for life in the phyllosphere (Andrews and Harris 2000). Furthermore, *Pantoea spp.* live in other extreme environments such as oil sands (Mitter et al. 2017) and seeds (Feng et al. 2006) indicating that they can live in difficult environments such as the phyllosphere. However, given the uncertainty of assigning taxonomy based on 16S rRNA sequencing alone, a follow-up, culture dependent study to determine the identity and functionality of these ASVs would be needed to confirm these potential functions in the *B. napus* phyllosphere.

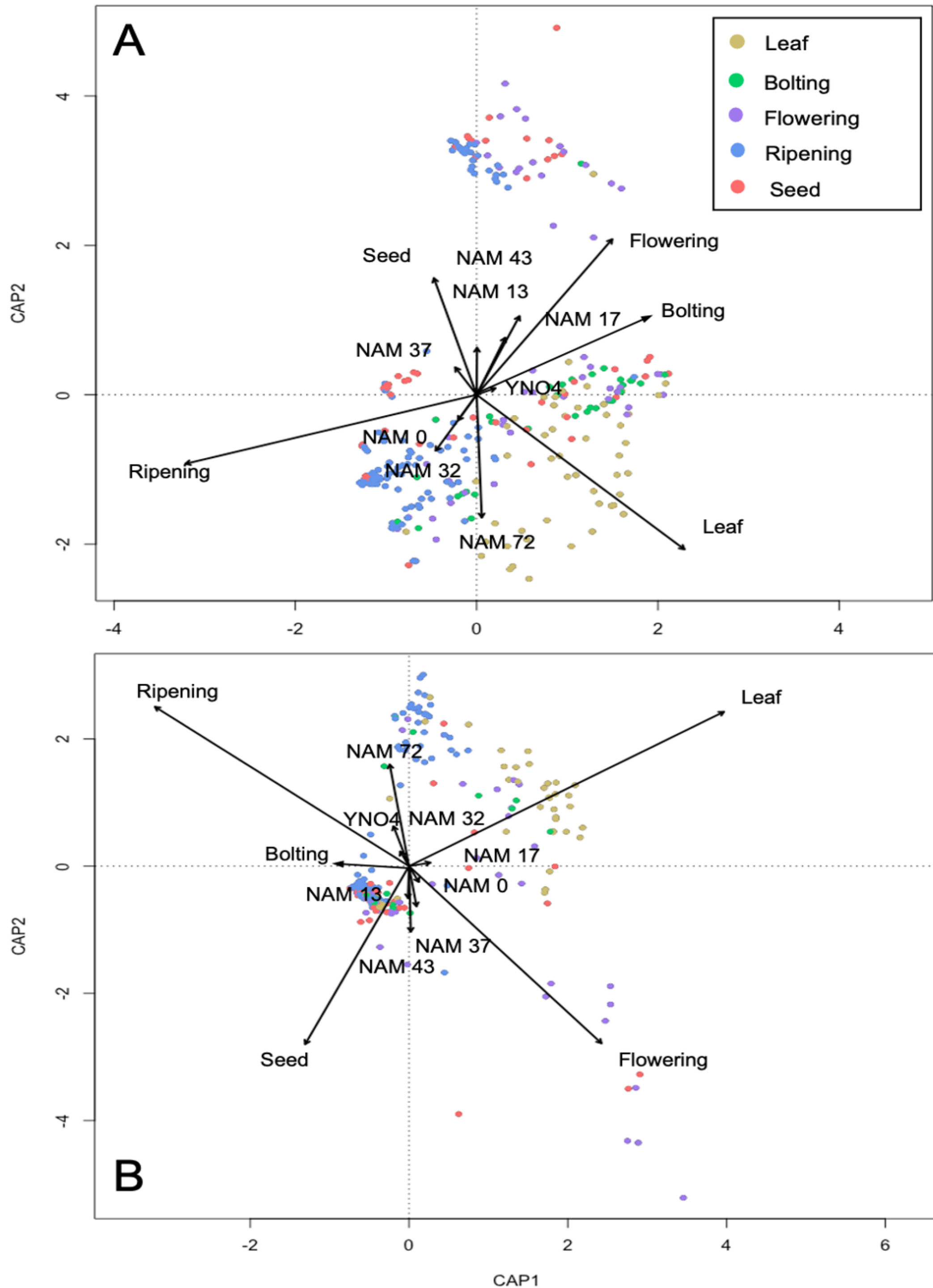


Figure 0.3 Distance-based redundancy analysis (dbRDA) of the weighted (28% of the variation) UniFrac distances (A) and unweighted (24% of the variation) UniFrac distances (B) of the Total bacterial community, across the entire 10-week sampling period, constrained by BBCH ($p = 0.001$) stage x *B. napus* line (NAM) (not significant) (biplots). Points are colored based on *B. napus* growth stage and correspond with the colors in Figure 1.

The most common ASV found was *Pseudomonas virdiflava.* This is a pathogenic species that causes foliar blights, root rots, and stem necrosis (Marina et al. 2008, Sarris et al. 2012). Furthermore, this species can cause disease in a wide variety of cultivated plants, including cantaloupe, celery and tomatoes. Typical pathology for infection caused by *Pseudomonas virdiflava* is the wilting and yellowing of leaves, which was seen in the *B. napus* leaves, but the plants did not have high disease levels, and this could have been normal leaf senescence. Culture dependent methods would need to be employed to verify if the observed wilting and yellowing leaves observed in the field was indeed caused by *Pseudomonas virdiflava* as its presence alone does not indicate the cause of disease.

The initially very high estimates of diversity during the first sampling week (Table 1) was likely caused by the plants being relatively small during the first sampling week as they were still in the leaf development stage, with two to six leaves. These plants had only recently emerged and may have been predominantly colonized by bacteria originating from the soil. The large number of unique or low abundance ASVs found during the first sampling week quickly disappeared in subsequent weeks as the leave matured (Fig. 2) and may have been selected against by the harsh conditions on the leaf surface. Both the Shannon-Weaver index and the Simpson index include estimates of observed diversity as well evenness but not abundance into the estimates, which likely lead to the over estimation of initial diversity as rare species are given equal weight (Kim et al. 2017). We did not see the same initial trend in the ACE metric likely because the ACE metric uses both evenness and abundance of rare ASVs to estimate the likelihood of a particular ASV to be present or not (Kim et al. 2017). A similar phenomenon was observed by Copeland et al. (2015) where the Shannon diversity index was very high initially and the community composition was similar to the soil, but as the growing season progressed, the diversity index decreased, and the leaves developed communities that were distinct from the soil.

Increasing bacterial diversity between the initial sampling week and the peak at flowering could have been caused by a number of factors. It is well established that while phyllosphere communities are separate from rhizosphere communities they do have considerable overlap in community composition (Wagner et al. 2014, Copeland et al 2015, Wagner et al. 2016). This increase in diversity leading up to flowering could stem from the deposition of soil dust from nearby farming activities. Vokou et al. (2012) found air samples and the phyllosphere of nine

different species shared a similar composition, suggesting that aerial deposition is a likely source of bacterial diversity on plants. Aerial deposition of soil dust would also explain the overlap in community composition between the rhizosphere and phyllosphere seen in other studies as the rhizosphere draws its community from the surrounding bulk soil which would also be the source of the dust. Additionally, this increase in diversity could be caused by visiting pollinators or insect herbivory. Humphrey et al. (2014) found that insect herbivory was positively correlated with bacterial diversity in the phyllosphere. The most likely explanation for the increasing diversity is a combination of aerial soil deposition, insect drivers and the plants selecting for a beneficial community.

Pre-flowering, the bacterial community on the leaves had greater diversity members (Figure 2, SI Table 2), compared to the Pod community. These functionally rich bacteria included members of the *Bradyrhizobiaceae* which are well documented for their widespread symbiosis with plants and their ability to fix nitrogen (Marcondes de Souza et al. 2014, Gopalakrishnan et al. 2015). There were other nitrogen-associated bacteria present pre-flowering as well, such as *Nitrosovibrio tenuis* which oxidized ammonium (Harms et al. 1976) and *Streptomyces mirabilis* which produces the enzyme nitroreductase and has shown antifungal and antibacterial activity (Bordoloi et al. 2001, Yang et al. 2012). Other functionally useful bacteria present on the leaves pre-flowering that disappear after flowering included *Burkholderia bryophila* which has been shown to have anti-fungal activity, carbon monoxide reduction capabilities and plant growth promoting properties (Vandamme et al. 2007, Weber and King 2012) which would be useful to the *B. napus* plant as many of its major pathogens are fungal (Canola Council of Canada 2020). *Methylobacterium* were also present on the leaves prior to flowering. This species can use both methane and methanol as a carbon source, both of which are byproducts of plant metabolism (Hanson and Hanson 1996, Nisbet et al. 2009, Dorokhov et al. 2018). Furthermore, *Methylobacterium* has the ability to form biofilms, making it well adapted for life in the phyllosphere (Hanson and Hanson 1996).

This bacterial diversity largely disappears after flowering (Figure 2, SI Table 2), with the majority of the core species found post flowering simply being additional AVSs of the core bacteria present throughout the entire growing season and a large dip in diversity seen between weeks six and seven. This decrease in diversity (Fig 2, Table 2) could have been caused by weeks five and six having relatively low precipitation and hotter temperatures (unpublished site

data). The recovery of some diversity in weeks eight and nine was likely caused by these weeks experiencing much cooler and wetter conditions, allowing the bacteria present the moisture needed to rebound. The phyllosphere is a harsh environment that can fluctuate rapidly in temperature, moisture and ultraviolet radiation levels, all of which will have an impact on the bacterial communities living on the surface of these leaves (Kinkel 1997, Vorholt 2012, Vacher et al. 2016). However, this diversity was not maintained during week ten as most of the leaves on the plants were undergoing senescence at this point. Despite this one anomalous large dip in diversity, the overall trend of decreasing diversity stands with one exception. Firmicute *Exiguobacterium* was not found in the pre-flowering core community and was abundant in the Pod community. *Exiguobacterium* is not widely studied creating large gaps in knowledge about this genus, however it has been shown to grow in difficult environments (Vishnivetskaya et al. 2009) which could be the plant preparing for transfer to the seed (Shade et al. 2017) or the community adapting to the quickly senescing leaves.

Plant growth stage, but not *B. napus* line, was the dominant factor in shaping the bacterial phyllosphere across all eight lines of *B. napus* sampled. There was a small, but abundant Core community present throughout the growing season, regardless of plant growth stage with rarer ASVs changing rapidly and seemingly without pattern. Initially, there was very high diversity, but this was likely caused by colonization by soil bacteria that quickly selected against due to the difficult nature of life in the phyllosphere. During the development stages, the bacterial community retained some of this diversity which included a higher potential for functional diversity. The bacterial community reached peak diversity during flowering and rapidly became much less diverse during the pod development and ripening stage, consisting primarily of ASVs from a small number of genera. These swift changes in both diversity, abundant and rare ASV composition suggests that while the plant has some control over the community composition, likely based on growth stage, there is also a probable large stochastic component to the community assembly processes. Community assembly process on the phyllosphere warrant further investigation as research into harnessing the microbiome for plant health intensifies. This work shows that the bacterial community present will change drastically throughout the growing season, and that multiple sampling points are necessary to get an accurate look at the microbiome and how it interacts with the plant as it develops.

4. BRASSICA NAPUS BACTERIAL ASSEMBLY PROCESSES VARY WITH PLANT COMPARTMENT AND GROWTH STAGE BUT NOT BETWEEN LINES

4.1 **Preface**

In this chapter I combine the bacterial sequencing data from eight lines a *Brassica napus* leaves, roots and rhizosphere to examine the assembly processes over the ten week sampling period. Dr. Steven Mamet assisted in the data processing and provided feedback on the manuscript. Dr. Helgason designed the experiment, secured funding and provided feedback on the manuscript. Dr. Siciliano designed the experiment, secured funding, and supervised all portions from sample collection to writing.

4.2 Abstract

Holobiont bacterial community assembly processes are an essential element to understanding the plant microbiome. To elucidate these processes, leaf, root, and rhizosphere samples were collected from eight lines of *Brassica napus* in Saskatchewan over the course of ten weeks. We then used ecological null modeling to disentangle the community assembly processes over the growing season in each plant part. The root was primarily dominated by stochastic community assembly processes, which defies previous knowledge of a highly selective root environment. Leaf assembly processes were primarily stochastic as well. In contrast, the rhizosphere was a highly selective environment. The dominant rhizosphere process leads to more similar communities. Assembly processes in all plant compartments were dependent on plant growth stage with little line effect on community assembly. The foundations of assembly in the leaf were due to the harsh environment leading to dominance of stochastic effects whereas the stochastic effects in the root interior likely arise due to competitive exclusion. Assuming strong selection could promote beneficial bacteria, engineering canola microbiomes to resist disease, which are typically aerially born, should focus on the flowering period whereas microbiomes to enhance yield should likely be engineered post-flowering as the rhizosphere is undergoing strong selection.

4.3 Introduction

Projected rapid increases in climate variability and global population (Gilbert et al. 2010) make the need for crops with resilient microbiomes ever more pressing (Ryan et al. 2009). Canola (*Brassica napus* L.) is a globally important oilseed crop with high resource demands, making it an ideal target for microbiome engineering. In addition to its high-quality oil, canola has been increasingly used as high-quality animal feed and for the production of biofuels. However, canola requires large nitrogen inputs and is susceptible to common crop diseases like *Fusarium* wilt, both of which could be addressed through more targeted microbiome manipulations. Previous studies of canola associated microbiomes focused primarily on the roots and rhizosphere (Copeland et al. 2015, Cordero et al. 2020, Gopalakrishnan et al. 2015, Glaeser et al. 2020), specific microbial isolates (Wassermann et al. 2020), or the microbiome of canola and other common crops (Hilton et al. 2018, Schlatter et al. 2019). Microbiome-centered approaches increase plant tolerance to abiotic stresses, disease, and low nutrients (Coleman-Derr and Tringe 2014, Fierer et al. 2010) though these benefits may be helped or hindered by microbial community assembly processes. Thus, a clear understanding of microbial community assembly is needed before we can create a sustainable microbiome that increases crop yield stability (Dini-Andreote and Raaijmakers 2018).

Two broad processes—deterministic and stochastic—influence community assembly of species (Fierer et al. 2010).Deterministic processes rely on ecological filters, such as homogenizing (more closely related communities than expected by random chance) or heterogenous (more distantly related communities than expected) selection (Dini-Andreote and Raaijmakers 2018). Stochastic processes include dispersal events and drift or diversification (Hubbell 2001) grouped into homogenizing dispersal and dispersal limitation, where dispersal refers to the movement of species from one habitat to another and drift is the random division, death, or diversification (mutation) of individuals within a community (Dini-Andreote and Raaijmakers 2018, Hubbell 2001). Homogenizing dispersal includes high rates of dispersal between habitats leading to similar communities. Dispersal limitation can lead to high rates of community turnover and more dissimilar communities. Disentangling the community assembly processes in microbial communities is essential to fully understanding how these communities' function. For example, Ning et al. (Ning et al. 2020) found that homogeneous selection of soil

microbiome in a grassland was correlated with drought and higher plant productivity under warmed conditions.

The relative influence of stochastic and deterministic processes in community dynamics, vary through space and time (Ning et al. 2020, Stegen et al. 2012, 16]. Productivity and resource availability (Stegen et al. 2017) are among several factors that influence the relative importance of stochastic versus deterministic processes (Kardol et al. 2013). As crop plants develop and alter their environment it is reasonable to expect an increase in the relative influence of deterministic processes (Chase et al. 2010), as selective pressures filter the initial microbial community (Ning et al. 2020). If microbial communities can be linked to improved crop performance, crop development programs may be able to leverage the microbiome at specific stages of phenological development to improve plant performance. For example, Wagner et al. (2014) found that in *Boechera stricta* (Drummond's rockcress), microbes could alter plant flowering time—an important canola breeding target correlated with yield stability. Understanding how the community assembles before flowering would allow the potential manipulation of this community to optimize flowering time. A useful metric to disentangle community assembly processes is to use a null model framework based on the phylogeny of the microbial communities (Dini-Andreote and Raaijmakers 2018, Stegen et al. 2012, Stegen et al. 2017). The phylogeny is repeatedly randomized to give a distribution of theoretical phylogenies that could occur if no selection processes were acting upon the community (Stegen et al. 2017). If the observed phylogeny falls two standard distributions outside of the mean null model distribution, then we can conclude that some selection process is acting upon the real community (Stegen et al. 2017). This framework allows for a more accurate estimation of ecological processes shaping microbial communities.

We selected eight phenologically diverse founder lines of a *B. napus* Nested Association Mapping (NAM) panel, to evaluate if bacterial community assembly in plant organs could be altered via breeding programs. We hypothesized that 1) community assembly processes differ among plant structures due to environmental differences and 2) assembly processes would vary with *B. napus* line and 3) the root surface and the leaves would have the strongest deterministic assembly processes leading to more homogeneous communities, whereas the rhizosphere would be dominated by stochastic community assembly processes leading to more heterogenous communities. The leaves, roots, and rhizosphere soil of eight lines of *B. napus* were sampled

weekly over the course of ten weeks beginning three weeks after planting when the plants were at the five to six leaf stage. All weeks after this are reported in weeks after planting (WAP). We then used a null-model framework as well as ordination approaches to elucidate the assembly processes governing bacterial community assembly throughout the growing season.

4.4 Materials and Methods

4.4.1 Field Collections

In May 2017, eight lines of *B. napus* (Mason et al. 2017, Clarke et al. 2016) were seeded at the Agriculture and Agri-Food Canada (AAFC) research farm outside of Saskatoon, Saskatchewan, Canada (52.1718° N, 106.5052° W). These lines of *Brassica napus* are part of the AAFC canola breeding program created by nested associating mapping, referred to as NAM lines (Clarke et al. 2016). They ranged in seed origin and color, fiber content, erucic acid content, and seed glucosinolate levels (SI table 1). Due to the low erucic acid content, several of these lines are not canola, but remain under the *B. napus* classification. Bazghaleh et al. (2020) (Bazghaleh et al. 2020) described the experimental design extensively, but briefly, the experiment was a randomized complete block design consisting of three replicate blocks (6.1 m long by 1.8 m wide) with each *B. napus* line arranged randomly within each block. All lines were planted on May 29, 2017. The site received 127.9 mm of precipitation throughout the growing season with a mean air temperature of 16.4 °C. Both the mean temperature and precipitation were slightly below average for the region. Leaf, root, and rhizosphere samples were collected from each of the eight lines in each block every week for ten weeks beginning on June 20, 2017, until August 22, 2017. The collections began three weeks after planting when the plants were are the 4-6 leaf stage. Root and rhizosphere samples were collected from the same individual plant, however due to the destructive sampling methods, leaf samples were collected from different plants within the plot.

Root and rhizosphere samples were collected by combining three canola plants from each plot using a sterilized trowel to a depth of approximately 10 cm. Edge rows were avoided to avoid possible contamination with other lines or weeds. Roots with attached rhizosphere soil were placed in a bag, closed, and placed on ice. All samples were stored at 4 °C until processing. Upon processing aboveground material was removed and soil not attached to the roots was

collected and stored at -80 °C for further analysis. The roots, with adhering rhizosphere soil, were then transferred to a flask containing 100 ml of sterile 0.05M NaCl buffer and shaken at 180 rpm for 15 minutes. After shaking, the roots were removed, rinsed with deionized water, and weighed. A subsample of root material was taken using a flame sterilized scalpel and frozen at -80 °C for later DNA extraction. The buffer and soil mixture were transferred to centrifuge tubes and centrifuged at 5000 rpm for 15 minutes at room temperature. The pellet containing the rhizosphere soil was transferred to 1.5 ml tubes and frozen at -80 °C for future DNA extraction. A total of 28 root and rhizosphere samples (8 lines by three blocks, with 3 randomly selected duplicate biological samples) were collected each week over the ten week sampling period.

Leaf samples were selected by avoiding leaves with visible signs of disease, insect damage, or senescence. Additionally, plants on the edge of plots were avoided as these plants were visibly dusty. During flowering, *B. napus* rapidly drops petals and leaves with heavy flower contamination were also avoided. During the seed development and ripening stages when leaf senescence was advanced, leaves with large amounts of necrotic tissue were avoided. Leaf samples were placed into sterile whirl-pak bags (Nasco, Wisconsin, USA) and placed onto ice. Leaf samples from the same NAM line, but from different blocks were not combined and plants were not destructively sampled as only a single or two leaf were sampled. Samples were then returned to the lab and stored at – 80 °C until further processing. A total of 28 leaf (8 lines by three blocks, with 3 randomly selected duplicate biological samples) were collected each week over the ten week sampling period.

4.4.2 DNA Extraction and Amplification

DNA was extracted from 50 mg root tissue using Qiagen PowerPlant extraction kit (Hilden, Germany) following manufacturer instructions. DNA was extracted from 250 mg rhizosphere soil using Qiagen PowerSoil extraction kit following manufacturer instructions. Frozen, brittle leaves were crumbled manually in the whirl-pak and a 0.05 g subsample was taken and extracted using Qiagen PowerPlant extraction kit (Hilden, Germany) following manufacturer instructions. Extraction duplicates were included. All root and rhizosphere samples were spiked with a known concentration of *Aliivibrio fischeri* as an internal standard (Smets et al. 2016). Initially, this was also done with the leaf samples, but after sequencing it was found that likely due to the naturally low bacterial abundances on leaves, the majority of samples only contained *A. fischeri* and little host bacteria. Leaf samples were re-extracted without the spike

which greatly improved bacterial amplification. After extraction, DNA was tested for quantity and quality following the standard Qubit protocol (Thermo Fisher Scientific, Waltham Massachusetts).

Root and rhizosphere DNA from soil was standardized to 5 ng/µl prior to amplification. DNA from roots were standardized to 1.5 ng/µl prior to amplification. The V4 region of the 16S rRNA was amplified using the primer set 342F with Illumina adapters (5’ - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CTA CGG GGG GCA GCA G - 3’) and the 806R (5’ - GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGA CTA CCG GGG TAT CT - 3’) (Mori et al. 2014). The PCR reaction mix (25 µl total) contained 2.5 µl DreamTaq Buffer (Thermo Fisher Scientific, Waltham Massachusetts), 2.5 µl dNTP mix (Invitrogen, Carlsbad, California), 1 µl of each primer, 0.25 µl DreamTaq (Thermo Fisher Scientific, Waltham Massachusetts), 17.75 µl nuclease free water, and 2 µl of the standardized template DNA. The PCR conditions were 95 °C for 5 minutes as an initial denaturization, followed by 95 °C for 30 seconds, 54 °C for 30 seconds, 72 °C for 30 seconds for 35 cycles, and a final elongation of 72 °C for 7 minutes. Negative controls and PCR duplicates were included

Template DNA from leaf samples was standardized to 4 ng/µl prior to amplification. Bacterial diversity in leaves was assessed by amplifying the V4 region of the bacterial 16S rRNA using the primer set 515F with Illumina adapters (5’ – TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA - 3’) and the 806R (5’ - GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGA CTA CCG GGG TAT CT - 3’) (Walters et al. 2015). The 515F/806R primers were selected after failed attempts to amplify with the same primers as the root and rhizosphere. While there are individual primer biases, the 515F/806R were deemed the best possible replacement primers after many leaf amplification failures, as the 342F/806R primer pair covers the entire fragment length of the leaf primer set (Mori et al. 2014). The PCR reaction mix consisted of 7 µl Invitrogen Platinum SuperFi PCR master mix (Thermo Fisher Scientific, Waltham, Massachusetts), 0.1 µl of each primer (10 µM stock), 3 µl (5 µM stock) plastid peptide nucleic acid blocker (pPNA), 2 ul (5 µM stock) mitochondrial peptide nucleic acid blocker (mPNA) (PNA Bio, California, USA), 10.3 µl nuclease free water, and 2 µl of the standardized template DNA. PNAs were included to block the amplification of host DNA, plant mitochondria, and chloroplasts, which are a common

contaminant from plant tissues (Ray et al. 2000, Von Wintzingerode 2000). The PCR conditions were 95 °C for 5 minutes as an initial denaturization, followed by 95 °C for 30 seconds, 78 °C for 10 seconds, 54 °C for 45 seconds, 72 °C for 60 seconds for 35 cycles, and a final elongation of 72 °C for 7 minutes. Negative controls and PCR duplicates were included.

PCR product was purified to eliminate primers and impurities using 1:1 ratio of Nucleomag NGS clean-up and size select (D-mark Biosciences, Scarborough, Ontario). Randomly selected technical duplicates were included during DNA extraction, amplification, and sequencing stages adding in 56 duplicates, bringing the total sample size up to 326. After purification, samples were indexed following the Illumina protocol, purified again to remove excess index primers, quantified and standardized to 4 nM, and pooled. Pooled libraries were then sequenced using the Illumina MiSeq platform using V3 chemistry. Leaf samples were sequenced separately from root and rhizosphere samples. A total of 307 root, 307 rhizosphere soil, and 326 leaf samples were sequenced. Leaf sequencing runs included more technical duplicates than root/rhizosphere runs to assure amplification due to previous sequencing failure. Quality assurance/control samples included field duplicates, DNA extraction duplicates, library preparation duplicates, and sequencing duplicates.

4.4.3 Data Processing

A total of 12,813,586 reads were produced for rhizosphere samples with an average of 41,874 per sample. For roots, a total of 73,911 were produced with an average of 241 reads per sample. For leaves, 10,839,325 reads were produced with an average of 18,186 reads per sample were produced. Sequences were imported into QIIME2 v. 2019.1 (Bolyen et al. 2019) and primers were removed using cutadapt v. 2020.2.0 (Martin et al. 2011). Reads were then processed into amplicon sequence variants (ASVs) (Janssen et al. 2018) and chimeras were removed using Deblur (Amir et al. 2017), resulting in 1,968 ASVs for leaves, 8,987 ASVs for rhizosphere samples, and 990 ASVs for root samples. ASVs were classified using a 342F/806R-trained (root/rhizosphere) or a 515F/806R-trained (leaves) V3/V4 SILVA 132 database (Quast et al. 2013). For leaf samples, host mitochondria and chloroplasts were removed after classification. Host DNA ranged from 6% to 100% of the read in each sample with an average of 32% across samples. Mitochondria and chloroplasts were also removed after classification for root and rhizosphere samples, however they comprised a very low percentage of the overall reads. Reads

classified as archaeal, eukaryotes or unassigned at the kingdom level were removed from all samples, but were not abundant overall. The abundance and taxonomy artifacts produced in QIIME2 were exported to BIOM format (McDonald et al. 2012) for processing in R v. 3.5.3 (R Core Team). Global singletons or ASVs with a sum of zero were removed. Phylogenetic trees were created using the fragment insertion method in QIIME2 (Quast et al. 2013). Root and rhizosphere abundances were standardized to the *Aliivibrio fischeri* spike.

4.4.4 Statistical Analysis

Each plant compartment represents a very different environment, meaning that the bacterial communities in each will experience different assembly processes. Due to this, and the necessary use of different primer sets, each plant compartment was analyzed separately, and no direct comparisons were made between plant compartments. Each analysis was repeated three times, for the leaf, root and rhizosphere communities.

Abundance-based Coverage Estimate (ACE), the Simpson index were calculated using the estimate_richness function on phyloseq (Oksanen et al. 2007) and Pielou's evenness were calculated using the vegan package v. 0.5.1 (McMurdie et al. 2013). Permutational analysis of variance (PERMANOVA) was performed using the adonis function in the vegan package in R (McMurdie et al. 2013). Bray Curtis distance matrices were calculated among samples from the same plant compartment (e.g. root) for each time point using the using the phyloseq package v. 1.34.0 (McMurdie et al. 2013).

The BBCH-scale (BBCH is not an acronym, but the name of the scale) is a scale used to uniformly identify and quantify the phenological stages of plant development, with scales developed for species specific development (Lancashire et al. 1991). All *B. napus* lines were assigned BBCH weekly using the Canola Council of Canada BBCH guide (Canoal Council of Canada 2020) and averaged. This was done because despite identical planting times, the eight *B. napus* lines did exhibit some differences in plant development. Sampling week 3 and 4 (WAP) took place during the leaf development stage for most *B. napus* lines sampled, with bolting during week 5. Peak flowering was reached for most lines during sampling week 6, with seed development occurring in the following two weeks. The last four weeks of sampling were characterized by ripening of the *B. napus* seed pods.

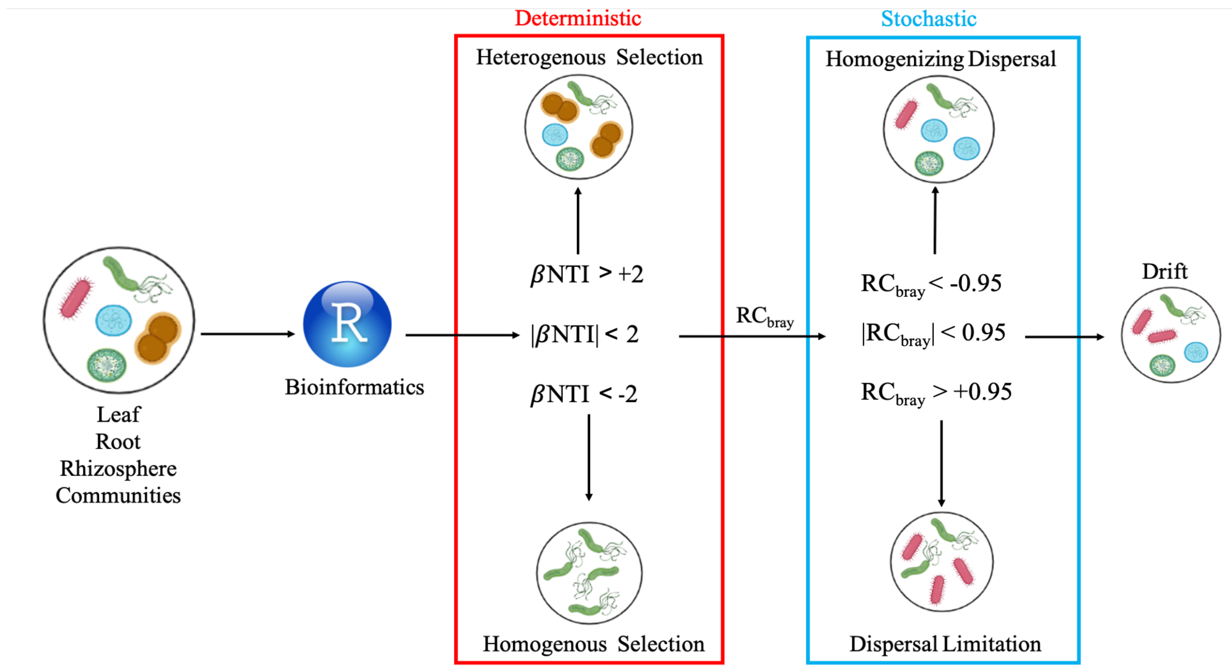


Figure 4.1 A conceptual diagram of the determination of the assembly processes. Leaf, root and rhizosphere communities were sampled, sequenced and processed (See methods). Following processing, amplicon sequence variants (ASVs) were imported to R (R Core Team 2018). A null model was generated using 999 randomizations from all ASVs present in that community. All pairwise comparisons with a |βNTI| > 2 are classified as deterministic with βNTI > +2 indicating heterogenous selection and βNTI < -2 indicating homogeneous selection. Observations with values |βNTI| < 2 and RC_{bray} > +0.95 were classified as dispersal limitation and observations with values |βNTI| < 2 and RC_{bray} < -0.95 were classified as homogenizing dispersal. Pairwise comparisons within |βNTI| < 2 and |RC_{bray}| < 0.95 indicated drift or diversification assembly processes were occurring.

Community assembly processes were approached using the null model framework (Vellend 2010, Stegen et al. 2012). Net Relatedness Index (NRI) were calculated by using the ses.mpd function (abundance.weighted=TRUE) in the picante package v. 1.8.2 (Kembel et al 2010). NRI is the number is number of standard deviations that the observed phylogeny differs from the null mean pairwise distance (MPD) after 999 iterations (Webb 2001). An NRI value of < -2 indicates that the community is phylogenetically more dispersed than expected where as an NRI value of > +2 indicates that the community more phylogenetically clustered the expected. Similarly, Nearest Taxa Indices (NTI) were calculated using the ses.mntd function (abundance.weighted=TRUE) in the picante package (Kembel et al. 2010). NTI is the number of standard deviations that the mean nearest taxon distance (MNTD) (Webb 2001). differs from the null MNTD after 999 iterations. An NTI value of -2 indicates that the community is more

distantly related than expected where as an NTI value of +2 indicates that the community more closely related than expected. While these metrics are similar, NRI is more sensitive to tree wide trends of clustering and evenness whereas NTI is more sensitive to these trends closer to the phylogeny tips (Stegen et al. 2013).

Following Stegen et al. (2013), selection pressures were quantified using βNTI metric in the picante package (comdist, abundance.weighted= TRUE) and Bray-Curtis-based Raup-Crick (RC_{bray}) in the iCAMP package v. 1.2.9 (Kembel et al. 2010, Ning et al. 2020). RC_{bray} is the probability that a given community is more dissimilar (+1) or less dissimilar (-1) than expected by chance (Chase et al. 2011). Like the previous metrics, RC_{bray} uses successive iterations to determine these probabilities. βNTI measures the difference between the observed βMNTD and the null βMNTD. The null distributions for both metrics were generated weekly for each plant compartment using 999 randomizations. $|\beta NTI| > 2$ indicates that deterministic selection dominates community assembly processes at a 5% significance level (Stegen et al. 2012). βNTI values > 2 were classified as heterogenous selection. βNTI values less than two were classified as homogeneous selection. Observations $|\beta NTI| < 2$ indicated predominance of stochastic, rather than deterministic processes ($p<0.025$). Pairwise comparisons between βNTI and RC_{bray} were done to determine the stochastic processes dominating bacterial community assembly (Fig. 1). Observations with values $|\beta NTI| < 2$ and $RC_{bray} > +0.95$ were classified as dispersal limitation and $|\beta NTI| < 2$ and $RC_{bray} < -0.95$ classified as homogenizing dispersal (Ning et al. 2020, Lin et al. 2012, Vellend 2010, Webb 2001). Pairwise observations not having values of $|\beta NTI| < 2$ or $|RC_{bray}| < 0.95$ were categorized as drift or diversification (Fig. 1). This could indicate that this population is weakly experiencing any of the previously mentioned processes or that the community is undergoing drift, which is the random division, death or mutation (diversification) of individual community members.

To examine which factors influenced deterministic selection processes distance-based redundancy analysis (dbRDA) (Legendre and Andersson 1999) was performed on weighted UniFrac distance matrices (Lozupone and Knight 2005) using the capscale function in the vegan package in R (Oksanen et al. 2019). The UniFrac distances were calculated using the phyloseq package (McMurdie and Holmes 2013). UniFrac distances were used to preserve the phylogenetic relationships in the communities. Leaf, root, and rhizosphere dbRDAs were constrained by BBCH, week prior mean temperature and precipitation, sampling day mean

temperature and precipitation and NAM line. All code available at https://github.com/jbell364/Canola-Selection.

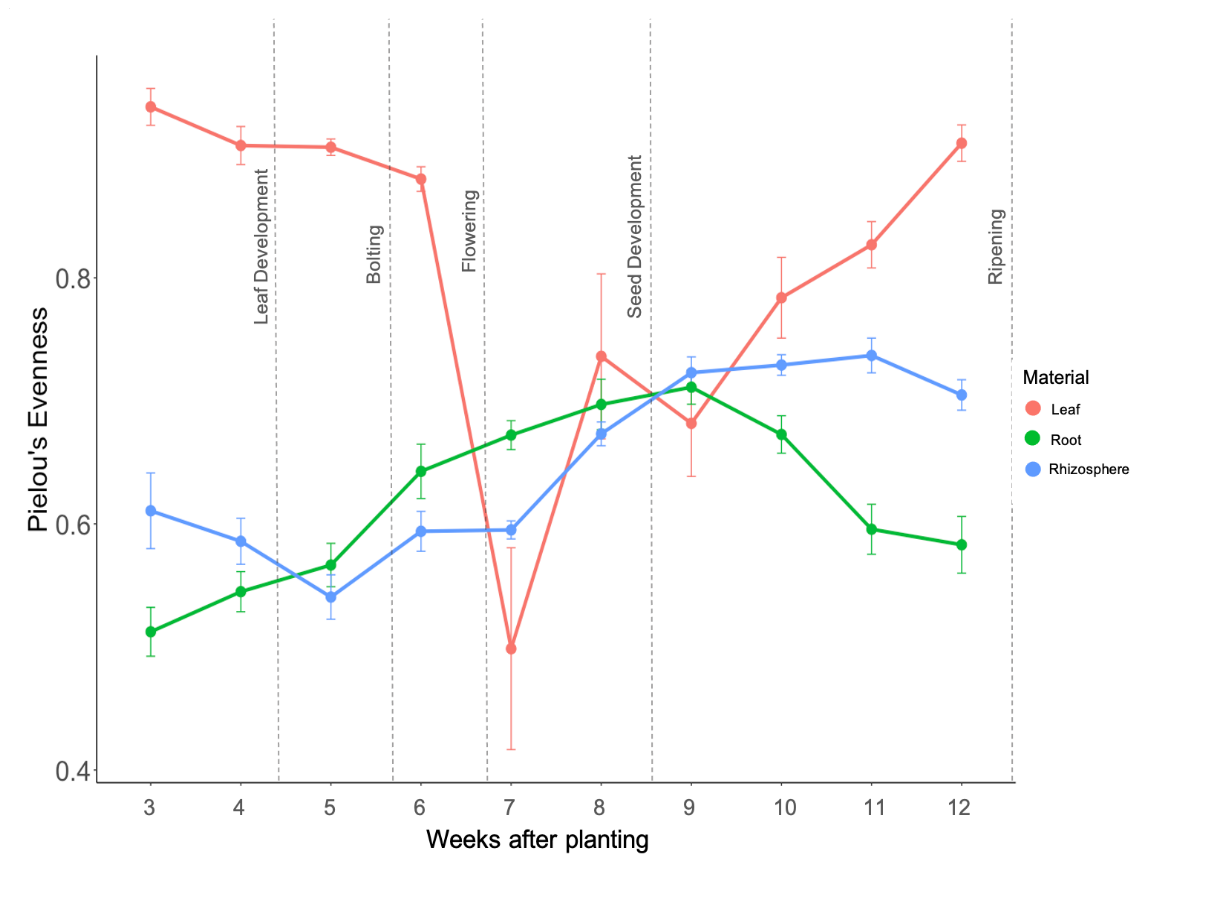


Figure 4.2 Pielou's evenness for the leaf (red), root (green), and rhizosphere (blue) over the ten-week sampling period. Each point represents 27 samples, and the error bars are the standard error. Growth stage is indicated by the dashed lines. The larger the number the more even the community.

4.5 Results

Pielou's evenness (Fig. 2) (Pielou 1966) was the lowest during flowering for both root and leaf. Interestingly, rhizosphere diversity was at its lowest during flowering but increased steadily after flowering. However, much like the leaf and root communities, Pielou's evenness was the lowest for rhizosphere communities during flowering (Fig. 2, SI Table 1). Both the Abundance-based Coverage Estimate (ACE) (Chao and Lee 1992), and the Simpson index (Jost

2007) for the leaf and root bacterial communities reached their peaks during weeks six and seven, or when the plants were flowering (SI Table 1).

Bacterial communities on the leaf, root, and rhizosphere were primarily composed of Proteobacteria with the largest class being Gammaproteobacteria (SI Fig. 1). In leaves, Gammaproteobacteria ranged from a high of 75% during week nine to a low of 40% during week twelve. In leaves, the second largest group consisted of classes not found in the root and rhizosphere communities (SI Fig. 1) but consisted primarily of Bacteroidetes (7%), Acidobacteria (6%), and Firmicutes (6%). In the root, Gammaproteobacteria comprised greater than 50% of the community in every week except week three. After Gammaproteobateria, the dominant class in the roots was Bacteroidia. Gammaproteobacteria was also the dominant class in the rhizosphere, ranging from 30% during week three to 79% during week six (SI Fig. 1). Similar to the root communities, Bacteroidia was the second most dominant class present in the rhizosphere.

The influence of *Brassica napus* line (NAM line) on bacterial community composition was inconsistent in each plant compartment and showed no clear trend throughout the growing season. Specifically, NAM line was never a significant explanatory variable for leaf communities. For root bacterial communities, NAM line was a significant explanatory variable only during weeks four and seven ($P = 0.01$ and 0.001, $R^2 = 0.30$ and 0.2, respectively). NAM line was a significant explanatory variable for six out of the ten sampling weeks for rhizosphere bacterial communities ($P < 0.05$, $R^2 = 0.23 – 0.32$, SI Table 3) but there was no consistent time period in which NAM line was or was not significant. Finally, even when NAM line was significant, it rarely explained much of the variation (SI Table 2).

Growth stage (BBCH) was consistent influence on all phylogenetic metrics. BBCH was a significant ($P<0.001$) explanatory variable for NTI, NRI (Table 1), and βNTI (SI Table 2). Interestingly, NAM line was significant for root NTI values ($P=0.03$) values but not for root NRI values nor root βNTI values (SI Table 3). Similarly, NAM line was significant for rhizosphere βNTI values, but not rhizosphere NTI nor NRI values. There were no significant interactions between NAM line and BBCH growth stage for any compartment.

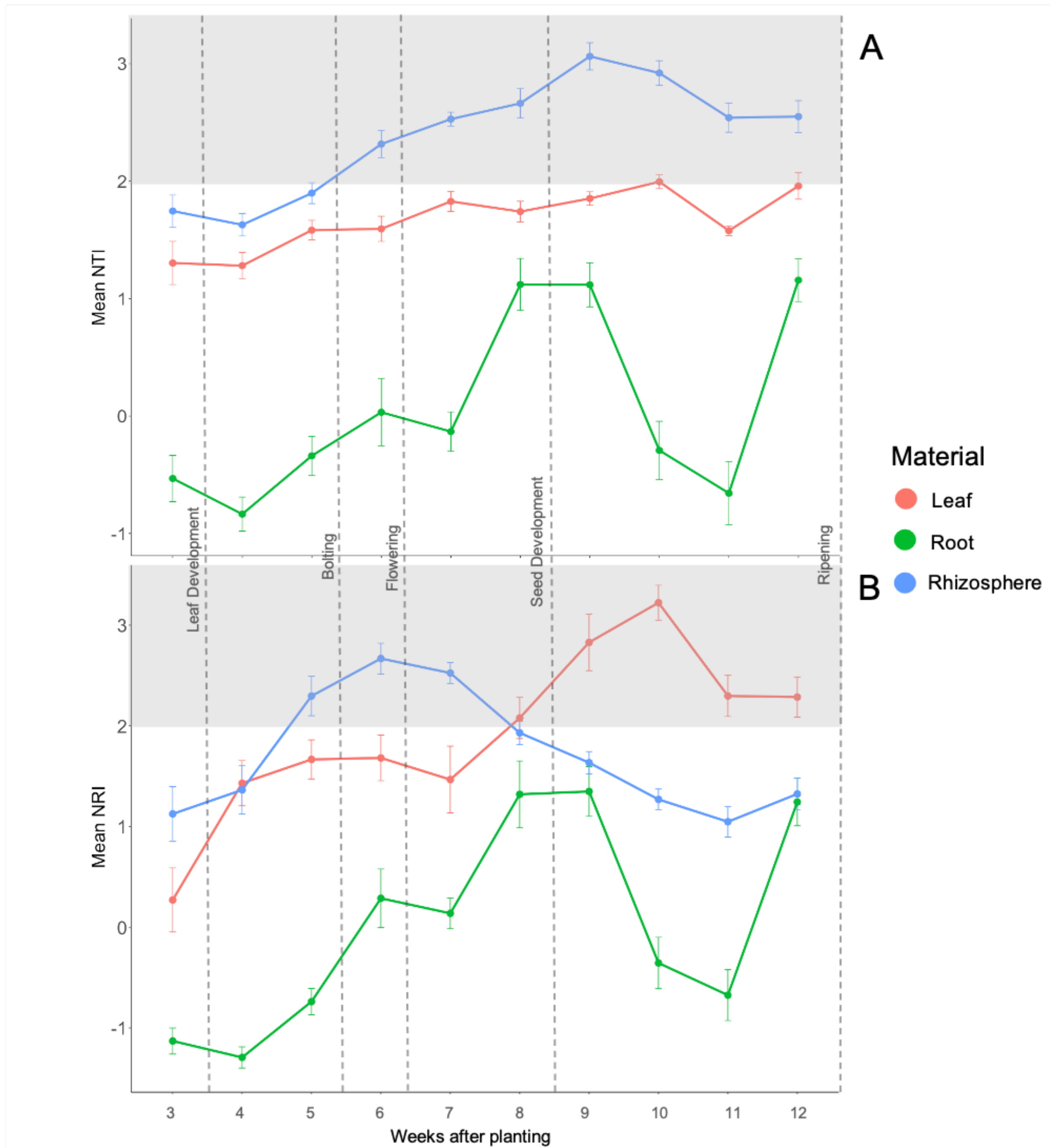


Figure 4.3 (A) Mean Nearest Taxon Index (NTI) for leaf, root and rhizosphere samples over the ten week sampling period. (B) Mean Net Relatedness Index (NRI) for leaf, root, and rhizosphere samples over the ten week sampling period. Each point represents 27 samples, and the error bars are the standard error. Growth stage is indicated by the dashed lines. Positive values indicate more phylogenetic clustering than expected by chance, whereas negative values indicate phylogenetic overdispersion. The gray shaded area indicates a significant ($p > 0.05$) phylogenetic clustering compared to the null hypothesis.

The leaf communities were always more clustered than expected, especially after flowering, suggesting that there were selection pressures occurring. Mean leaf NRI values were consistently greater than zero throughout the entire growing season indicating an increasing trend of phylogenetic clustering (Fig. 3B) (Kembel 2009). However, leaf NRI values did not differ from the null hypothesis ($|NRI| < 2$; $P > 0.05$) until weeks five to twelve ($P \leq 0.05$). Leaf NTI did not differ from the null hypothesis until week nine ($P \leq 0.05$; Fig. 3A).

In the root, no strong clustering or over dispersion was detected. Mean Root NTI values were consistently different than zero, though did not differ from the null hypothesis ($P > 0.05$; Figure 3A). Root NRI values showed similar trends as NTI values in that they were consistently greater than zero and did not differ from the null hypothesis (Fig. 3B). However, BBCH ($P < 0.001$) was significant for both NTI and NRI values and NAM line ($p=0.0308$) significant for NRI values.

Rhizosphere NTI values showed stronger clustering of the bacterial communities than the rhizosphere NRI values. Rhizosphere NTI values were greater than zero and differed from the null hypothesis ($P => 0.05$; Fig. 3A). Rhizosphere NRI were greater than zero but only differed from the null hypothesis in weeks six through nine (Fig. 3B). BBCH was significant ($P<0.001$) for both rhizosphere NTI and NRI values and NAM line was not significant.

βNTI values had consistent trends to NRI and NTI values (Fig. 4). After week five, from flowering to ripening, rhizosphere $\beta NTI > -2$ ($P > 0.001$) indicating homogenous selection was occurring. Root βNTI values only differed from the null hypothesis during week nine ($P > 0.01$) indicating homogenous selection was occurring during this week. Similarly, leaf βNTI values only differed from the null hypothesis ($P>0.001$) during week six, or flowering, however, unlike the root and rhizosphere, the leaf $\beta NTI < +2$, which denotes heterogenous selection.

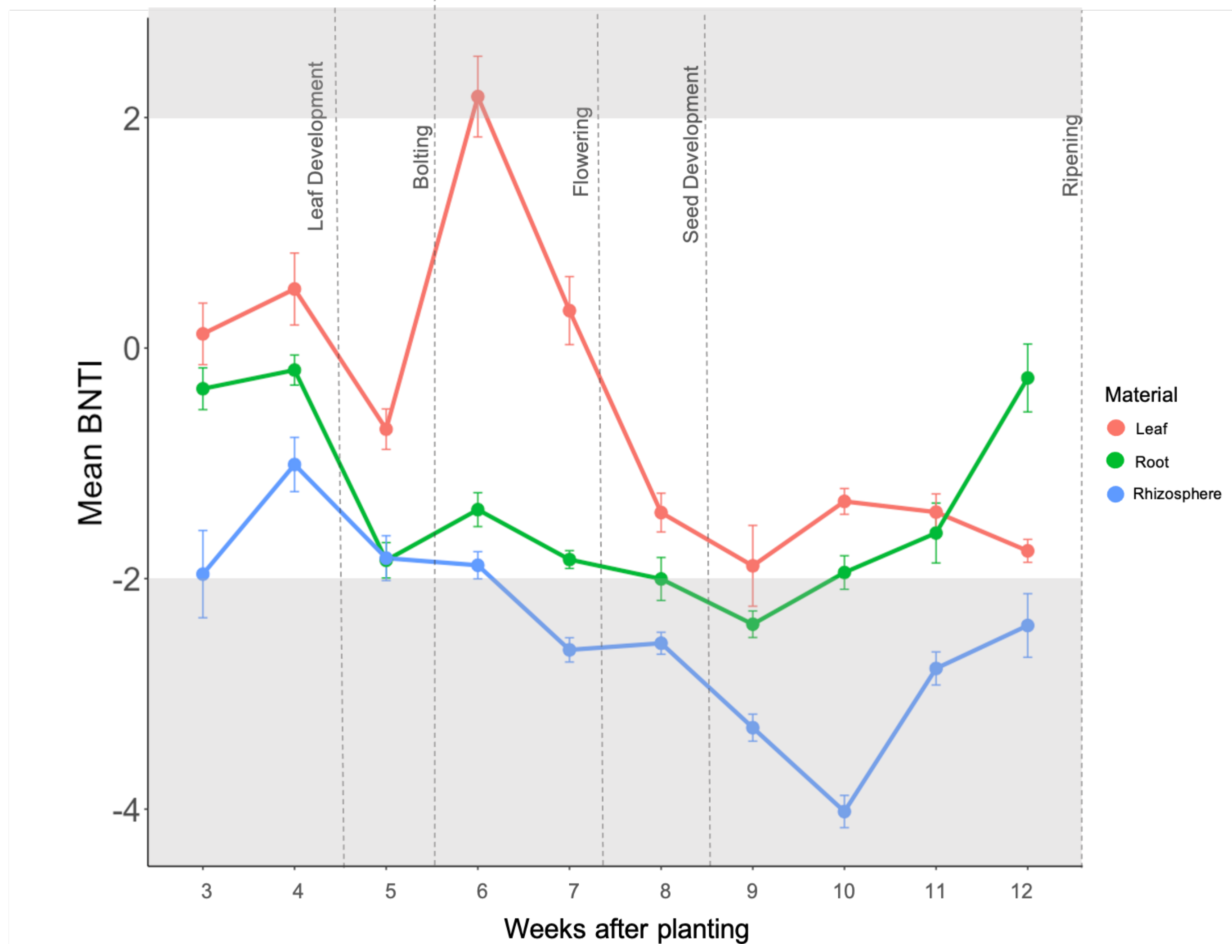


Figure 4.4 Mean βNTI for leaf, root, and rhizosphere samples over the ten week sampling period. Each point represents 27 samples, and the error bars are the standard error. Growth stage is indicated by the dashed lines. Positive values indicate heterogenous selection is occurring whereas negative values indicate homogeneous selection. The gray shaded area indicates a significant deviation from the null hypothesis.

The primary assembly process in leaves was drift/diversification (Fig. 5A) with only weeks six and seven not being dominated by drift/diversification. Interestingly, after week seven, selection in the leaves moved from heterogenous selection to homogeneous selection. Drift also dominated bacterial community assembly in the root until week seven when the dominant process became homogeneous selection (Fig. 5B). Homogeneous selection remained the dominant process until week twelve, when drift dominated again. Homogeneous selection was the dominant process in all weeks in the rhizosphere with the exception of weeks four and six (Fig. 5C). Rhizosphere bacterial communities experienced a noteworthy amount of dispersal

limitation, which occurred in weeks three, four and six with dispersal limitation as the dominant process during week four (62%). Dispersal limitation was seen in the roots, but this process made up less than ten precent in every week except weeks six, seven, and nine.

To assess which environmental factors could be acting as abiotic filters causing homogenous selection, dbRDAs were done on the leaf, root and rhizosphere (Fig. 6) and were constrained by BBCH, week prior mean temperature and precipitation, sampling day mean temperature and precipitation and NAM line. These filters captured most variation in the leaf (19.3%, Fig. 6A), followed by the root (18.4%, Fig. 6B) with the lowest amount of variation explained in rhizosphere communities (13.7%, Fig. 6C). Interestingly, while capturing a decent amount of the variation in each plant compartment, none of the factors were significant nor did the amount of variation captured account for the high levels of deterministic selection seen, especially in the rhizosphere. This suggests the presence of a high number of unmeasured filters, which could be both biotic (inter- or intraspecies interactions) or abiotic (soil factors, relative humidity, etc.).

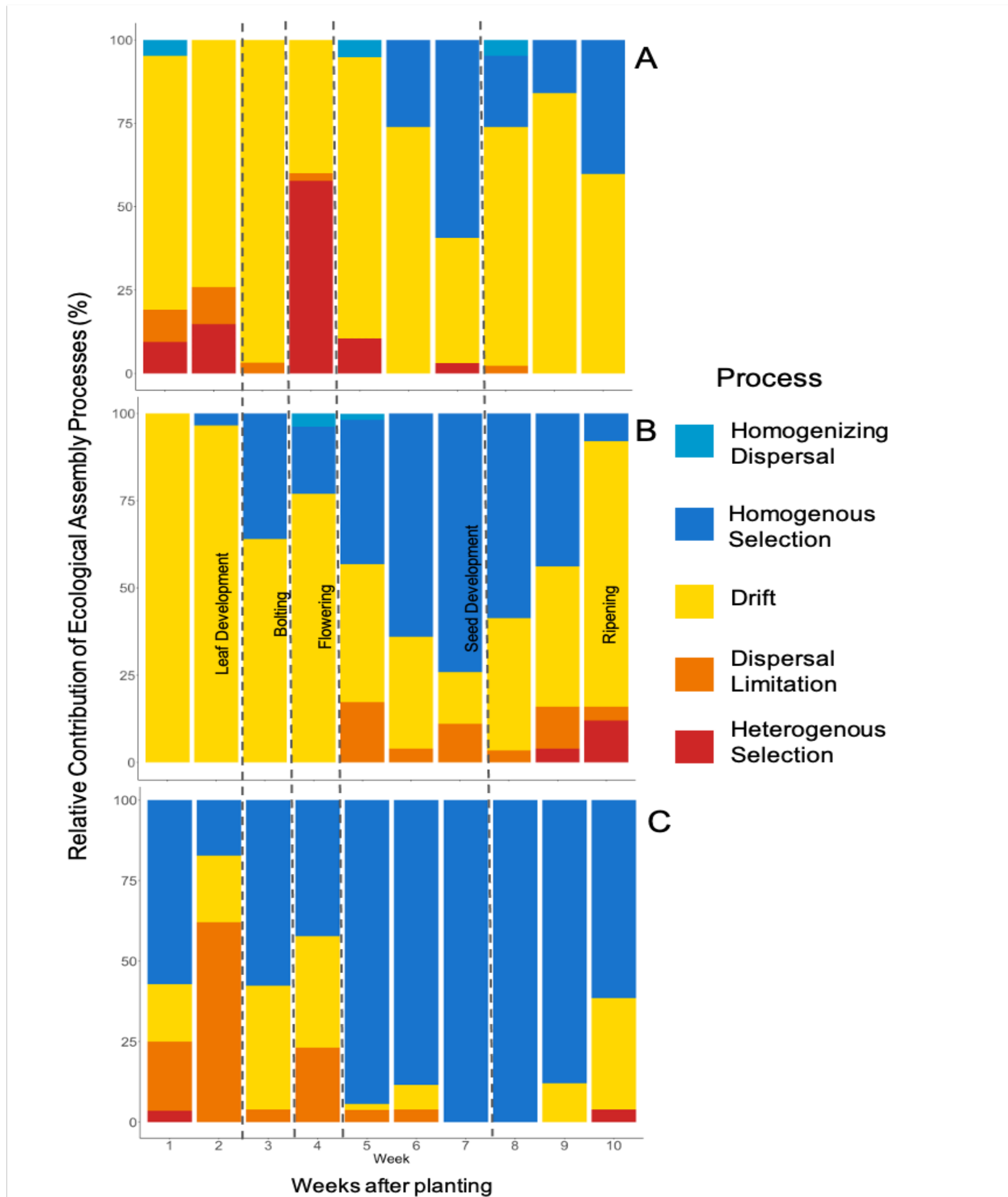


Figure 4.5 Ecological assembly processes in the bacterial communities present in the leaf (A), root (B) and rhizosphere (C) across all ten sampling weeks. Deterministic processes were classified as heterogenous selection (βNTI > 2) or homogeneous selection (βNTI < -2). Stochastic processes were classified as homogeneous dispersal (|βNTI| < 2 and RC_{bray} < -0.95) or dispersal limitation (|βNTI| < 2 and RC_{bray} > +0.95). Pairwise observations within the confines of |βNTI| < 2 and |RC_{bray}| < 0.95 were classified as drift/diversification. Growth stage is indicated by the dashed lines.

Figure 4.6 Distance-based redundancy analysis (dbRDA) of the weighted UniFrac distances for the leaf (A, pink), root (B, green) and rhizosphere (C, blue) for the entire ten week sampling period. Each has been constrained by BBCH, week prior mean temperature and precipitation, sampling day mean temperature and precipitation and NAM line. The amount of variation captured for each is in the left-hand corner.

4.6 Discussion

The root is generally thought to be a highly selective environment (Edwards et al. 2015, van der Heijden and Schlaeppi 2015), however deterministic selection accounted for more than 50% of the community assembly processes in only three out of the ten weeks, which was not what we hypothesized. Root communities were not more or less clustered than expected by chance (NTI and NRI assessments) and strong selection processes were not occurring (βNTI) suggesting that root selection processes are not as strong as previously thought. Using a different approach, ie dbRDA, we came to the same conclusion. If the selection processes were primarily deterministic, as we hypothesized, the root assembly processes would have been like the processes observed in the rhizosphere. One reason the root may have been seen as a highly selective environment is because it consistently less diverse than the rhizosphere soil (Philippot et al. 2013), van der Heijden and Schlaeppi 2015, Wagner et al. 2016). Our work suggests that this lack of diversity found in the root, relative to the rhizosphere, may arise from the priority effect or competitive exclusion. When a bacterial species is able to establish itself in or on the root, it could maintain that niche solely through competitive exclusion (Tan et al. 2015), not allowing more bacterial species to establish and increase diversity (Jacoby and Kopriva 2019). If competitive exclusion is the primary reason roots lack diversity, then it would follow that the main community assembly process is drift/diversification, as the community would not change significantly throughout the growing season once the species has established and excluded others. Alternatively, the stable root community could be an example of the priority effect where the order and timing of arrival dictates the species composition of the root (Fukami and Najajima 2011). If assembly in the root is being affected by the priority effect, the dominance of Gammaproteobacteria in the root could be an indication of this. Gammaproteobacteria appeared quickly and its relative abundance did not change much over the ten-week sampling period. Most likely, the stable root community and the predominance of drift as the main assembly process is a combination of both competitive exclusion and the priority effect.

The leaf is a harsh environment with high prokaryotic mortality and daily disturbance events from changes in temperature, moisture, and UV radiation (Vorholt 2012, Vacher et al. 2016). Given these difficult conditions, it follows that the major selection pressure is the neutral process of drift/diversification rather than a more plant driven, deterministic process. Given these

severe conditions it could be possible that no single process was able to dominate due to the high mortality rates and frequent disturbance. Temperature and precipitation accounted for more variation in the leaf community than the root and rhizosphere. Both precipitation events, as well as large temperature fluctuations, would be recurrent disturbance events for the leaf community, causing stochastic processes to dominate as deterministic processes would be halted. Additionally, the root and rhizosphere is more protected from these recurrent disturbances, which would allow for deterministic processes to continue, which is what was observed.

The rhizosphere effect has been well documented (Smalla et al. 2001, Gregory 2006, Philiippot et al. 2013) wherein the rhizosphere exhibits changes in bacterial richness when compared to the bulk soil. Given the rhizosphere effect is consistent and drastic, there must be deterministic selection processes at work. We saw this reflected in the root where homogeneous selection comprised more than 50% of the selection processes for all weeks except four. In fact, in weeks nine to eleven, homogeneous selection comprised almost all of the selection processes occurring in the rhizosphere. The dominance of homogenous selection could have been caused by the larger root system which exerted more selection pressure, both of which are correlated with growth stage, which has been documented previously (Ceja-Navarro et al. 2021). The increase in beneficial bacteria during and after flowering has been documented (Bell et al. 2020) so the *B. napus* plants are likely selecting for beneficial species here to increase seed set and ripening. During seed development and ripening the plant likely undergoes an increased demand for water and nutrients. To meet these demands, the rhizosphere community would have to shift in order to increase nutrient cycling, hence the dominant deterministic process is homogeneous selection.

One of the hypotheses of this study was that assembly processes would vary with *B. napus* (NAM) line but we were not able to show this. The NAM lines selected for this study were chosen to emphasize differences in various characteristics in hopes of understanding how NAM line shaped the microbiome (Bazghaleh et al. 2020). Despite this careful selection, NAM line had the smallest effect on assembly processes after plant compartment and growth stage. In the rhizosphere, where NAM line had the most consistent effect, the influence of line was not consistent throughout the growing season suggesting that it did not have a stable influence on the rhizosphere. This is contrary to other studies which have shown a large effect of plant line on microbial community structure (Edwards et al. 2015, Colemam-Derr et al. 2016, Dombrowski

et al. 2017). The lack of line differences could be a specific effect of *B. napus*. Previous work on these same NAM lines has shown that there is no consistent effect of NAM line on phyllosphere bacterial communities nor on the seed microbiome (Morales Moreira et al. 2021, Bell et al 2020). Copeland et al. (2015) did not note any effect of canola line on the phyllosphere nor rhizosphere as well. Only genetically modified *B. napus* demonstrated line level differences in the microbiome but these did not persist between growing season (Dunfield and Germida 2001, Siciliano and Germida 1999, Dunfield and Germida 2003). This suggests that for the microbiome of canola, environment and plant growth stage will impact microbial community assembly processes more than differences in canola line.

Growth stage consistently accounted for differences in NTI, NRI, and βNTI (Table 1, SI Table 4) in contrast with variable *B. napus* line (NAM) influence. Growth stage effect outweighs that of NAM lines that are independent of growth stage alterations. Plants undergo large physiological shifts throughout their lifecycles (Nitsch 1965, Mohan and Rao 1984, Shu et al. 2010) which then correspond to changes in the plant microbiome (Smalla et al. 2001, Wagner et al. 2016, Copeland et al. 2015, Hilton et al. 2018, Gregory 2006). Changes in community assembly processes caused by shifts in plant phenology that result from breeding selection would change not only the composition of the plant associated communities through deterministic selection. However, shifts in phenology could also change the community dynamics, as one species may have an advantage over other species under these new selection pressures. These changes could alter the benefits that plant associated communities confer and open a route for more successful microbiome manipulation.

Plant breeders manipulate plant phenology, or growth stage (Piao et al. 2019), which is the largest determinant of bacterial community assembly processes on *B. napus*. Manipulating plant phenology as well as the environment conditions through inputs has been suggested as a means of engineering more robust plant microbiomes (Ryan et al. 2009, Quiza et al. 2015). Periods of time when the microbiome is undergoing strong selection will make good targets for microbiome engineering as strong selection likely means the plant is selecting for the most fit microbial communities. If the breeding goal is disease reduction, given that most canola diseases are transmitted aerially, it would be wise to focus on the leaf microbiome manipulation. The leaf bacterial community reaches maximum diversity and experiences the strongest selection during the flowering period. Any efforts to manipulate the bacterial microbiome on the leaf

should be done before or during when the plant flowers; alternatively, the flowering period could be extended to cultivate more of these beneficial bacteria. Similarly, if the breeding target is larger yields, then focusing on the rhizosphere communities after flowering would likely be the most beneficial. The rhizosphere communities are undergoing strong selection after flowering, which could mean the plant is selecting for beneficial relationships to improve seed production and ripening. Focusing breeding efforts on this time period could impact these processes. Additionally, further study needs to be done on the root exudation patterns occurring during the seed development and ripening periods to determine why the selection pressure is highest during these periods. Root exudation patterns could then serve as a mechanism to engineer beneficial root and rhizosphere communities. A better understanding of the assembly processes of plant microbiomes will allow for the most targeted manipulation and hopefully lead to more robust microbiomes which can improve agricultural sustainability.

5. SMOOTH BROME INVASION ALTERS MICROBIAL COMMUNITY ASSEMBLY PROCESSES AND ECOSYSTEM SERVICES

5.1 Preface

I analyzed soil and fungal sequencing data in this chapter to look at the effects of the invasive grass, *Bromus inermis* on microbial community assembly processes and ecosystem services at the Kernen prairie. Dr. Lamb designed the experiment, secured funding and provided feedback on the manuscript. Dr. Siciliano designed the experiment and supervised all part of the chapter from sample collection to writing.

5.2 Abstract

Invasive plants alter soil microbial communities and ecosystem services reducing the Earth's carrying capacity for humans. Many ecosystem services are underpinned by soil microbial communities, and these communities arise from assembly processes that are likely altered by invasion. We evaluated the hypothesis that invasive effects on grassland ecosystem services arise from changes in microbial community assembly processes caused by invasion. We sampled 515 plots undergoing invasion by smooth brome (*Bromus inermis*) at a native Rough Fescue prairie located near Saskatoon, Saskatchewan, Canada. Each week, for 26 weeks, we monitored invasion effects on vascular plant communities, ecosystem services, as well as bacterial and fungal community structures. We used the on-going *Bromus inermis* invasion to disentangle the effects of invasion from season on community structure. Invasive effects on ecosystem services interacted with seasonal (plant green-up, peak biomass, and plant senescence) effects but consistently disrupted ecosystem service provision. Invasion increased heterogenous selection in fungal communities but otherwise had minor effects on assembly process. Only ~20% of community composition could be ascribed to deterministic filters which hindered our ability to conclusively link invasion to assembly processes. Assembly processes explained changes in ecosystem services with bacterial assembly accounting for 2.5% of food, 4% of climate, 9% of conservation and 5.5% of fertility services. Fungal communities were less

consistent in their effects on ecosystem services with only food (3%) and water (4%) services influenced by assembly. After seasonality (27%), bacterial assembly processes (4%) accounted for the largest effect on ecosystem services, overshadowing invasion (2%) and fungal assembly (2%). At this 130-hectare site, after seasonal effects, bacterial assembly processes had the largest effect on ecosystem services with plant invasion placing a distant third.

5.3 Introduction

Plant invasion impacts ecosystem services, which are broadly defined as the benefits human derive from natural ecosystems (Daily et al. 1997). While the effect of invasive plant species on ecosystems can vary, it almost always decreases native diversity and abundance (Vilà et al. 2011). Interestingly, invasive species usually increase overall plant biomass causing greater nutrient and water demands which then causes the native species to be competitively excluded, leading to biodiversity loss (Vilà et al. 2011). Altering nutrient and water demands, coupled with changes in native biomass and abundance all have cascading effects on the soil which in turn will impact ecosystem services such as climate regulation, water purification and food production (Zhang et al. 2019). Changes in ecosystem services have lasting impacts on human health such as disease regulation, contamination of water and soil, decreased recreation and in the case of smooth brome invasion, the loss of agricultural capacity (Pyšek and Richardson). The mitigation and restoration of damaged ecosystem services can be extremely costly (Pejchar and Mooney 2009). For example, in 2017, the United States government spent an estimated three billion dollars on prevention, control and eradication of invasive species (Crafton and Angadjivand 2018).

The abundance and ranges of invasive plant species are expected to increase with climate change (Tylianakis et al. 2008). Invasive species can have severe impacts on the local flora, which in turn causes changes in soil microbial communities and the ecosystem services that these local floras and microfauna communities provide (Van der Putten et al. 2013). Any change plant community structure or composition will change the soil microbial communities by altering: (i) the input of organic matter (Ehrenfeld 2003, Van Der Putten et al. 2007), (ii) the quality and quantity of root exudations (Broz and Vivanco 2007, Knapp and Kovács 2012), and (iii) rates of nutrient cycling (Ehrenfeld 2003). While there has been work done to elucidate how invasive plants change soil properties (Gibbons et al. 2017), how these changes affect soil microbial

communities has yet to reach a consensus (Bunn et al. 2015, Zhang et al. 2019). It is possible that invasive plants will cause idiosyncratic changes in the soil microbial communities, i.e. each site/species combination will cause unique changes in the soil microbial community. However, it is also likely that the combination increased invasive biomass coupled with the loss of native biodiversity that causes changes in soil structure, organic matter input and nutrient cycling which will then alter the fundamentals processes by which microbial communities assemble.

Microbial community assembly processes are dominated by two broad forces, deterministic and stochastic processes (Fierer et al. 2010). Deterministic processes act on communities through ecological filters and can be further subdivided into two additional categories. These subdivisions are: homogenizing selection wherein the communities are more closely similar communities than expected by random chance, or heterogenous selection wherein communities are more distantly related than expected (Dini-Andreote and Raaijmakers 2018). Stochastic processes can be subdivided into three different processes: homogenizing dispersal (no impediments to dispersal), heterogenous dispersal (some impediment to dispersal, can lead to more dissimilar communities) and drift (random division, death, or mutation) (Hubbell et al. 2001, Dini-Andreote and Raaijmakers 2018). Given that both deterministic and stochastic processes are reliant on ecological and environmental factors, plant invasion will likely alter local microbial community assembly processes

The null-model framework used to assess microbial phylogenies is fairly recent it has several benefits over other methods to examine microbial community assembly processes. Firstly, it allows for the integration of both stochastic and deterministic assembly processes (Stegen et al. 2013) whereas previous methods classified unexplained variance as stochastic assembly processes (Zhou and Ning 2017). Secondly, this method uses microbial phylogenies to estimate microbial community assembly processes versus species abundance and richness estimates. Phylogenies estimate assembly processes much more accurately (Gerhold et al. 2015) because they can detect co-existence and therefore potential niche differentiation. The more phylogenetically clustered a community is, the more likely strong selection is occurring because ecological traits that conserve niche breadth are often highly conserved (Webb 2000, Martiny et al. 2015). If selection was not occurring, it would be more likely that the community would be experiencing overdispersion, as there would be no need for the conservation of traits nor niches, leading to more similar communities than expected by chance

While the null-model method is a better option for estimating assembly processes, it does have limitations linked to sampling effort and trait inference (Stegen et al. 2012, Zhou and Ning 2017). The null-model methods relies on the phylogeny of the regional species pool (Webb 2000, Stegen et al. 2012, 2013, 2015) and the estimation of the regional species pool will be a function of sampling effort. Further, regional species pool estimates are not directly comparable with other studies unless sampling efforts were identical. Secondly, the null-model relies on linking microbial traits associated with fitness in an environment with the sequencing target. Thus, the null-model assumes that 16S based phylogenies are linked to traits and thereby fitness. While horizontal gene transfer is a factor in many bacterial communities, many bacterial traits that confer specific advantages such as methanogenesis or other unusual metabolism strategies are deeply conserved (Martiny et al. 2015). Finally, given the difficulty of sequencing and constructing fungal phylogenies, the same assumptions of trait conservation are not as robust nor are most surveys of fungal diversity complete (Blackwell et al. 2011). However, despite its limitation, the null-model framework remains a strong estimate of microbial community assembly processes.

Smooth brome (*Bromus inermis*) is commonly planted as a forage grass in Western Canada despite being an invasive species that has been widely studied and shown to decrease native diversity and alter soil communities (Piper et al. 2015a, Piper et al. 2015b, Mamet et al. 2017, Slopek and Lamb 2017, Bahm et al. 2011, Salesman and Thomsen 2011, Stoz et al. 2019, Otfinowski et al. 2007, Chagnon et al. 2018). Smooth brome invasion greatly reduces native plant diversity (Piper et al. 2015a, Piper et al. 2015b, Mamet et al. 2017, Mamet et al. 2019, Li et al. 2018), which in turn causes changes in soil microbial communities' structure and function as well as altering soil nutrient cycling. The changes in plant and microbial communities and their subsequent alteration of soil nutrient cycling will have long-term effects of the ecosystem services provided by prairies in Western Canada. Any changes caused by smooth brome invasion will likely help to perpetuate the invasion, impeding efforts to restore native prairie plant populations.

To examine how smooth brome impacts microbial community assembly processes and ecosystem services, 515 plots of varying levels of invaded biomass were sampled over a 26 week period (13-19 plots each week), spanning the entire growing season in 2014 (Bell et al. 2020). For every plot sampled, a range of ecosystem services were measured including greenhouse gas

emissions, glyphosate degradation and extracellular enzymes, as well as plant surveys and biomass data were collected, and soil bacteria and fungi were sequenced. We hypothesized that as invasive biomass increases, selection pressure in soil microbial communities would differ from those in native plots. Given previous work (Piper et al. 2015a, Piper et al. 2015b, Mamet et al. 2017) we hypothesized that we would see the large shift in microbial community assembly processes in bacterial communities rather than fungal communities. Additionally, we hypothesized a large magnitude of difference between the selection pressures in native vs invaded plots, would correlate with ecosystem service differences between native and invaded plots.

5.4 Methods

5.4.1 Field Site and sampling

The methods for this project have been extensively described previously (Bell et al. 2020). Briefly, samples were collected at Kernen Prairie which is a 130-ha remnant rough fescue prairie on the edge of the city of Saskatoon, Saskatchewan, Canada (52°10‘‘ N, 106° 33‘‘ W). A total of 515 samples were collected from May 15, 2014, continuing through November 5, 2014. The site has grassland and low shrub communities, as well as a small number of aspen (*Populus tremuloides*) bluffs and ephemeral wetlands. Native grass species include Plains Rough Fescue (*Festuca hallii*), Wheatgrass (*Elymus lanceolatus*), and Needlegrass (*Hesperostipa curtiseta*). Common native broadleaf species include Northern Bedstraw (*Galium boreale),* Pasture Sage (*Artemisia frigida),* and Prairie Rose (*Rosa arkansana)*. Low shrub communities are dominated primarily by Western Snowberry *(Symphoricarpos occidentalis).* The site is undergoing multiple invasions by species including the forage grasses Smooth Brome (*Bromus inermius*) and Kentucky Bluegrass (*Poa pratensis*), and the forbs Canada Thistle (*Cirsium arvense*), perennial sow thistle (*Sonchus arvensis*), and Absinthe (*Artemisia absinthum*) (Slopek and Lamb 2017). Microtopography, soil water availability, prescribed fire, and grazing history are primary influences on the plant community structure of the prairie (Looman 1969, Romo 2003, Gross and Romo 2010, Gross and Romo 2019).

Samples were collected week for 26 weeks starting May 15, 2014, continuing through November 5, 2014. An average of 13 samples were collected each week; however some weeks

have less sampling points due to adverse weather conditions or sampling crew size. Soil temperature and moisture was taken in the field using a 5TE Decagon probe (Pullman, Washington). A 1 cm diameter, 15 cm depth core of soil was taken with a push corer in each plot for future DNA work and frozen at -80 °C until processing. Two cores were collected (5 cm diameter, 30 cm depth) with a slide hammer corer, and soil was passed through a 4.5 mm sieve. Sieved soil was then collected for soil aggregate analysis and nutrient analysis. Soil was stored at 4 °C until processing. A subsample was taken and stored at – 80 °C for DNA extraction.

Table 5.1 The mean five ecosystem services for the entire sampling period with standard error in parenthesis by Invasion Level. The portion of invaded biomass for each Invasion Level is in parenthesis. Significant differences are denoted by superscripted letters and was determined using a one-way ANOVA with results listed below the means.

Invasion Level	Forage	Water	Climate	Soil Conservation	Nutrient Cycling
Native (0-14%)	79.5 (5.2)^{b}	6.47 (0.2)^{a}	10.39 (0.2)^{a}	8.21 (0.2)	8.47(0.2)^{a}
Mid (15-49%	85.01 (4.2)^{b}	6.13 (0.2)^{a}	9.72 (0.2)^{b}	7.81 (0.2)	8.06 (0.2)^{b}
Invaded (50-100%)	127.8 (6.6)^{a}	5.04 (0.2)^{b}	10.51 (0.2)^{a}	8.14 (0.2)	8.41 (0.3)^{a}
Df	2	2	2	2	2
Sum of Square	250937	168	61	16	61
Mean of Square	125468	83.82	30.29	7.92	30.92

F	25.89	8.87	4.87	0.82	4.87
P	< 0.001	< 0.001	0.008	0.44	0.008

Plant species and litter were collected from a 50 by 50 cm quadrat centered on each plot. In each plot, vascular plant species were clipped separately, dried at 60 °C and weighed. Dry plant biomass was summed into native, invasive, and total biomass and by functional group to obtain a plot measures of plant biomass. Litter was also collected and weighed. Plant biomass was adjusted for live versus senesced biomass beginning on August 15th. When clipping, percent greenness of the biomass was estimated for each species. Plant community evenness was calculated using the E_{var} index (Tuomisto 2012). Forage quality (poor, fair, good) was assigned for each species using the descriptions using established descriptions (Tannas 2003. Tannas 2003).

Samples were initially classified as native or invaded based on an early spring survey, however individual plots were later reclassified as native or invaded based on the proportion of invaded biomass present. Plots with greater than 50% invaded biomass were classified as invaded giving 154 invaded samples. Plots with less than 15% invaded biomass were classified as native plots resulting in 189 native samples. Samples with 15-49% invaded biomass were classified as mid-level invaded (mid) resulting in 175 mid samples. The dataset was then further subdivided into three seasons based on plant biomass with green-up encompassing weeks 1-8 (May 15 – July 3), peak biomass from weeks 9-18 (July 14- September 10) and senescence from weeks 19-26 (September 24 – November 5).

5.4.2 Ecosystem Services

Climate Regulation

Following Lamb et al. (2011) concentrations of soil carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) were measured using vented surface chambers, attached to a DX-4015 Fourier transform infrared trace gas analyzer (FTIR-TGA, Gasmet Technologies Oy, Helsinki, Finland) (Bell et al. 2020).

Water purification

Glyphosate degradation was measured using ^{13}C-labelled glyphosate measured on a Picarro G2201-I analyzer. The concentration of $^{13}CO_2$ from the degradation of glyphosate was calculated in nmol per g soil per day (Bell et al. 2020).

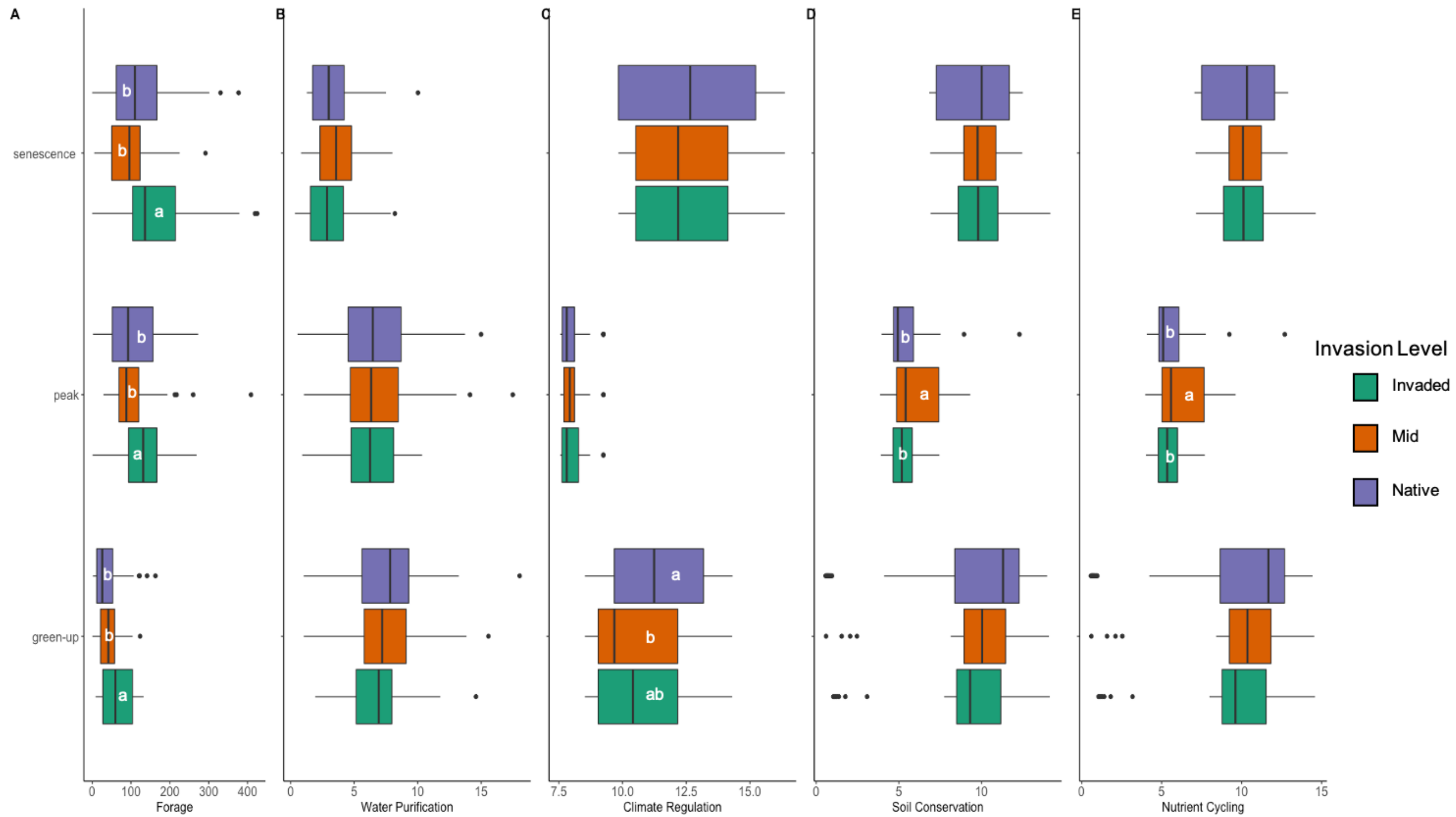


Figure 5.1 Boxplots of the five ecosystem services: Forage Production (A), Water Purification (B), Climate Regulation (C), Soil Conservation (D) and Nutrient Cycling (E) by Season and Invasion Level. The box boundaries represent the first and third quartiles of the distribution and the median is the horizontal line of raw data. The whiskers represent 1.5 times the interquartile range. Significant differences within a season are denoted in white letters.

Nutrient Cycling

Soil phosphatase and arylsulfatase analyzed following established methods (Eivazi, F. & Tabatabai 1977, Whalen and Warman 1996). Phosphatase was analyzed using 10 mM p-nitrophenyl and arylsulfatase was analyzed using 100 µL of 10 mM p-nitrophenyl (Bell et al. 2020). Both reactions were read at 405 nm using an iMark microplate reader (Bio Rad, Hercules, California). Soil dehydrogenase was analyzed following Trevors (184). A solution 0.4% Iodonitrotetrazolium chloride solution was used as a substrate and methanol was added to quench the reaction (Bell et al. 2020). Samples were read at 480 nm using an iMark microplate reader (Bio Rad, Hercules, California).

Potential nitrification was measured using methods from Carter and Gregorich (2008). Briefly a test media which was prepared with 4 mM $(NH_4)_2SO_4$ as a growth substrate, 15 mM $NaClO_3$ as an oxidation inhibitor, and 1 mM $KHPO_4$ as a buffer with 4M KCl to terminate the reaction. Nitrite production was measured using a colorimetric method (Bell et al. 2020). The plate was then read at 543 nm using an iMark microplate reader (Bio Rad, Hercules, California).

Microfaunal feeding rates were measured using bait lamina sticks (Hamel et al. 2007). Sticks were scanned and the average number of the sixteen holes consumed were counted over the three-week period (Kratz 1998, Bell et al 2020).

Soil Conservation

Soil moisture content was measured using a Mettler Toledo Moisture Analyzer MJ33 (Colombus, Ohio). Soil pH was measured by adding 10 ml 0.01 M $CaCl_2$ to 5 g air-dried soil and then measured using a pH meter. Total organic carbon and nitrogen and inorganic nitrogen was extracted by shaking 10 grams of field fresh soil with 50 ml of 2M KCl (Carter and Gregorich 2008, Bell et al. 2020). Inorganic and total phosphorous were measured using a modified sodium bicarbonate protocol (Carter and Gregorich 2008, Bell et al. 2020). The amount of total and inorganic phosphate was determined colorimetrically using the ammonium molybdate-antimony potassium tartrate-ascorbic acid method (Carter and Gregorich 2008, Bell et al. 2020). Organic phosphorus was calculated as the difference between total extractable and extractable inorganic phosphorus. Wet aggregate stability was measured using a method adapted from Soil Sampling and Methods of Analysis (Carter and Gregorich 2008, Bell et al. 2020) using an oscillating dual-layered sieve machine modified (Six et al. 2000).

5.4.3 DNA extraction, amplification and sequencing

DNA was extracted from 250 mg rhizosphere soil, soil in direct contact with roots, using Qiagen PowerSoil extraction kit (Hilden, Germany) following manufacturer instructions. Extraction duplicates were included. After extraction, DNA was tested for quantity following the standard Qubit protocol (Thermo Fisher Scientific, Waltham Massachusetts). Prior to amplification, DNA was standardized to 1 ng/µl. Bacterial diversity was assessed by amplifying the V4 region of the bacterial 16S rRNA was amplified using the primer set 515F with Illumina adapters (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA 3') and the 806R (5' - GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GGA CTA CCG GGG TAT CT - 3') (Mori et al. 2014). Fungal diversity was assessed by amplifying using the primer set ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA–3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC–3') (Walters et al. 2015). For exact reaction conditions see Bell et al. (2020). In all cases, PCR product was purified to eliminate primers and impurities using 1:1 ratio of Nucleomag NGS clean-up and size select (D-mark Biosciences, Scarborough, Ontario). After purification, samples were indexed following the Illumina protocol (Illumina 2013), purified again to remove excess index primers, quantified and standardized to 4 nM, and pooled. Pooled libraries were then sequenced using the Illumina MiSeq platform.

5.4.4 Data Processing

For bacteria, a total of 8,506,841 reads were produced with an average of 12,008 reads per sample. Sequences were imported into QIIME2 v 2019.1 (Bolyen et al. 2018) and primers were removed using *cutadapt* (Martin 2011). Reads were then processed into amplicon sequence variants (ASVs) using DADA2 (Callahan et al. 2016) resulting in 263,940 ASVs with an average of 13,055 ASVs per sample. ASVs were then classified using a 515/806 trained a 342F/806R-trained V3/V4 SILVA 132 database (Quast et al. 2013). For fungal sequences, a total of 12,371,309 reads were generated with an average of 21,478 reads per sample. Fungal primers were also imported into QIIME2, primers were removed using cutadapt and sequences were sorted into ASVs using DADA2 resulting in a total of 17,374 ASVs with an average of 4,735

ASVs per sample. Fungal ASVs were then classified using the Unite Database (Abarenkov et al. 2010).

Table 5.2 Effect size of bacterial and fungal assembly on ecosystem services compared to season and invasion[a].

	Forage	Water	Climate	Soil Conservation	Nutrient Cycling	Average Effect Size[c]
Bacteria	2.61%	NS[b]	3.89%	8.98%	5.60%	3.89%
Fungi	2.74%	3.77%	0.58%	NS	NS	1.78%
Season	19.38%	23.67%	54.77%	11.51%	38.08%	27.33%
Invasion	5.82%	NS	0.51%	NS	NS	1.71%

[a]Estimated by the linear model Ecosystem service~bacterial mean βNTI * fungal mean βNTI * Season *Treatment for each of the five ecosystem services.

[b] Not significant at the $p<0.05$ for that ecosystem service. Detailed ANOVA table presented in SI Table 3.

[c]Calculated as the arithmetic mean of all effect sizes, regardless of significance.

5.4.5 Statistical Analysis

Potential spatial autocorrelation was examined using Moran's I (Legendre, and Legendre 1984). Moran's I was calculated using the *Moran.I* function in the R v 4.0.3 (R Core Team 2018) package *ape* (Paradis and Schliep 2019). There was spatial autocorrelation, especially in forage biomass measurements, however it was inconsistent and likely due to the inherent heterogenous nature of prairie species distributions and invasion patchiness (Bell et al. 2020). Due to these factors the spatial autocorrelation was not corrected for the current analysis. Temporal autocorrelation was examined using the *lm* and *acf* functions (base R). There was no temporal autocorrelation detected within each season.

To examine the effect of Invasion Level on bacterial and fungal community composition, permutational multivariate analysis of variance (PERMANOVA) was conducted on Bray-Curtis distances matrices using the *adonis* function in the package *vegan* (Oksanen et al. 2019). Given the significant interaction of both Invasion Level and Season as well as the inherent seasonality of grassland ecosystems, the decision was made to include the original separation into three different seasons for all analysis to better isolate the effects of Invasion Level in addition to examining the entire sampling period.

Bacterial and fungal assembly processes were assessed using the βNTI and RC_{bray} metrics. A |βNTI| value greater than two indicates that deterministic selection is occurring, whereas a |βNTI| value less than two indicates stochastic processes are dominating. Mean βNTI and RC_{bray} values were calculated for each sampling plot by taking the average of all pairwise observations for a plot. For both metrics, the null distribution was set within each Season and Invasion Level for both bacteria and fungi. Selection pressures were quantified following Stegen et al. (2013), with the use of the βNTI metric in the picante package (comdist, abundance.weighted= TRUE) and Bray-Curtis-based Raup-Crick (RC_{bray}) in the iCAMP package v. 1.2.9 (Kembel et al 2010, Ning et al. 2020). The difference between the observed βMNTD and the null βMNTD is the βNTI metric with the null distribution generated using 999 randomizations. Values of $|\beta NTI| > 2$ indicate that deterministic selection is occurring at a 5% significance level (Stegen et al. 2012). βNTI values > 2 were classified as likely $p<0.025$) heterogenous selection. βNTI values less than two were classified as homogeneous selection. Observations $|\beta NTI| < 2$ indicated that stochastic processes were likely ($p<0.025$) occurring. The RC_{bray} metric is the probability that an ASV occurs in a sample compared to the distribution and abundance of the ASV across all samples and it uses successive iterations to determine these probabilities. Pairwise comparisons between βNTI and RC_{bray} were done to determine the stochastic processes dominating bacterial community assembly. Observations with values $|\beta NTI| < 2$ and $RC_{bray} > +0.95$ were classified as dispersal limitation and $|\beta NTI| < 2$ and $RC_{bray} < -0.95$ classified as homogenizing dispersal (Ning et al. 2020, Lin et al. 2012, Vellend 2010, Web 2000). Pairwise observations not falling within the constraints of$|\beta NTI| < 2$ or $|RC_{bray}| < 0.95$ were categorized as drift. Values on falling within the limits of$|\beta NTI| < 2$ or $|RC_{bray}| < 0.95$ suggest that a population is weakly experiencing any of the processes or that the community is undergoing drift, which is the random division, death or mutation of individual community members.

To examine which factors influenced deterministic selection processes distance-based redundancy analysis (dbRDA) (Legendre and Andersson 1999) was performed on weighted UniFrac distance matrices (Lozupone and Knight 2005) using the capscale function in the vegan package in R (Oksanen et al. 2019). The UniFrac distances were calculated using the phyloseq package (McMurdie and Holmes 2013). UniFrac distances were used to preserve the phylogenetic relationships in the communities. All dbRDAs were constrained by invasive biomass, water extractable organic carbon, soil pH, field moisture and temperature and 53 µm aggregate size.

Nutrient cycling was combined into a single measure by taking the silhouette widths (Borcard et al. 2011) of bait lamina and all extracellular enzymes (phosphatase, arylsulfatase, dehydrogenase and potential nitrification) to give each measure equal weight. The silhouette widths were then used to create a distance matrix of the Euclidean distances. The mean of all Euclidean distances was taken for a given plot giving a mean value for nutrient cycling. Similarly, soil conservation was calculated in the exact same manner, but using aggregate weights, field moisture, pH and soil nutrients values.

To examine if ecosystem services varied with Invasion Level and section pressure (βNTI), ANCOVAs (analysis of covariance) were used with the basic model consisting of Ecosystem Service ~ bacterial βNTI*fungal βNTI *Season*Invasion Level. For the entire sampling season, only the Invasion Level by βNTI interaction was included. The decision was made to include both fungal and bacterial βNTI values, as well as their associated interaction, as this was the most biologically realistic. However, models that did not include the bacterial βNTI by fungal βNTI were also run to examine the individual effects of bacterial and fungal assembly processes on ecosystem services. Ecosystem services were analyzed by Season and Invasion Level using the *aov* function (base R) as well as by the entire sampling period. Analysis of covariance (ANCOVA) was done to assess the response of ecosystem services to mean βNTI values of both fungi and bacteria, and Invasion Level using the *lm* and *anova* functions (base R). All interactions were included as well.

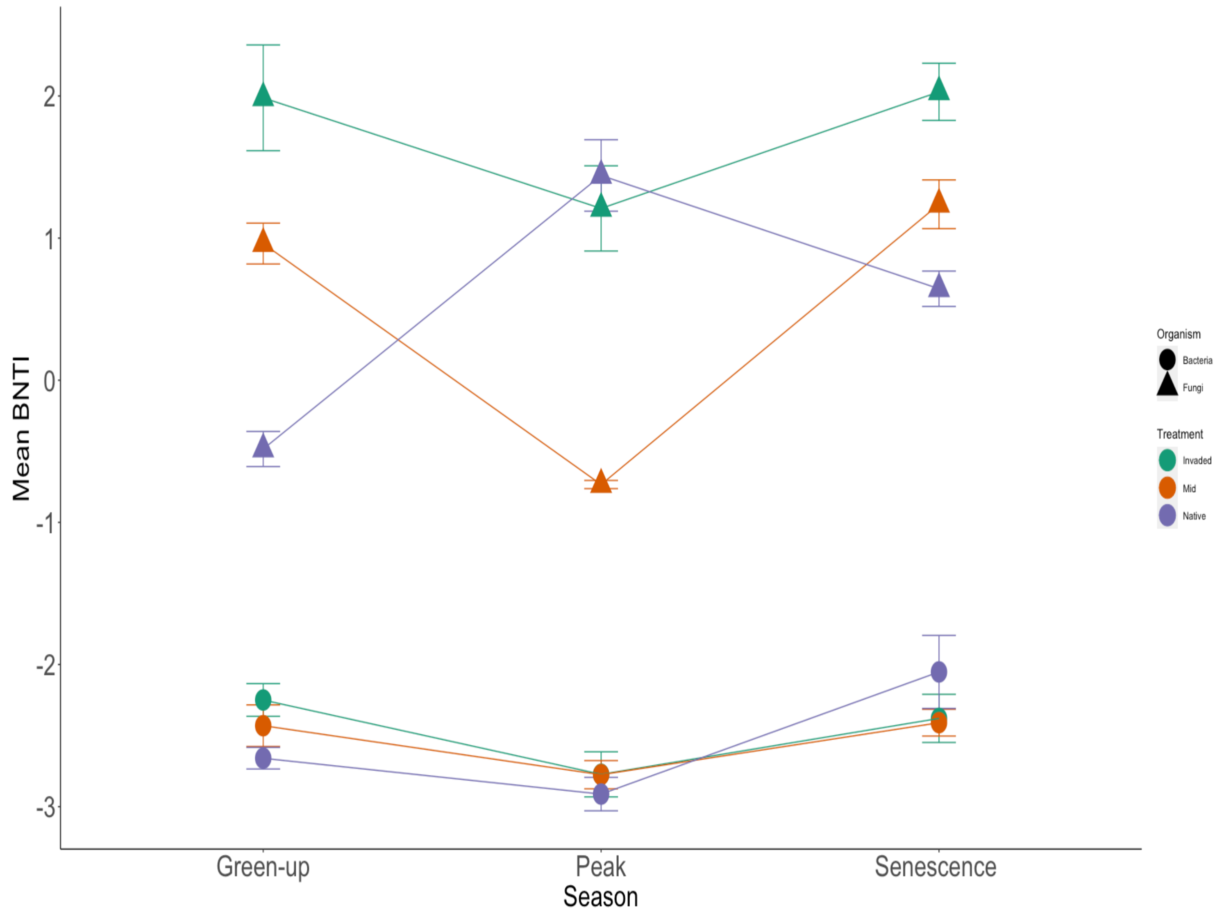


Figure 5.2 Mean βNTI values for bacterial (circles) and fungal (triangles) communities across all three seasons. Native plots are purple, Mid plots are orange and Invaded plots are green. Each point represents at least 34 plots and error bars represent standard error. Positive values indicate heterogenous selection is occurring whereas negative values indicate homogeneous selection. The gray shaded area indicates a significant deviation from the null hypothesis.

5.5 Results

Season was the main driver of both bacterial ($p<0.001$) and fungal ($p<0.001$) community composition with Invasion Level also altering community composition but not significantly interacting with Season (SI Table 1). The over-riding importance of Season on soil community composition is reflected in the relatively small effects of Invasion seen on bacterial βNTI compared to Season (Figure 2). For example, Invasion Level was only significant for bacterial assembly during Senescence ($p = 0.04$) with a mean βNTI difference of 0.3 compared to a

Green-up/Peak difference of 0.1. Season and Invasion were trending ($p = 0.056$) in fungal community composition which is reflected in the large differences between fungal assembly processes in Green-Up and Senescence, but not Peak.

Across the entire growing season, invasion altered ecosystem services (Table 1). Invasion increased forage biomass, as expected. Additionally, invasion altered climate regulation, soil conservation, water purification and nutrient cycling. The invasive effects of *B. inermis* interacted with seasons for ecosystem services linked to climate regulation, soil conservation and nutrient cycling in the largest impact of invasion seen during the green up season (Figure 1). The Green Up season marks an 8 week period in which a northern grassland transitions from a snow covered, ~0°C to a vibrant ecosystem at ~30°C where the perennial vegetation becomes active again and begins to grow. Thus, at Peak season, climate regulation, soil conservation and nutrient cycling, all show a concerted alteration, but the effects of invasion are muted during Peak. As the ecosystem approaches freeze up and Senescence, invasion once again influences climate regulation but has little effect on soil conservation or nutrient cycling. In contrast, Forage and Water Purification have consistent Invasion effects across all three Seasons.

Bacterial assembly processes were consistently a significant factor in ecosystem service provision (Table 2). As one would expect based on Figure 1, Season had the largest influence accounting for ~27% of ecosystem effect size. What was unexpected, was the non-significant and minor influence of *Bromus inermis* invasion on all ecosystem services except forage biomass. For example, *B. inermis* invasiveness's effect size was only 0.34% for nutrient cycling compared to 5.6% for bacterial assembly processes. Fungal assembly played an equally important role in forage production as bacterial assembly, but interestingly were a dominant effect in water purification whereas bacteria were minor and non-significant (0.06%). On average, bacterial assembly had effect sizes roughly double that of fungal assembly or *B. inermis* invasion.

Homogenous selection accounted for greater than 50% of bacterial assembly processes, with a strong Seasonal increase in selection pressure during Peak (Fig 3 A-C). Invasion processes did not interact with this Seasonal effect but increased in processes ascribed to dispersal limitation which ranged from 6 to 23% of assembly processes. A similarly strong Seasonal effect, and weaker Invasion effect on bacterial community composition was reflected in the PERMANOVA analysis (SI Table 1). Interestingly, Native plots had a much larger increase

in the proportion of heterogenous selection and homogenizing dispersal in during the Senescence period than the Mid and Invaded plots from 0% during Peak to 5.5% during Senescence.

The primary selection pressure, > 50%, in all fungal communities across all seasons were the stochastic processes of drift and dispersal limitation (Figure 3). Unlike bacteria, Invasion Level had a much more pronounced effect on assembly processes with native plots having the highest level of deterministic selection pressure, namely heterogenous selection across all three Seasons. Heterogenous selection, ~ 10%, was much lower during Green-Up in Invaded plots and during Peak in Mid plots compared to the other seasons. The Season by Invasion level interaction was also reflected in the community composition.

Bacterial deterministic assembly pressures were greater in Invaded (~22%) compared to Native (16%) with the smallest effect seen during Senescence (Fig 4). Aggregate size, soil pH, field moisture and temperature, soil carbon and invasive biomass explained more than 15% of deterministic assembly processes for all bacterial communities. Soil pH consistently had the most explanatory power of soil community composition followed closely by soil moisture. In Mid invaded plots, temperature and 53 µm aggregate weight were also significant constraints on microbial community composition. Invasive biomass constrained bacterial community in Native plots during Green-up season and Invaded plots during Peak biomass.

In contrast to bacteria, fungal deterministic assembly pressures were greater in Native (~18%) compared to Invaded (13%) with the greatest effect seen during Senescence (SI Fig 3). Aggregate size, soil pH, field moisture and temperature, soil carbon and invasive biomass explained wide amount of fungal community variation from the lowest at 8% in Invaded-Peak Biomass plots to the highest at 28% in Native-Senescence plots (SI Fig 3). Not unsurprisingly, aggregate size was more important for fungal communities than bacteria and was significant ($p > 0.05$) for Invaded plots during Green-up, Mid plots (SI Fig B, E, H) during Peak Biomass and Senescence as well as Native plots during Senescence. Soil pH and moisture were less consistently important for fungal communities.

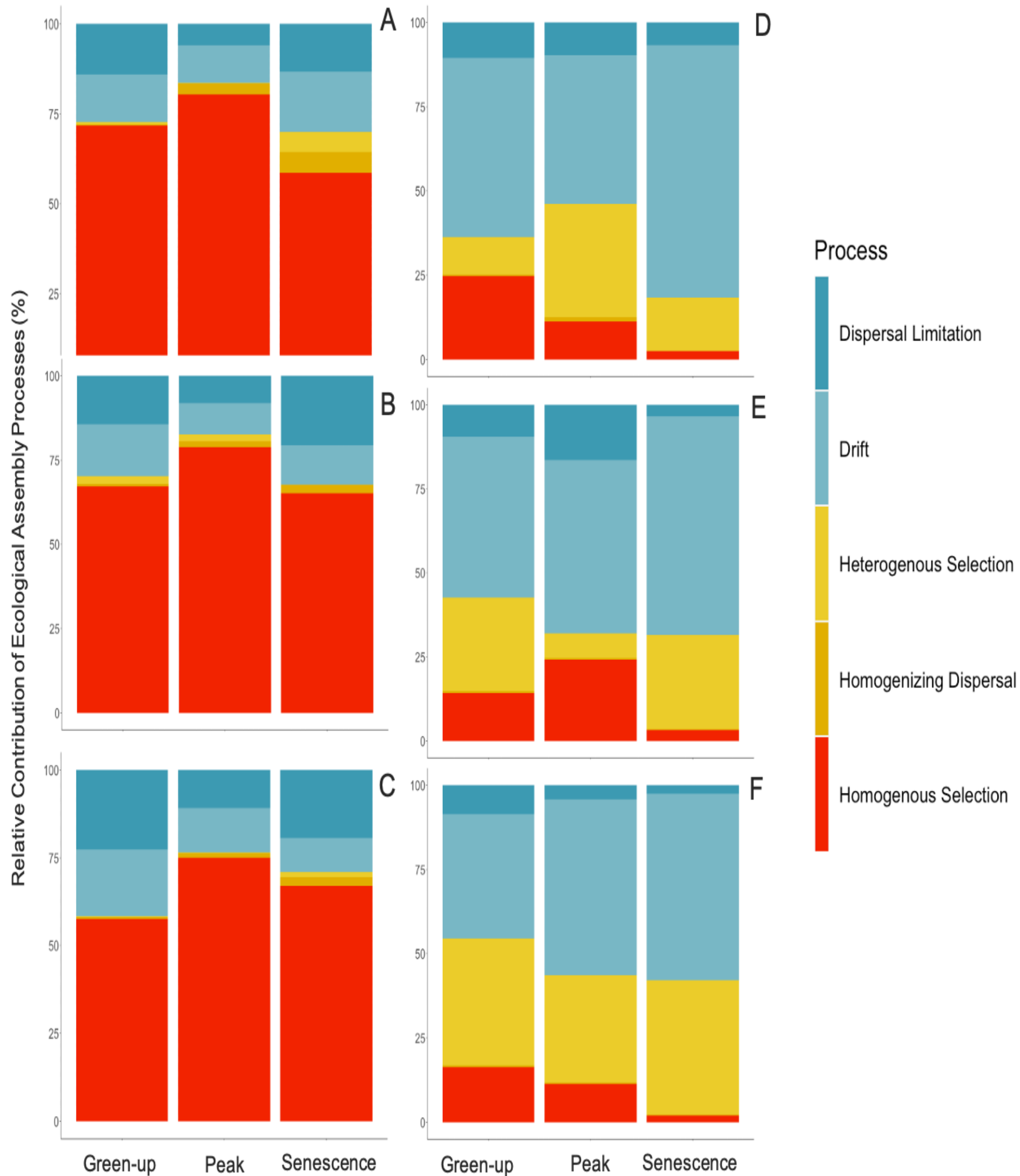


Figure 5.3 Ecological assembly processes in the bacterial (A-C) and fungal (D-F) communities across all seasons in Native plots (A, D), Mid plots (B, E) and Invaded Plots (C, F). Deterministic processes were classified as heterogenous selection (βNTI > 2) or homogeneous selection (βNTI < -2). Stochastic processes were classified as homogeneous dispersal (|βNTI| < 2 and RC_{bray} < -0.95) or dispersal limitation (|βNTI| < 2 and RC_{bray} > +0.95). Pairwise observations within the confines of |βNTI| < 2 and |RC_{bray}| < 0.95 were classified as drift.

5.6 Discussion

The largest determinant of variation in ecosystem services as well as microbial community assembly processes was Season. Northern grasslands are highly seasonal, experiencing large fluctuations in temperature, moisture, and plant biomass (Falge et al. 2002) which account for the large seasonal shift observed in this study. This is consistent with previous results that show that ecosystem services provided by grasslands change substantially with seasons (Paruelo et al. 2016). While it may seem obvious that seasonality affects ecosystem services, alterations in the seasonality of some of these services could change the severity of invasion. For example, Prevéy and Seastedt (2014) found that altering the timing of precipitation, something that is expected to happen with climate change (Alexander et al. 2001), increased the amount of invasive grass cover and lowered summer soil moisture and nutrients (soil conservation). This could mean that with the changing in seasonal cycles caused by climate change, the impact of invasive species could be amplified, especially as the growing season in temperate areas is expected to lengthen (Christiansen et al. 2011).

After season, bacterial assembly processes dominated the provision of ecosystem services linked to climate regulation, soil conservation and nutrient cycling. All three of these services are tightly linked to bacterial activity, such as CH_4 and N_2O emissions or the excretion of phosphatases. We had hypothesized that *B. inermis* invasion would alter assembly processes, leading to changes in bacterial communities which would then lead to altered ecosystem processes. Instead, we observed a process in which *B. inermis* invasion had a small ~2% direct effect on ecosystem services, and a modest effect on bacterial community assembly. Only ~20% of community composition could be ascribed to deterministic filters which hindered our ability to conclusively link invasion to assembly processes.

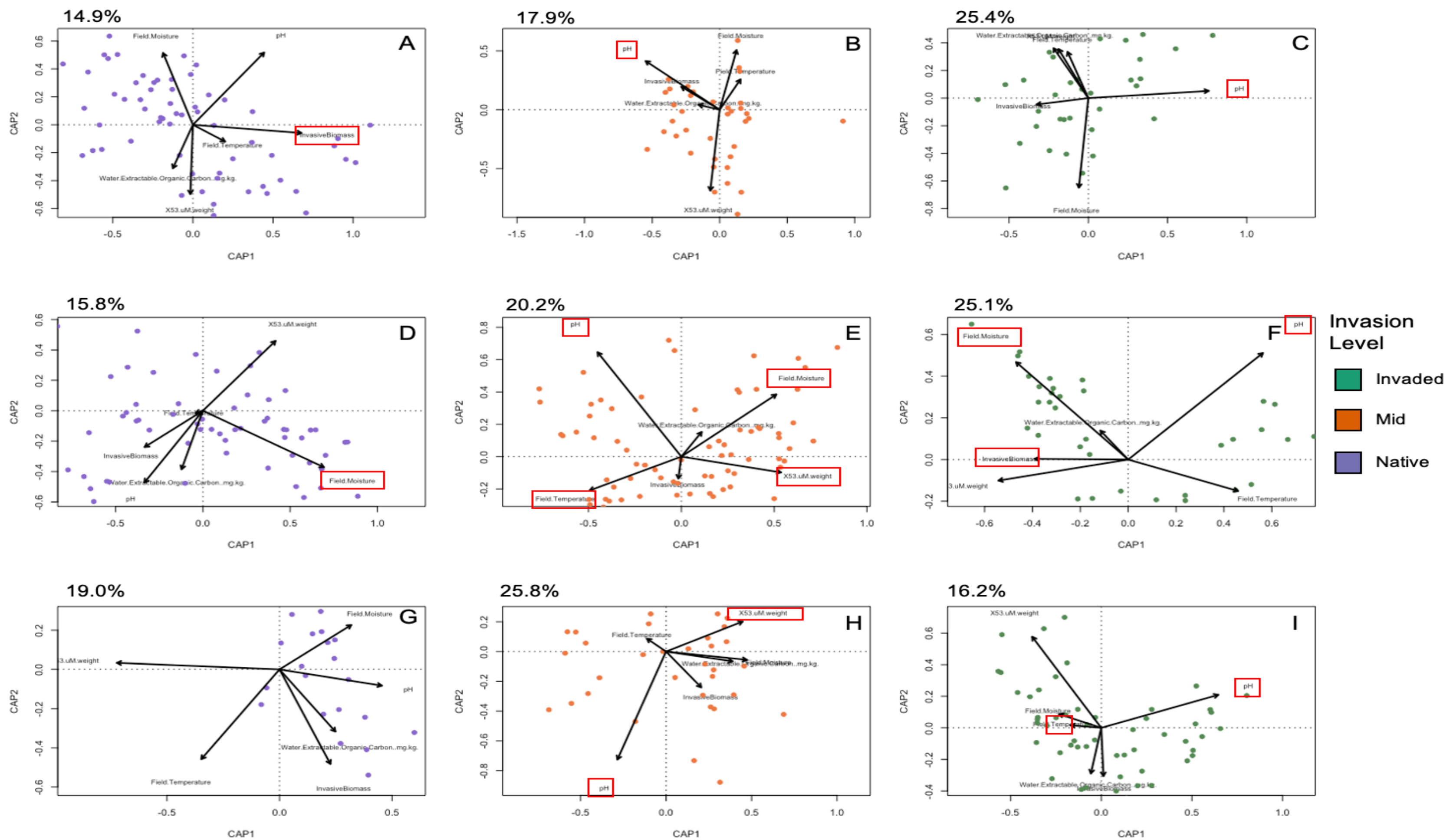


Figure 5.4 Bacterial distance-based redundancy analysis (dbRDA) of the weighted UniFrac distances during Green-up (A-C), Peak Biomass (D-F), and Senescence (G-I) for Native plots (A, D, G), Mid plots (B, E, H), and Invaded plots (C, F, I). All dbRDAs were constrained by were constrained by 53 µm aggregate weight, soil pH, field moisture, invasive biomass, field temperature and water extractable organic carbon content. Significant terms are indicated by a red box around the term.

Despite the small effects of invasion on bacterial assembly, bacterial assembly had a large effect ~4% on ecosystem services. One might conceptualize this as an amplification of the invasive effect, in which plant invasion only slightly changes overall bacterial community composition and assembly, but in so doing amplifies the effects on ecosystem services. In turn, the feedback from altered ecosystems promotes *B. inermis* invasion, leading to more slight changes in assembly (Fig. 5). These amplification loop example, *B. inermis* homogenizes the local plant community which invasion alters local nitrogen and nutrient cycling (Piper et al. 2015, *inermis* homogenization of local plant communities which would alter nutrient cycling as well (Stotz et al. 2019, Li et al. 2018).) as well as fungal/bacterial networks (Mamet et al 2017.) leading to an increase in bacterial richness (Piper et al. 2015). Invasive species can perpetuate their invasion through the alteration of local nutrient cycling, microbial and plant communities, and soil conditions (Mack et al. 2000, Ehrenfeld 2003, Vilà et al. 2011) and it is becoming increasingly clear that this is the mechanism that *B. inermis* uses to continue to spread. Changes in soil microbial communities and nutrient cycling caused by *B. inermis* could have long term impacts which can cause a loss of agricultural capacity and other ecosystem services.

The largest determinant of both bacterial and fungal assembly processes was season. However, within each season, Invaded plots had a higher proportion of dispersal limitation than Native plots. The greater water requirements of *B. inermis* leading to drier soils would impede movement of bacteria or fungal spores along water pores and root surfaces (Watt et al. 2006). Soil moisture was a significant determinant of both bacterial and fungal community composition throughout the growing season. This impediment of movement caused by drier soil may be the explanation of rare bacterial species increasing in abundance and increasing intra-trophic interactions as dispersal would be limited leading to more dissimilar communities (Piper et al. 2015). The higher proportion of fungal heterogeneous selection in Invaded plots as well as the higher proportion of homogenous selection in Mid fungal plots is consistent with this conceptualization of how *B. inermis* alters soil microbial communities (Piper et al. 2015, Piper et al. 2015, Mamet et al. 2017, Mamet et al. 2019).

Across Season and Invasion Level, fungal communities weakly experienced deterministic selection (Stegen et al 2015). Furthermore, the amount of variation captured by the dbRDAs was smaller for fungal communities, suggesting the environmental filters, which act as strong selection pressures (Nemergut 2013), did not have as large of an influence on fungal

communities as they did on bacterial communities. As noted in the introduction, the extension of the null-model hypothesis to fungal communities is not as strongly supported as it is for bacterial communities and thus, interpretations of fungal assembly processes should be considered as

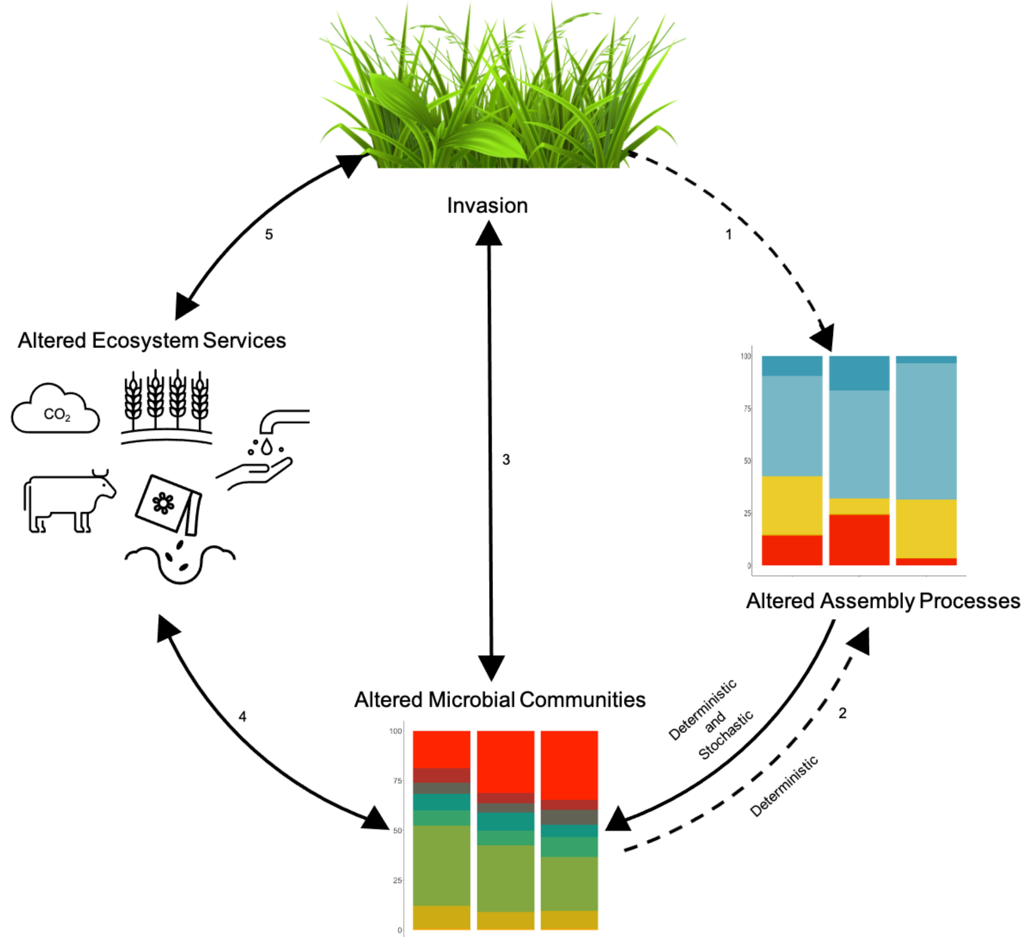


Figure 5.5 A conceptual feedback loop wherein the invasion can alter the existing soil microbial community assembly processes leading to differences in diversity, evenness and functionality (1. Lamb et al. 2011). Stochastic and deterministic processes then impact community structure which can feedback through the deterministic processes of inter and intraspecific competition to perpetuate the changed community (2. Van der Putten et al. 2007). Changes in soil microbial community structure can both perpetuate the invasion (3. Inderjit and van der Putten 2010) and alter local ecosystem services (4. Hawkes et al. 2005). Altered ecosystem services can then create plant-soil feedbacks which will also perpetuate the invasion (5. Levine et al. 2006).

tentative. The different mean βNTI values by Invasion Level as well as the differences in community composition with Season and Invasion Level does suggest that *B. inermis* is influencing the soil fungal communities. Additionally, both the Mid and Invaded plots had a

larger proportion of heterogenous selection that Native plots suggesting that the fungal communities here are undergoing selection for more distinct communities (Zhou and Ding 2017). Invasive grasses have been shown repeatedly to disrupt native fungal communities and nutrient cycling (Egerton-Warburton et al. 2007, Hawkes et al. 2006, Bunn et al. 2015). For example, Phillips et al. (2019) found that the invasive grass *Bromus diandrus* and *Avena fatua* altered the composition of fungi and nitrogen cycling in chaparral ecosystems. The influence of *B. inermis* can also be seen in the mean βNTI values as well. Mid plots, or plots with 15-49% invaded biomass, diverged from both Native and Invaded plots across all seasons but particularly during Peak Biomass when the influence of *B. inermis* would have been at its strongest. This deviation of the Mid Plots from both the Native and Invaded plots suggests that the presence of *B. inermis* is disrupting normal assembly processes. This is consistent with previous work which has shown that *B. inermis* leads to a reduction in fungal diversity (Mamet et al. 2017).

Here we showed that bacterial assembly processes are a significant factor in ecosystem service provision, more important than invasion status or fungal assembly processes. However, the large stochastic component in microbial communities, ca. 80%, as well as spatial scale (Graham et al. 2016), hinders attributing ecosystem services to specific changes in microbial community structure and assembly processes. At this 130-hectare site, after seasonal effects, bacterial assembly processes had the largest effect on ecosystem services with plant invasion placing a distant third. We are perplexed by this, as we, believed that above plant invasive biomass would be dominant factor, after season on the ecosystem services. Instead, as is often the case, nature has surprised and suggested that these grassland dynamics are more subtle than we ever imagined. Invasive plants change plant-soil feedbacks in a manner that will perpetuate the invasion (Ven der Putten et al. 2013, Zhang et al. 2019) as well as evidence that invasive species drastically alter ecosystem services (Pejchar and Mooney 2009, Pyšek et al. 2010, Vilà et al. 2011, Walsh et al. 2016). But this mechanism of altering ecosystem services is not occurring by altering bacterial assembly. Instead, bacterial assembly processes are influencing ecosystem services in a manner, independent of *B. inermis* biomass. Perhaps in this case, the invasion whole is much greater than the sum of its invasive parts?

Data Availability

All raw sequence files can be found at the National Center for Biotechnology Information (NCBI) under Bioproject PRJNA580515. Compiled soil physical and chemical properties, along with plant survey data and other ecosystem services can be found in the Dryad repository, along with the ASVs estimates (doi:10.5061/dryad.1ns1rn8q7).

6. SYNTHESIS AND CONCLUSIONS

6.1 Dissertation Overview

Microbial community assembly processes are the ecological pressures that shape microbial community composition and distribution (Kraft and Ackerly 2014). More colloquially, assembly processes are how communities grow and change over space and time. Harnessing the microbiome to improve crop performance and increase yield is necessary to improve agricultural sustainability but we need understand how the microbiome assembles first (Chaparro et al. 2012. Busby et al. 2017). Once we understand the shaping forces, we can manipulate them to select for more robust microbial communities. Along with agricultural intensification, climate change is expected to increase disturbance and cause plants to shift their ranges. These changes will also alter soil microbial assembly processes and having a more complete understanding of them will help with remediation efforts (Van der Putten et al. 2013, Graham et al. 2016). Understanding how natural ecosystems compare to disturbed systems not only provides a baseline for remediation efforts, it will also provide a more complete understanding of how microbial communities operate in general.

The key findings from my research are that no matter the degree of complexity of the system, time is the most important factor shaping microbial community assembly processes. Time is represented by the growth of plants which will occur on a cyclical and annual basis. With each new growing season, a new cycle of time will begin again, and the assembly processes will start anew. At the simplest level examined, the canola leaf, growth stage, or in other words, time, was the key factor shaping the microbial community. At the next level of complexity, the canola root and rhizosphere we saw the same dominance of growth stage, with the strongest selection pressures occurring during seed set and ripening. Finally, at the highest level of complexity, soil

microbial communities in a grassland undergoing invasion, the primary factor shaping both community assembly processes and ecosystem services was season, or once again, time. Overall, this dissertation highlighted that time is the primary force driving microbial community assembly processes and plant or other environmental factors are secondary.

6.2 Synthesis of Findings

This dissertation was designed to look at the impact of plants on microbial community assembly processes at increasing levels of complexity. The first two studies were designed to examine the effect of line differences of *Brassica napus* on the associated microbiome. We used eight different lines that were selected with an emphasis on their differences. For these two studies, we sampled the leaves, roots, and rhizosphere of the eight lines over a period of ten weeks. The final study was designed to examine the effects of *Bromus inermis* invasion on soil microbial community assembly processes and ecosystem services. Despite the intention to examine the effects of plants, we found that at each level of complexity, from the simplest (the canola leaf) to the most complex (the grassland) the dominant factor shaping microbial community assembly processes was time. In the first two studies, time was represented by *B. napus* growth stage. In the final study, time was represented by the seasonal differences in the grassland.

The leaf surface is an exceptionally harsh environment for microbial communities. These communities must contend with large daily fluctuations in UV stress, water and nutrient availability as well as huge shifts in temperature (Vacher et al. 2016). Due to these stressors, the phyllosphere is less diverse than soil microbial communities and is primarily dominated by bacteria (Vorholt 2012, Bulgarelli et al. 2013). The lower level of diversity makes this nutrient poor environment the least complex environment on the plant in which to study assembly processes. Additionally, because of these factors, we found that the primary force shaping microbial communities in the phyllosphere in *B. napus* was drift or diversification. The constant fluctuations in environmental conditions likely act as daily disturbances which does not allow of any sort of community stability to be achieved. Due to the near constant disruption, deterministic processes like inter- or intraspecific competition would not be able to be fully realized as bacterial species are simply trying to survive the disturbance.

B. napus growth stage was the primary factor shaping the bacterial community in the phyllosphere. There was a strong effect of growth stage on community composition and assembly processes. Plant growth stage has been shown previously to be an important factor shaping microbial communities (Smalla et al. 2001, Schlatter et al. 2019, Copeland et al. 2015). There was a distinct shift in community composition of the core bacteria before and after flowering (Bell et al. 2020). This change in composition was again reflected in the second study, where we found a shift from heterogenous selection before flowering to homogenous selection after flowering. The switch in selection pressures and the following change in community composition was most likely due to the rapidly senescing leaves on the *B. napus* plants. A shift from more function diversity to a more homogenous community, which probably represents saprotrophs colonizing the dying leaf, was observed (Bell et al. 2020).

The root and rhizosphere are more diverse than the phyllosphere. However, despite the increase in diversity, drift was the primary force shaping root communities during the first four weeks of sampling. We also observed a dominance of Gammaproteobacteria from the first sampling date. This could suggest that there are priority effects wherein the assembly is governed by the arrival order of species (Fukami and Nakajima 2011). Once the Gammproteobacteria had established themselves, they could have stopped other species from colonizing the root through competitive exclusion (Tan et al. 2015). Mostly likely the unexpectedly large portion of drift seen in the roots is a combination of both priority effects and competitive exclusion. Unlike the root, the rhizosphere followed expectations and was primarily dominated by deterministic effect, namely homogenous selection. This was most likely the rhizosphere effect in action (Philippot et al. 2013, Smalla et al. 2001, Gregory 2006).

While the leaf, root and rhizosphere all exhibited different selection pressures as well as varying community composition, growth stage and not NAM was the primary determinant of both composition and selection. This was surprising because the NAM lines had been specifically selected to emphasize genetic differences and plant line is known to affect microbial community composition (Edwards et al. 2015, Colemam-Derr et al. 2016, Dombrowski et al. 2017). The lack of line differences is likely a B. napus specific effect as previous work has shown little difference in canola line (Copeland et al. 2015, Morales Moreira et al. 2021). Despite no NAM differences, we did find a strong influence of canola growth stage in all plant compartments. Growth stage, or time, has also shown to affect the microbiome (Colemam-Derr

et al. 2016, Dombrowski et al. 2017, Copeland et al. 2015, Hilton et al. 2018). This is not surprising, as plants undergo large physiological changes throughout their lifecycles which cause changes in leaf structure and root exudate patterns as well as other changes that would impact the microbiome (Nitsch 1965, Mohan Ram and Rao 1984, Shu et al. 2010).

The plant microbiome provides many benefits for the plant including disease suppression and nutrient cycling (Bulgarelli et al. 2013) and breeding plants to have a more robust microbiome has been suggested to achieve more sustainable agriculture (Ryan et al. 2009). Assuming that the plant is actively selecting for beneficial microbes, periods of time where strong selection processes are seen could make good targets for plant breeding efforts. For example, altering flowering start or duration might allow the phyllosphere community to maintain the potential functional diversity that was observed (Bell et al. 2020) which could provide increased disease resistance to the plants as most canola diseases are aerially transmitted.

In the most complex system analyzed, the Kernen prairie, time was once against the largest determinant of microbial community assembly and structure, as well as ecosystem services. This system was much more complex than the previously analyzed agricultural systems because it is a natural ecosystem that has much higher plant diversity and more complex intertrophic interactions. Time in this system, while the largest determinant, is more complex than in agriculture systems. The prairie consists of many perennial species which green-up and grow much more slowly than planted crops. Furthermore, there is no firm end date to the growing season as unless used as pasture, the prairie will not be harvested like agricultural systems, but rather will naturally senescence as temperatures and day length decline. Due to these differences, time here is not represented as crop growth stage, but rather as season. More specifically, this study is split into Green-up, Peak Biomass and Senescence.

Initially we hypothesized that the amount of invasive biomass would be the primary determinant of differences in assembly, composition, and ecosystem services between native and invaded plots. However, once again, it was time, or season that played the largest role. While the amount of invasive biomass present did interact with season, most differences observed in both assembly processes and ecosystem services was attributed to Season and not to Invasion Level. Deterministic selection in the form of homogenous selection was strongest during the Peak biomass season, when the influence of the plants would be at their strongest. However, despite this strong selection, we saw few differences between the Invasion Levels. In

fungal communities, selection processes were disrupted by Invasion Level. Invaded plots experienced a higher overall proportion of heterogenous selection, which steadily declined from Green-up to Senescence. Fungi in native plots resembled bacterial more, in that fungi in these plots, experienced the strongest deterministic selection during Peak Biomass. Mid level plots resembled Invaded plots more closely, suggesting that Invasion is having a disruptive effect on fungal communities. However, despite differences in fungal assembly processes caused by Invasion Level, the largest factor shaping both bacterial and fungal assembly and composition was time in the form of seasonal differences.

Ecosystem services varied largely from season to season as well. Season captured an average of 27% of the variation in ecosystem services. Surprisingly, the factor that captured the most variation after Season was bacterial assembly processes which accounted for ~4%. The amount of invasive biomass only accounted for ~2% of the variation in ecosystem services, which was like the amount captured by fungal assembly processes. This was contrary to our hypothesis where we expected invasive biomass to play a larger role in shaping both ecosystem services and microbial community composition and assembly. While invasive biomass was not the most important factor, invasive species have been shown to alter both soil microbial communities and ecosystem services (Van der Putten et al. 2013), both of which can perpetuate the invasion, so the effects of invasion still warrant study.

6.3 Future Research Directions

Future agricultural and natural studies on soil microbial community assembly processes would benefit from additional sampling time points. While sampling and processing can be both expensive and time consuming, the main factor driving assembly processes in this dissertation was time. Most studies consist of very limited time points, making it hard to infer assembly processes accurately. Assembly processes do not occur instantaneously, and limited sampling points can only offer a snapshot. Ideally, especially in the study of climate change, long term data consisting of annual repeated sampling would take place. Long-term sampling coupled extensive environmental data would allow for a much more complete understanding of microbial community assembly processes and the factors that shape them. Additionally, with an increasingly unstable climate (Alexander et al. 2001), a better understanding of inter-annual

variation which is likely to become more prevalent will help us understand and eventually predict microbial community assembly processes. Relevant environmental data should be included with increase sampling to determine, at least partially, what is influencing the observed assembly processes. Ideally, this would include measurements such as soil nutrients and texture as well as plant cover and meteorological data.

In addition to more sampling, future projects should implement study and manipulation of root exudates. Root exudation are likely the primary factor in recruitment and retention in rhizosphere community assembly (Chaparro et al. 2013). Working closely with plant breeders to manipulate the quantity and quality of root exudation could provide useful clues on how this vital food source impacts microbial community assembly. Moreover, mesocosm experiments wherein no plant is present, but an isolated root exudate compound is added could help further disentangle how specific compounds impact assembly processes and composition as well as ecosystem services such as microbial respiration and decomposition. For natural ecosystems, plots or a greenhouse study could be implemented where commonly found species in are planted in a full factorial design from monoculture to the most diverse which would include all focus plants. This design would allow for a more complete understanding of how individual plants and combinations of plants influence assembly processes and ecosystem services. For both the mesocosm and greenhouse experiments, multiple sampling points should be included across the growing season for the plants or approximating the same time period for the soil only mesocosms. For agricultural studies, the inclusion of multiple plant species will not be necessary as the majority of crops are grown in monocultures. However, multiple lines should be included, and their root exudation patterns should be both examined and if possible manipulated.

Coupling 16S rRNA data with qPCR and RNA sequencing would provide an even more complete picture of microbial community assembly processes. While 16S rRNA data can give an idea of what is there, it is impossible to know if these bacteria are alive or how abundant they are. qPCR data would provide an estimation of the bacteria numbers in the sample and RNA data would give an idea of what is currently active. The 16S rRNA will give an approximation of the selection pressures that the community is undergoing. The qPCR and RNA sequencing data will give an idea of how the community is responding to these pressures. For example, if the community is experiencing heterogenous selection at high rates we would expect the community to become more similar and perhaps less diverse. Are the numbers of bacterial going down or are

certain species becoming more dominant? The qPCR data would help answer these questions. Are there genes associated with antibiotic resistance and stress that are elevated? This could be a sign of increased competition which could be the cause of the homogenous selection observed from the 16S rRNA data. This increase in competition might lead to more similar communities as only the fittest would survive, validating the homogenous selection previously observed. This could be applied to answer any number of questions on how microbial communities respond to various disturbances, imposed or natural. For example, tracking plant invasions from year to year, a site undergoing restoration, soil microbial communities after a recent fire or at a field site undergoing moisture or temperature manipulation. They would also work well for agricultural manipulations such as fertilizer or tillage trials or intercropping experiments. Combining all the techniques will provide us with a much more thorough understanding of how microbial communities assemble which can hopefully lead to the more efficient manipulation and management of microbial communities.

7. REFERENCES

Alexander, L. V., X. Zhang, T.C. Peterson, J. Caesar, B. Gleason, et al. 2006. Global observed changes in daily climate extremes of temperature and precipitation. J. Geophys. Res. Atmos. 111(5): 1–22.

Amir, A., M. Daniel, J. Navas-Molina, E. Kopylova, J. Morton, et al. 2017. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. Am. Soc. Microbiol. 2(2): 1–7.

Bahm, M. A., Barnes, T. G. & Jensen, K. C. Restoring Native Plant Communities in Smooth Brome (Bromus inermis)– Dominated Grasslands. 2011. *Invasive Plant Sci. Manag.* 4, 239 250.

Bao, Y., Feng, Y., Stegen, J. C., Wu, M., Chen, R., Liu, W., Zhang, J., Li, Z., & Lin, X. 2020. Straw chemistry links the assembly of bacterial communities to decomposition in paddy soils. *Soil Biology and Biochemistry*, *148*(September 2019), 107866.

Bartoli, C., O. Berge, C.L. Monteil, C. Guilbaud, G.M. Balestra, et al. 2014. The Pseudomonas viridiflava phylogroups in the P.syringae species complex are characterized by genetic variability and phenotypic plasticity of pathogenicity-related traits. Environ. Microbiol. 16(7): 2301–2315.

Batool, F., Y. Rehman, and S. Hasnain. 2016. Phylloplane associated plant bacteria of commercially superior wheat varieties exhibit superior plant growth promoting abilities. Front. Life Sci. 9(4): 313–322.

Bazghaleh, N., S.D. Mamet, J.K. Bell, Z.M. Moreira, Z.M. Taye, et al. 2020. An intensive multilocation temporal dataset of fungal communities in the root and rhizosphere of Brassica napus. Data Br. 30: 105467.

Beier, S., A.F. Andersson, P.E. Galand, C. Hochart, J.B. Logue, et al. 2020. The environment drives microbial trait variability in aquatic habitats. Mol. Ecol. (January): 1–13.

Bell, Jennifer K., Steven D. Siciliano, and Eric G. Lamb. 2020. "A Survey of Invasive Plants on Grassland Soil Microbial Communities and Ecosystem Services." *Scientific Data* 7 (1): 1–8.

Bhattacharyya, P.N., and D.K. Jha. 2012. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World J. Microbiol. Biotechnol. 28(4): 1327–1350.

Bolyen, E., J.R. Rideout, M.R. Dillon, N.A. Bokulich, C. Abnet, et al. 2018. QIIME 2 : Reproducible , interactive , scalable , and extensible microbiome data science. PeerJ.

Borcard, D., Gillet, F., Legendre, & Legendre, P. (2011). Numerical Ecology with R. In *Springer*.

Bordoloi, G.N., B. Kumari, A. Guha, M. Bordoloi, R.N.S. Yadav, et al. 2001. Isolation and structure elucidation of a new antifungal and antibacterial antibiotic produced by Streptomyces sp. 201. Biosci. Biotechnol. Biochem. 65(8): 1856–1858.

Broz, A. K., Manter, D. K. & Vivanco, J. M. Soil fungal abundance and diversity: Another victim of the invasive plant Centaurea maculosa. *ISME J.* **1**, 763–765 (2007).

Bulgarelli D, Schlaeppi K, Spaepen S, et al 2013. Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–38.

Bunn, R. A., Ramsey, P. W. & Lekberg, Y. Do native and invasive plants differ in their interactions with arbuscular mycorrhizal fungi? A meta-analysis. *J. Ecol.* **103**, 1547–1556 (2015).

Busby PE, Soman C, Wagner MR, et al 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. PLoS Biol 15:1–14.

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. Nature 427:731–733.

Canola Council of Canada, 2020-05-25. https://www.canolacouncil.org/canola encyclopedia/crop-development/growth-stages/

Cao, J., X. Jia, S. Pang, Y. Hu, Y. Li, et al. 2020. Functional structure, taxonomic composition and the dominant assembly processes of soil prokaryotic community along an altitudinal gradient. Appl. Soil Ecol. 155(May): 103647.

Caporaso JG, Lauber CL, Walters WA, et al 2011 Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci 108:4516–4522.

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol. Fertil. Soils 48:489–499.

Carter, M. R., and E. G. Gregorich, editors. Soil Sampling and Methods of Analysis. 2008. Page Soil Sampling and Methods of Analysis, Second Edition. 2nd edition. CRC Press, Boca Raton.

Carvalho, C. R., Dias, A. C. F., Homma, S. K., & Cardoso, E. J. B. N. 2020. Phyllosphere bacterial assembly in citrus crop under conventional and ecological management. *PeerJ*, *2020*(6), 1–20.

Ceja-Navarro, J.A., Y. Wang, D. Ning, A. Arellano, L. Ramanculova, et al. 2021. Protist diversity and community complexity in the rhizosphere of switchgrass are dynamic as plants develop. Microbiome 9(1): 1–18.

Chagnon, P. L., Brown, C., Stotz, G. C. & Cahill, J. F. Soil biotic quality lacks spatial structure and is positively associated with fertility in a northern grassland. *J. Ecol.* **106**, 195–206 (2018).

Chase, J. M., & Myers, J. A. 2011. Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*(1576), 2351–2363.

Christiansen, D.E., S.L. Markstrom, and L.E. Hay. 2011. Impacts of climate change on the growing season in the United States. Earth Interact. 15(33): 1–17.

Clarke, W. E. *et al.* 2016. A high - density SNP genotyping array for Brassica napus and its ancestral diploid species based on optimised selection of single - locus markers in the allotetraploid genome. *Theor. Appl. Genet.* **129**, 1887–1899.

Coleman-Derr, D., and S.G. Tringe. 2014. Building the crops of tomorrow: Advantages of symbiont-based approaches to improving abiotic stress tolerance. Front. Microbiol. 5(JUN): 1–6.

Copeland, J.K., L. Yuan, M. Layeghifard, P.W. Wang, and D.S. Guttman. 2015. Seasonal community succession of the phyllosphere microbiome. Mol. Plant-Microbe Interact. 28(3): 274–285.

Costanza, R., D'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R. V., Paruelo, J., Raskin, R. G., Sutton, P., & van den Belt, M. 1997. The value of the world's ecosystem services and natural capital. *Nature TA - TT -*, *387*(6630), 253–260.

Costanza, R., de Groot, R., Braat, L., Kubiszewski, I., Fioramonti, L., Sutton, P., Farber, S., & Grasso, M. (2017). Twenty years of ecosystem services: How far have we come and how far do we still need to go? *Ecosystem Services*, *28*, 1–16.

Cordero, J., J.R. de Freitas, and J.J. Germida. 2020. Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. Can. J. Microbiol. 66(1): 71–85.

Cormack, R. M., Legendre, L., & Legendre, P. (1984). Numerical Ecology. *Biometrics*, *40*(1), 280.

Coutinho, T.A., and S.N. Venter. 2009. Pantoea ananatis: An unconventional plant pathogen. Mol. Plant Pathol. 10(3): 325–335.

Coyte KZ, Rao C, Rakoff-Nahoum S, Foster KR 2021. Ecological rules for the assembly of microbiome communities. PLoS Biol 19:1–19.

Crafton, E. R., & Angadjivand, S. (2018). *Invasive Species: A Brief Overview*. 2. www.crs.gov%7C7-5700

Daily, G.C. et al. (1997) Ecosystem services: benefits applied to human societies by natural ecosystems. Issues Ecol. 2, 1–16.

Dini-Andreote, F., J.C. Stegen, J.D. Van Elsas, and J.F. Salles. 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proc. Natl. Acad. Sci. U. S. A. 112(11): E1326–E1332.

Dini-Andreote, F., and J.M. Raaijmakers. 2018. Embracing Community Ecology in Plant Microbiome Research. Trends Plant Sci. 23(6): 467–469.

Dorokhov, Y.L., E. V. Sheshukova, and T. V. Komarova. 2018. Methanol in plant life. Front. Plant Sci. 871(November): 1–6. doi: 10.3389/fpls.2018.01623.

Dumbrell, A. J., Nelson, M., Helgason, T., Dytham, C., & Fitter, A. H. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. *International Society for Microbial Ecology*, *4*(3), 337–345.

Dunfield, K.E., and J.J. Germida. 2003. Seasonal Changes in the Rhizosphere Microbial Communities Associated with Field-Grown Genetically Modified Canola (Brassica napus). Appl. Environ. Microbiol. 69(12): 7310–7318.

Dunfield, K.E., and J.J. Germida. 2001. Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified Brassica napus. FEMS Microbiol. Ecol. 38(1): 1–9.

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., Eisen, J. A., & Sundaresan, V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences*, *112*(8), E911–E920

Egerton-Warburton, L.M., N.C. Johnson, and E.B. Allen. 2007. Mycorrhizal community dynamics following nitrogen fertilization: A cross-site test in five grasslands. Ecol. Monogr. 77(4): 527–544.

Ehrenfeld JG. 2003. Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes. Ecosystems 6:503–523.

Evans S, Martiny JBH, Allison SD. 2017. Effects of dispersal and selection on stochastic assembly in microbial communities. ISME J 11:176–185.

Falge E, Bayreuth U, Baldocchi DD, et al. 2002. Lincoln Seasonality of ecosystem respiration and gross primary production as derived from FLUXNET measurements. Agric For Meterology 113:53–74

Feng, Y., D. Shen, and W. Song. 2006. Rice endophyte Pantoea agglomerans YS19 promotes host plant growth and affects allocations of host photosynthates. J. Appl. Microbiol. 100(5): 938–945.

Fierer N, Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 103:626–631.

Fierer, N., Nemergut, D., Knight, R. & Craine, J. M. 2010. Changes through time: Integrating microorganisms into the study of succession. *Res. Microbiol.* **161**, 635–642.

Fine, P.V.A., and S.W. Kembel. 2011. Phylogenetic community structure and phylogenetic turnover across space and edaphic gradients in western Amazonian tree communities. Ecography (Cop.). 34(4): 552–565.

Freilich, S., R. Zarecki, O. Eilam, E.S. Segal, C.S. Henry, et al. 2011. Competitive and cooperative metabolic interactions in bacterial communities. Nat. Commun. 2(1).

Friesen, M.L., S.S. Porter, S.C. Stark, E.J. Von Wettberg, J.L. Sachs, et al. 2011. Microbially mediated plant functional traits. Annu. Rev. Ecol. Evol. Syst. 42.

Fukami, T., and M. Nakajima. 2011. Community assembly: Alternative stable states or alternative transient states? Ecol. Lett. 14(10): 973–984.

Gerhold P, Cahill JF, Winter M, et al (2015) Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). Funct Ecol 29:600–614.

Gregory, P.J. 2006. Roots, rhizosphere and soil: The route to a better understanding of soil science? Eur. J. Soil Sci. 57(1): 2–12.

Gibbons, S. M., Lekberg, Y., Mummey, D. L., Sangwan, N., Ramsey, P. W., & Gilbert, J. A. (2017). Invasive Plants Rapidly Reshape Soil Properties in a Grassland Ecosystem. *MSystems*, *2*(2).

Gopalakrishnan S, Sathya A, Vijayabharathi R, et al. 2015. Plant growth promoting rhizobia: challenges and opportunities. 3 Biotech 5:355–377.

Gkarmiri, K., S. Mahmood, A. Ekblad, S. Alström, N. Högberg, et al. 2017. Identifying the active microbiome associated with roots and rhizosphere soil of oilseed rape. Appl. Environ. Microbiol. 83(22): 1–14.

Glaeser, S.P., I. Gabur, H. Haghighi, J.O. Bartz, P. Kämpfer, et al. 2020. Endophytic bacterial communities of oilseed rape associate with genotype-specific resistance against Verticillium longisporum. FEMS Microbiol. Ecol. 96(1): 1–15.

Gopalakrishnan, S., A. Sathya, R. Vijayabharathi, R.K. Varshney, C.L.L. Gowda, et al. 2015. Plant growth promoting rhizobia: challenges and opportunities. 3 Biotech 5(4): 355–377.

Graham EB, Knelman JE, Schindlbacher A, et al. 2016. Microbes as engines of ecosystem function: When does community structure enhance predictions of ecosystem processes? Front Microbiol 7:1–10.

Gross, D. V & Romo, J. T. 2010 Burning history, time of burning, and year effects on plant community structure and heterogeneity in fescue prairie. *Botany* **88**, 1–12.

Gross, D. V. & Romo, J. T. 2019. Temporal changes in species composition in Fescue Prairie: relationships with burning history, time of burning , and environmental. Plant Ecology. **208**, 137–153.

Haichar, F. el Z., Santaella, C., Heulin, T., & Achouak, W. 2014. Root exudates mediated interactions belowground. *Soil Biology and Biochemistry*, *77*, 69–80.

Hall SJ, Asner GP. 2007. Biological invasion alters regional nitrogen-oxide emissions from tropical rainforests. Glob. Change Biol. 13:2143–60

Hanson, R.S., and T.E. Hanson. 1996. Methanotrophic bacteria. Microbiol. Rev. 60(2): 439–471.

Harms, H., H.P. Koops, and H. Wehrmann. 1976. An ammonia-oxidizing bacterium, Nitrosovibrio tenuis nov. gen. nov. sp. Arch. Microbiol. 108(1): 105–111.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., & Widmer, F. 2015. Distinct soil microbial diversity under long-term organic and conventional farming. *ISME Journal*, *9*(5), 1177–1194.

Hawkes, C. V., Wren, I. F., Herman, D. J., & Firestone, M. K. (2005). Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecology Letters*, *8*(9), 976 985.

Hawkes, C. V., J. Belnap, C. D'Antonio, and M.K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant Soil 281(1–2): 369–380.

Hilton, S., A.J. Bennett, D. Chandler, P. Mills, and G.D. Bending. 2018. Preceding crop and seasonal effects influence fungal, bacterial and nematode diversity in wheat and oilseed rape rhizosphere and soil. Appl. Soil Ecol. 126(December 2017): 34–46

Hu, F.P., J.M. Young, and M.J. Fletcher. 1998. Preliminary description of biocidal (syringomycin) activity in fluorescent plant pathogenic Pseudomonas species. J. Appl. Microbiol. 85(2): 365–371.

Hubbell, S.P., 2001. The Unified Neutral Theory of Biodiversity and Biogeography (MPB- 32).

Illumina. 16S Metagenomic Sequencing Library. *Illumina.com* 1–28 (2013).

Inderjit, van der Putten WH (2010) Impacts of soil microbial communities on exotic plant invasions. Trends Ecol Evol 25:512–519.

Jacoby, R.P., and S. Kopriva. 2019. Metabolic niches in the rhizosphere microbiome: New tools and approaches to analyse metabolic mechanisms of plant-microbe nutrient exchange. J. Exp. Bot. 70(4): 1087–1094.

Jangid, K., Williams, M. A., Franzluebbers, A. J., Sanderlin, J. S., Reeves, J. H., Jenkins, M. B., Endale, D. M., Coleman, D. C., & Whitman, W. B. 2008. Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biology and Biochemistry*, *40*(11), 2843–2853.

Jiao, S., Yang, Y., Xu, Y., Zhang, J., & Lu, Y. 2020. Balance between community assembly processes mediates species coexistence in agricultural soil microbiomes across eastern China. *ISME Journal*, *14*(1), 202–216.

Jones DL, Nguyen C, Finlay RD. 2009. Carbon flow in the rhizosphere: Carbon trading at the soil-root interface. Plant Soil 321:5–33.

Jost, L. 2007. Partitioning diversity into independent alpha and beta components. Ecology 88(10): 2427–2439.

Kembel, S. W. Disentangling niche and neutral influences on community assembly: Assessing the performance of community phylogenetic structure tests. *Ecol. Lett.* **12**, 949–960 (2009).

Kembel, S. W. *et al.* Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* **26**, 1463–1464 (2010).

Kim, B.R., J. Shin, R.B. Guevarra, J.H. Lee, D.W. Kim, et al. 2017. Deciphering diversity indices for a better understanding of microbial communities. J. Microbiol. Biotechnol. 27(12): 2089–2093.

Knief, C., A. Ramette, L. Frances, C. Alonso-blanco, and J.A. Vorholt. 2010. Site and plant species are important determinants of the Methylobacterium community composition in the plant phyllosphere. ISME J.: 719–728.

Kraft and Ackerly. 2014 Ecology and the environment. Chapter 3: Assembly of Plant Communities: Ecol Environ 69-88.

Knapp, P. A. 1996. Cheatgrass (Bromus tectorum L) dominance in the great basin desert. History, persistence, and influences to human activities. *Global Environmental Change*, *6*(1), 37–52.

Kuramae EE, Yergeau E, Wong LC, et al. 2012. Soil characteristics more strongly influence soil bacterial communities than land-use type. FEMS Microbiol Ecol 79:12–24.

Lamb, E. G. *et al.* A High Arctic soil ecosystem resists long-term environmental manipulations. *Glob. Chang. Biol.* **17**, 3187–3194 (2011).

Lamb, E. G., Kennedy, N., & Siciliano, S. D. (2011). Effects of plant species richness and evenness on soil microbial community diversity and function. *Plant and Soil*, *338*(1), 483–495.

Lancashire, P.D., H. Bleiholder, T. Van Den Boom, P. Langelüddeke, R. Stauss, et al. 1991. A uniform decimal code for growth stages of crops and weeds. Ann. Appl. Biol. 119(3): 561–601.

Langenheder, S., and E.S. Lindström. 2019. Factors influencing aquatic and terrestrial bacterial community assembly. Environ. Microbiol. Rep. 11(3): 306–315.

Legendre, P., and M. J. Andersson. (1999). Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecological Monographs 69:1–24.

Leff, J. W., Jones, S. E., Prober, S. M., Barberán, A., Borer, E. T., Firn, J. L., Harpole, W. S., Hobbie, S. E., Hofmockel, K. S., Knops, J. M. H., McCulley, R. L., La Pierre, K., Risch, A. C., Seabloom, E. W., Schütz, M., Steenbock, C., Stevens, C. J., & Fierer, N. 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(35), 10967–10972.

Lekberg, Y., Gibbons, S. M., Rosendahl, S., & Ramsey, P. W. 2013. Severe plant invasions can increase mycorrhizal fungal abundance and diversity. *ISME Journal*, *7*(7), 1424–1433.

Levine, J. M., Pachepsky, E., Kendall, B. E., Yelenik, S. G., & Lambers, J. H. R. 2006. Plant soil feedbacks and invasive spread. *Ecology Letters*, *9*(9), 1005–1014.

Li, Z. *et al.* 2018. Plant belowground diversity and species segregation by depth in a semi-arid grassland. *Écoscience* **25**, 1–7.

Liu, W., J. Russel, H.L. Røder, J.S. Madsen, M. Burmølle, et al. 2017. Low-abundant species facilitates specific spatial organization that promotes multispecies biofilm formation. Environ. Microbiol. 19(7): 2893–2905.

Looman, J. The Fescue grasslands of Western Canada. 1969. *Veg. Acta Geobot.* **19**, 128–145.

Lozupone, C., and R. Knight. 2005. UniFrac: A new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology 71:8228–8235.

Lurie, E., Edwards, J., Eisen, J. A., Podishetty, N. K., Johnson, C., Bhatnagar, S., Santos-Medellín, C., & Sundaresan, V. 2015. Structure, variation, and assembly of the root-

associated microbiomes of rice. *Proceedings of the National Academy of Sciences*, *112*(8), E911–E920.

Mack, R., D. Simberloff, W.M. Lonsdale, H. Evans, M. Clout, et al. 2000. Biotic Invasions: Causes, Epidemiology, Global Consequences and Control. Ecol. Appl. 10(November 1999): 689–710.

Mamet, S. D., Lamb, E. G., Piper, C. L., Winsley, T. & Siciliano, S. D. 2017. Archaea and bacteria mediate the effects of native species root loss on fungi during plant invasion. *ISME J.* **11**, 1261–1275.

Mamet, S. D. *et al.* Structural equation modeling of a winnowed soil microbiome identifies how invasive plants re-structure microbial networks. 2019. *ISME J.* **13**, 1988–1996.

Marina, M., S.J. Maiale, F.R. Rossi, M.F. Romero, E.I. Rivas, et al. 2008. Apoplastic polyamine oxidation plays different roles in local responses of tobacco to infection by the necrotrophic fungus Sclerotinia sclerotiorum and the biotrophic bacterium Pseudomonas viridiflava. Plant Physiol. 147(4): 2164–2178.

Matthews TJ, Whittaker RJ. 2014. Neutral theory and the species abun- dance distribution: recent developments and prospects for unifying niche and neutral perspectives. Ecol Evol 4:2263–2277.Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. a, Green, J. L., … Staley, J. T. 2006. Microbial biogeography: putting microorganisms on the map. *Nature Reviews. Microbiology*, *4*.

Mason, A. S. *et al.* 2017. A user guide to the Brassica 60K Illumina Infinium ™ SNP genotyping array. *Theor. Appl. Genet.* **130**, 621–633.

Mariotte, P., Mehrabi, Z., Bezemer, T. M., De Deyn, G. B., Kulmatiski, A., Drigo, B., Veen, G. F. (Ciska., van der Heijden, M. G. A., & Kardol, P. 2018. Plant–Soil Feedback: Bridging Natural and Agricultural Sciences. *Trends in Ecology and Evolution*, *33*(2), 129–142.

Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. 2011. *EMBnet.journal* **17**, 10–12.

Martiny, J.B.H., S.E. Jones, J.T. Lennon, and A.C. Martiny. 2015. Microbiomes in light of traits: A phylogenetic perspective. Science (80-.). 350(6261).

Mbuthia, L. W., Acosta-Martínez, V., DeBryun, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., & Eash, N. 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. *Soil Biology and Biochemistry*, *89*, 24–34.

McDonald, D., J.C. Clemente, J. Kuczynski, J.R. Rideout, J. Stombaugh, et al. 2012. The Biological Observation Matrix (BIOM) format or: How I learned to stop worrying and love the ome-ome. Gigascience 464(1): 1–6.

McMurdie, P. J., and S. Holmes. 2013. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 8.

Millennium Ecosystem Assessment (Program). 2005. Ecosystems and human well being. Washington, D.C.: Island Press.

Mitter, E.K., J.R. de Freitas, and J.J. Germida. 2017. Bacterial root microbiome of plants growing in oil sands reclamation covers. Front. Microbiol. 8(MAY): 1–14.

Mohan Ram, H.Y., and I.V.R. Rao. 1984. Physiology of flower bud growth and opening. Proc. Plant Sci. 93(3): 253–274.

Morales Moreira, Z.P., B.L. Helgason, and J.J. Germida. 2020. Crop, genotype, and field environmental conditions shape bacterial and fungal seed epiphytic microbiomes. Can. J. Microbiol.: 1–35.

Morales Moreira, Z.P., B.L. Helgason, and J.J. Germida. 2021. Environment has a Stronger Effect than Host Plant Genotype in Shaping Spring Brassica napus Seed Microbiomes . Phytobiomes J.: PBIOMES-08-20-0.

Mori, H. *et al.* 2014. Design and experimental application of a novel non-degenerate universal primer set that amplifies prokaryotic 16S rRNA genes with a low possibility to amplify eukaryotic rRNA genes. *DNA Res.* **21**, 217–227.

Nemergut, Diana R., Steven K. Schmidt, Tadashi Fukami, Sean P. O'Neill, Teresa M. Bilinski, Lee F. Stanish, Joseph E. Knelman, et al. 2013. "Patterns and Processes of Microbial Community Assembly." *Microbiology and Molecular Biology Reviews* 77 (3): 342–56.

Newton AC, Fitt BDL, Atkins SD, et al. 2010. Pathogenesis, parasitism and mutualism in the trophic space of microbe-plant interactions. Trends Microbiol 18:365–373.

Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan, E. J., Mathesius, U., Poot, P., Purugganan, M. D., Richards, C. L., Valladares, F., & van Kleunen, M. 2010. Plant phenotypic plasticity in a changing climate. *Trends in Plant Science*, *15*(12), 684–692.

Ning, D. *et al.* 2020. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. *Nat. Commun.* **11**.

Nisbet, R.E.R., R. Fisher, R.H. Nimmo, D.S. Bendall, P.M. Crill, et al. 2009. Emission of methane from plants. Proc. R. Soc. B Biol. Sci. 276(1660): 1347–1354

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn, P. R. Minchin, R. B. O'hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs, and H. W. Maintainer. 2019. Package "vegan" Title Community Ecology Package.

Paradis E, Schliep K. 2019. "ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R." *Bioinformatics*, **35**, 526-528.

Paruelo, J.M., M. Texeira, L. Staiano, M. Mastrángelo, L. Amdan, et al. 2016. An integrative index of Ecosystem Services provision based on remotely sensed data. Ecol. Indic. 71: 145–154.

Pejchar, L., & Mooney, H. A. (2009). Invasive species, ecosystem services and human well-being. *Trends in Ecology and Evolution*, *24*(9), 497–504.

Pérez-Jaramillo, J. E., Mendes, R., & Raaijmakers, J. M. 2016. Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Molecular Biology*, *90*(6), 635–644.

Phillips, M.L., S.E. Weber, L. V. Andrews, E.L. Aronson, M.F. Allen, et al. 2019. Fungal community assembly in soils and roots under plant invasion and nitrogen deposition. Fungal Ecol. 40: 107–117.

Piccoli P, Bottini R. 2013. Terpene Production by Bacteria and its Involvement in Plant Growth Promotion, Stress Alleviation, and Yield Increase. Mol Microb Ecol Rhizosph 1:335–343.

Pielou, E.C. 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. 13(C): 131–144.

Piper, C. L., Siciliano, S. D., Winsley, T. & Lamb, E. G. 2015. Smooth brome invasion increases rare soil bacterial species prevalence, bacterial species richness and evenness. *J. Ecol.* **103**, 386–396.

Piper, C. L., Lamb, E. G. & Siciliano, S. D. 2015. Smooth brome changes gross soil nitrogen cycling processes during invasion of a rough fescue grassland. *Plant Ecol.* **216**, 235–246.

Ponomarova, O., N. Gabrielli, D.C. Sévin, M. Mülleder, K. Zirngibl, et al. 2017. Yeast Creates a Niche for Symbiotic Lactic Acid Bacteria through Nitrogen Overflow. Cell Syst. 5(4): 345-357.e6

Potts SG, Imperatriz-Fonseca V, Ngo HT, et al. 2016. Safeguarding pollinators and their values to human well-being. Nature 540:220–229.

Prevéy, J.S., and T.R. Seastedt. 2014. Seasonality of precipitation interacts with exotic species to alter composition and phenology of a semi-arid grassland. J. Ecol. 102(6): 1549–1561.

Pyšek, Petr, and David M. Richardson. “Invasive Species, Environmental Change and Management, and Health.” *Annual Review of Environment and Resources*, vol. 35, 2010, pp. 25–55.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, et al. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 41(D1): 590–596.

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

Ramirez, K. S., Craine, J. M., & Fierer, N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, *18*(6), 1918–1927.

Romero, F.M., F.R. Rossi, A. Gárriz, P. Carrasco, and O.A. Ruíz. 2019. A bacterial endophyte from apoplast fluids protects canola plants from different phytopathogens via antibiosis and induction of host resistance. Phytopathology 109(3): 375–383.

Romo, J. T. 2003. Reintroducing fire for conservation of fescue prairie association remnants in the northern Great Plains. *Canadian Field Naturalist* **117**, 89-99.

Ryan, P.R., Y. Dessaux, L.S. Thomashow, and D.M. Weller. 2009. Rhizosphere engineering and management for sustainable agriculture. Plant Soil 321(1–2): 363–383.

Saccá ML, Barra Caracciolo A, Di Lenola M, Grenni P. 2017 Ecosystem Services Provided By Soil Microorganisms. In: Lukac M, Grenni P, Gamboni M (eds) Soil Biological Communities and Ecosystem Resilience. Springer International Publishing, Cham, pp 9–24

Salesman, J. B. and M. Thomsen. 2011 "Smooth Brome (*Bromus inermis*) in tallgrass prairies: a review of control methods and future research directions. *Ecological Restoration* **29**, 374-381.

Schlatter, D.C., J.C. Hansen, W.F. Schillinger, T.S. Sullivan, and T.C. Paulitz. 2019. Common and unique rhizosphere microbial communities of wheat and canola in a semiarid Mediterranean environment. Appl. Soil Ecol. 144(July): 170–181.

Shade, A., and J. Handelsman. 2012. Beyond the Venn diagram: The hunt for a core microbiome. Environ. Microbiol. 14(1): 4–12.

Shade, A., and N. Stopnisek. 2019. Abundance-occupancy distributions to prioritize plant core microbiome membership. Curr. Opin. Microbiol. 49: 50–58.

Shafroth PB, Cleverly JR, Dudley TL, et al. 2005. Control of Tamarix in the western United States: Implications for water salvage, wildlife use, and riparian restoration. Environ Manage 35:231–246.

Sheng Y, Li G, Dong H, et al. 2021. Distinct assembly processes shape bacterial communities along unsaturated, groundwater fluctuated, and saturated zones. Sci Total Environ 761:143303.

Shu, Z., Y. Shi, H. Qian, Y. Tao, and D. Tang. 2010. Distinct respiration and physiological changes during flower development and senescence in two freesia cultivars. HortScience 45(7): 1088–1092.

Siciliano, S.D., and J.J. Germida. 1999. Taxonomic diversity of bacteria associated with the roots of field-grown transgenic Brassica napus cv. Quest, compared to the non-transgenic B. napus cv. Excel and B. rapa cv. Parkland. FEMS Microbiol. Ecol. 29(3): 263–272.

Slopek, J. I. & Lamb, E. G. 2017. Long-Term Efficacy of Glyphosate for Smooth Brome Control in Native Prairie. *Invasive Plant Sci. Manag.* **10**, 350–355.

Smalla, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, et al. 2001. Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis: Plant-Dependent Enrichment and Seasonal Shifts Revealed. Appl. Environ. Microbiol. 67(10): 4742–4751.

Smets, W., J.W. Leff, M.A. Bradford, R.L. McCulley, S. Lebeer, et al. 2016. A method for simultaneous measurement of soil bacterial abundances and community composition via 16S rRNA gene sequencing. Soil Biol. Biochem. 96: 145–151.

Smith TW, Lundholm JT. 2010. Variation partitioning as a tool to distinguish between niche and neutral processes. Ecography 33: 648–655.

Stegen, J. C., Lin, X., Konopka, A. E. & Fredrickson, J. K. Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J.* **6**, 1653–1664 (2012).

Stegen, J. C. *et al.* Quantifying community assembly processes and identifying features that impose them. *ISME J.* **7**, 2069–2079 (2013)

Stegen, J. C., Lin, X., Fredrickson, J. K. & Konopka, A. E. Estimating and mapping ecological processes influencing microbial community assembly. *Front. Microbiol.* **6**, 1–15 (2015).

Stotz, G. C., Gianoli, E. & Cahill, J. F. Biotic homogenization within and across eight widely distributed grasslands following invasion by Bromus inermis. *Ecology* **100**, 1–13 (2019).

Suding, K. N., Stanley Harpole, W., Fukami, T., Kulmatiski, A., Macdougall, A. S., Stein, C., & van der Putten, W. H. 2013. Consequences of plant-soil feedbacks in invasion. *Journal of Ecology*, *101*(2), 298–308.

Tan, S., Y. Gu, C. Yang, Y. Dong, X. Mei, et al. 2015. Bacillus amyloliquefaciens T-5 may prevent Ralstonia solanacearum infection through competitive exclusion. Biol. Fertil. Soils 52(3): 341–351.

Tannas K: 2003. Common Plants of the Western Rangelands, Vol. 1: Grasses, Grass-like Species. Edmonton: Agriculture, Food & Rural Development.

Tannas K: 2003. Common Plants of the Western Rangelands, Vol. 3: Forbs. Edmonton: Agriculture, Food & Rural Development.

Toju, H., Okayasu, K. & Notaguchi, M. 2019. Leaf-associated microbiomes of grafted tomato plants. Sci. Rep. 9 , 1787.

Tripathi, B.M., J.C. Stegen, M. Kim, K. Dong, J.M. Adams, et al. 2018. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. ISME J. 12(4): 1072–1083.

Trivedi P, Leach JE, Tringe SG, et al. 2020. Plant–microbiome interactions: from community assembly to plant health. Nat Rev Microbiol 18:607–621.

Tuomisto, H. 2012. An updated consumer's guide to evenness and related indices. *Oikos* **121**, 1203 1218.

Tylianakis, Jason M., Raphael K. Didham, Jordi Bascompte, and David A. Wardle. 2008. "Global Change and Species Interactions in Terrestrial Ecosystems." *Ecology Letters* 11 (12): 1351–63.

Uren NC. 2013. Root Exudates and Soil: Crucial for Molecular Understanding of Interactions in the Rhizosphere. Mol Microb Ecol Rhizosph 1:221–227.

Vacher C, Hampe A, Porté AJ, et al. 2016. The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. Annu Rev Ecol Evol Syst 47:1–24.

Van Der Putten, W. H., Klironomos, J. N. & Wardle, D. A. 2007. Microbial ecology of biological invasions. *ISME Journal* **1**, 28–37.

Van Der Putten WH, Klironomos JN, Wardle DA. 2007. Microbial ecology of biological invasions. ISME J. 1:28–37

Van der Putten, Wim H., Richard D. Bardgett, James D. Bever, T. Martijn Bezemer, Brenda B. Casper, Tadashi Fukami, Paul Kardol, et al. 2013. "Plant-Soil Feedbacks: The Past, the Present and Future Challenges." *Journal of Ecology* 101 (2): 265–76.

Vandamme, P., K. Opelt, N. Knöchel, C. Berg, S. Schönmann, et al. 2007. Burkholderia bryophila sp. nov. and Burkholderia megapolitana sp. nov., moss-associated species with antifungal and plant-growth-promoting properties. Int. J. Syst. Evol. Microbiol. 57(10): 2228–2235.

Vandenkoornhuyse, P., A. Quaiser, M. Duhamel, A. Le Van, and A. Dufresne. 2015. The importance of the microbiome of the plant holobiont. New Phytol. 206(4): 1196–1206.

Vellend, M. 2010. Conceptual Synthesis in Community Ecology. *The Quarterly Review of Biology*, *85*(2), 183–206.

Vilà, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarošík, V., Maron, J. L., Pergl, J., Schaffner, U., Sun, Y., & Pyšek, P. 2011. Ecological impacts of invasive alien plants: A meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters*, *14*(7), 702–708.

Vishnivetskaya, T.A., S. Kathariou, and J.M. Tiedje. 2009. The Exiguobacterium genus: Biodiversity and biogeography. Extremophiles 13(3): 541–555.

Vogel, C., N. Bodenhausen, W. Gruissem, and J.A. Vorholt. 2016. The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. New Phytol. 212(1): 192–207.

Vorholt J. 2012. Microbial life in the phyllosphere. Nat Rev Microbiol 10:828–40.

Walsh JR, Carpenter SR, Van Der Zanden MJ. 2016. Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proc Natl Acad Sci U S A 113:4081–4085.

Wassermann, B., D. Rybakova, C. Müller, and G. Berg. 2017. Harnessing the microbiomes of Brassica vegetables for health issues. Sci. Rep. 7(1): 1–12

Watt M, Silk WK, Passioura JB. 2006. Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. Ann Bot 97:839–855.

Walters, W. *et al.* 2015. Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. *mSystems* **1**, e0009-15.

Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: An example for rain forest trees. *Am. Nat.* **156**, 145–155.

Widder, S., Allen, R. J., Pfeiffer, T., Curtis, T. P., Wiuf, C., Sloan, W. T., Cordero, O. X., Brown, S. P., Momeni, B., Shou, W., Kettle, H., Flint, H. J., Haas, A. F., Laroche, B., Kreft, J.-U., Rainey, P. B., Freilich, S., Schuster, S., Milferstedt, K., … Soyer, O. S. 2016. Challenges in microbial ecology: building predictive understanding of community function and dynamics. *The ISME Journal*, *10*(11), 2557–2568.

WWF. 2018. Living Planet Report - 2018: Aiming higher. In *Environmental Conservation* (Vol. 26, Issue 04).

Walterson, A.M., and J. Stavrinides. 2015. Pantoea: Insights into a highly versatile and diverse genus within the Enterobacteriaceae. FEMS Microbiol. Rev. 39(6): 968–984.

Wassermann, B., D. Rybakova, C. Müller, and G. Berg. 2017. Harnessing the microbiomes of Brassica vegetables for health issues. Sci. Rep. 7(1): 1–12.

Wood, S.A. et al. 2015. Functional traits in agriculture: agro- biodiversity and ecosystem services. Trends Ecol. Evol. 30, 531–539

Yang, J., B. Xie, J. Bai, and Q. Yang. 2012. Purification and characterization of a nitroreductase from the soil bacterium Streptomyces mirabilis. Process Biochem. 47(5): 720–724.

Zhang, Pei, Bo Li, Jihua Wu, and Shuijin Hu. 2019. "Invasive Plants Differentially Affect Soil Biota through Litter and Rhizosphere Pathways: A Meta-Analysis." *Ecology Letters*.

Zhang, L., B. Ma, C. Tang, H. Yu, X. Lv, et al. Heterogeneity induced by pyrogenic organic matter in wildfire-perturbed soils mediates bacterial community assembly processes.

Zhou, J., and D. Nig. 2017. Stochastic Community Assembly : Does It. Microbiol. Mol. Biol. Rev. 81(4): 1–32.

APPENDICES

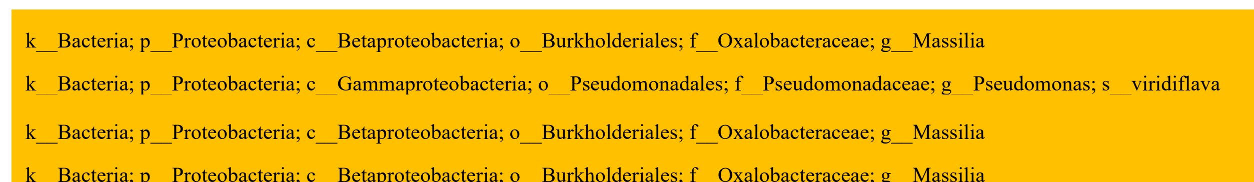
APPENDIX 1

Supplemental information for Chapter 3

Table A1.1: Summary of the NAM lines used in this study, including sources from Agrifood and Agriculture Canada (AAFC), United States Department of Agriculture (USDA) and Plant Gene Resources of Canada (PGRC) and seed trait qualities.

			Seed Quality Traits			
NAM Code	**Source**	**Origin**	**Seed Color**	**Fiber**	**Low Erucic**	**Low Glucosinolate**
NAM 0	AAFC	Canada	Black	Low	X	X
NAM 13		Germany	Black	High	X	X
NAM 17	AAFC	Canada	Black	Low	X	X
NAM 32	PGRC	South Korea	Black		X	
NAM 37		Australia	Black	High	X	
NAM 43	USDA	Bangladesh	Black			
NAM 72	AAFC	Canada	Yellow	Very Low	X	X
YN04	AAFC	Canada	Black	Very Low	X	X

Table A1.2: Summary of the taxonomoy of core phyllosphere members. Yellow represents the core, which is always present throughout the growing season. Orange represents the members present before or during flowering. Blue represents the members present after flowering, during pod development and ripening.



Taxonomy
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia

Core
Flowering Core
Pod Core

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Pantoea
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Pantoea
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Pantoea
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Pantoea
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Pantoea
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Sphingobacteriaceae; g__Pedobacter; s__
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__[Exiguobacteraceae]; g__Exiguobacterium; s__
k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__[Exiguobacteraceae]; g__Exiguobacterium; s__
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__[Exiguobacteraceae]; g__Exiguobacterium; s__
k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__[Exiguobacteraceae]; g__Exiguobacterium; s__
k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__[Exiguobacteraceae]; g__Exiguobacterium; s__
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__viridiflava
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Pantoea
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Pantoea
k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__fragi
k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Pantoea

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Massilia

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Stenotrophomonas

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Sphingobacteriaceae; g__Pedobacter; s__

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus; s__longiquaesitum

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Planctomycetes; c__Phycisphaerae; o__WD2101; f__; g__; s__

k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__

k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus; s__flexus

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Sinobacteraceae; g__; s__

k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Sphingobacteriaceae; g__Mucilaginibacter; s__composti

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Kaistobacter; s__

k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__Sphingobacteriaceae; g__Pedobacter; s__

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Propionibacteriaceae; g__Microlunatus; s__

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Mycobacteriaceae; g__Mycobacterium; s__vaccae

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__SC-I-84; f__; g__; s__

k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Chitinophagaceae; g__; s__

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Bradyrhizobiaceae

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Nitrosomonadales; f__Nitrosomonadaceae; g__Nitrosovibrio; s__tenuis

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Propionibacteriaceae; g__Propionibacterium; s__acnes

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Micrococcaceae; g__Arthrobacter

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Propionibacteriaceae; g__Microlunatus; s__

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Streptomycetaceae; g__Streptomyces; s__mirabilis

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Rhizobiaceae

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Propionibacteriaceae; g__Microlunatus; s__

k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Burkholderiaceae; g__Burkholderia; s__bryophila

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Propionibacteriaceae; g__Microlunatus; s__

k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus; s__longiquaesitum

k__Bacteria; p__Chloroflexi; c__Ellin6529; o__; f__; g__; s__

k__Bacteria; p__Chloroflexi; c__Ellin6529; o__; f__; g__; s__

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Rhodanobacter; s__

k__Bacteria; p__Actinobacteria; c__Thermoleophilia; o__Gaiellales; f__Gaiellaceae; g__; s__

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Bradyrhizobiaceae

k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Nitrosomonadales; f__Nitrosomonadaceae; g__Nitrosovibrio; s__tenuis

k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__

k__Bacteria; p__Planctomycetes; c__Phycisphaerae; o__WD2101; f__; g__; s__

k__Bacteria; p__Chloroflexi; c__Ellin6529; o__; f__; g__; s__

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae

k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__SC-I-84; f__; g__; s__

k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Rhizobiaceae

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Kaistobacter; s__

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae

k__Bacteria; p__Planctomycetes; c__Phycisphaerae; o__WD2101; f__; g__; s__

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Kaistobacter; s__

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Bradyrhizobiaceae

k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__SC-I-84; f__; g__; s__

k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus; s__muralis

k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__

k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus; s__flexus

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Methylobacteriaceae; g__Methylobacterium

k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Chitinophagaceae; g__; s__

k__Bacteria; p__Planctomycetes; c__Phycisphaerae; o__WD2101; f__; g__; s__

k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__

k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Chitinophagaceae; g__; s__

k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Chitinophagaceae; g__; s__

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Geodermatophilaceae

k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas

k__Bacteria; p__Chloroflexi; c__Ktedonobacteria; o__Ktedonobacterales; f__Ktedonobacteraceae

k__Bacteria; p__Chloroflexi; c__Ellin6529; o__; f__; g__; s__

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Kaistobacter; s__

k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__

k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Chitinophagaceae

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Kaistobacter; s__

k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Nocardioidaceae; g__Kribbella; s__

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Bradyrhizobiaceae

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Kaistobacter; s__

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Bradyrhizobiaceae

k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Bradyrhizobiaceae

k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Chitinophagaceae; g__; s__

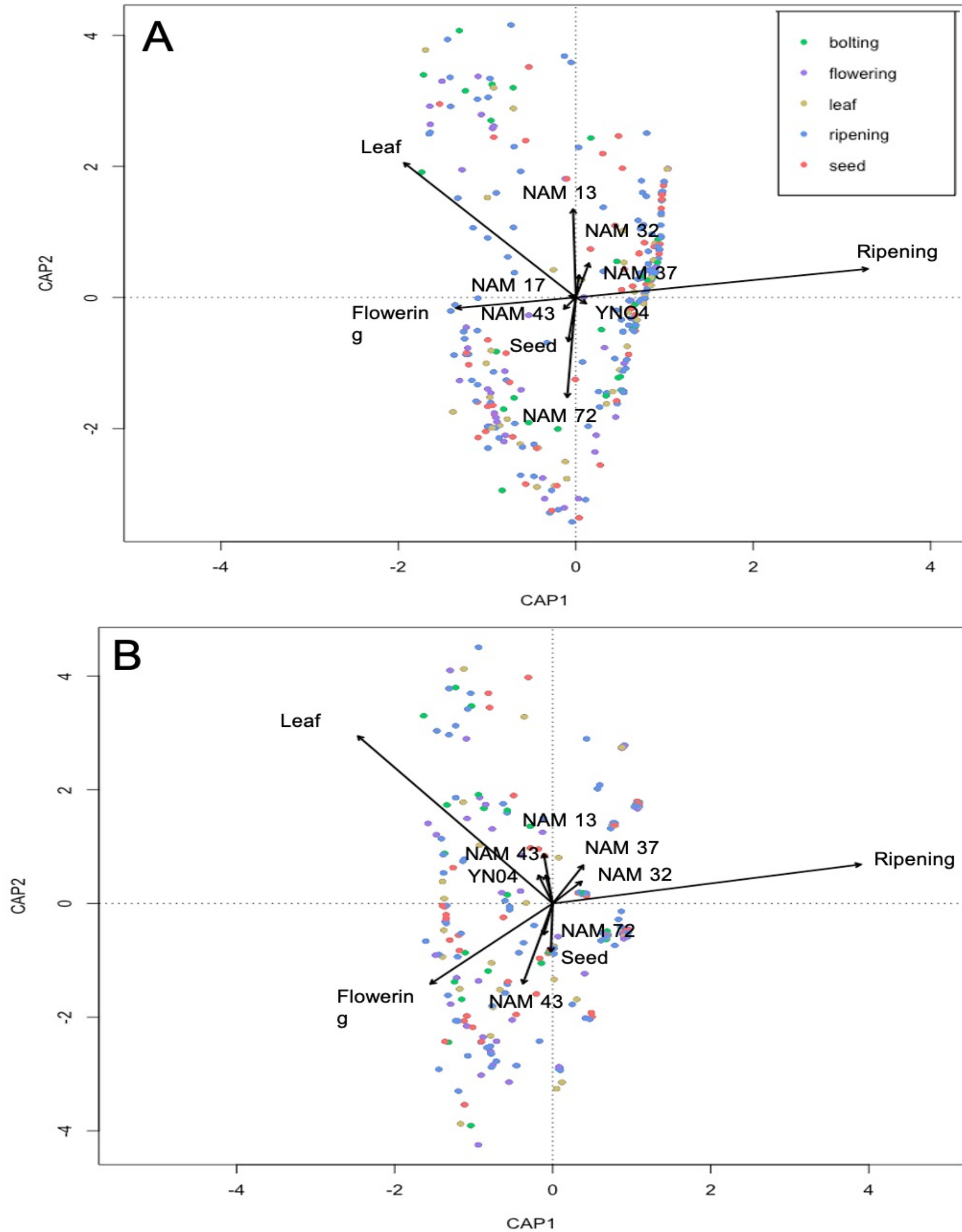


Figure A1.1: Distance-based redundancy analysis (dbRDA) of the weighted UniFrac distances (A) and unweighted UniFrac distances (B) of the core bacterial community, across the entire 10-week sampling period, constrained by BBCH stage x NAM (biplots). Points are colored based on canola growth stage.

Figure A1.2: Rarefaction curves of all samples

APPENDIX 2

Supplemental information for Chapter 4

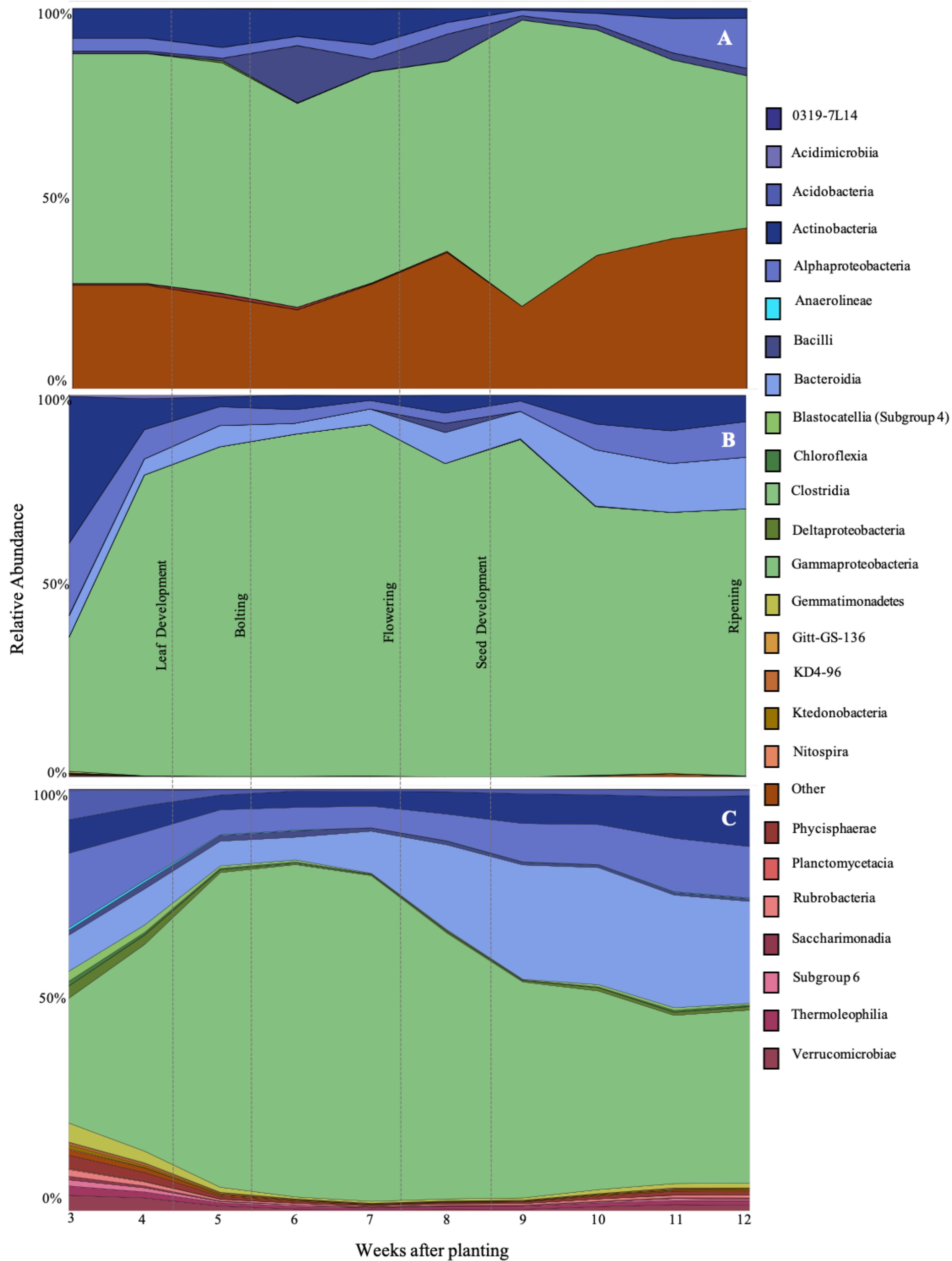


Figure A2.1: Relative abundance of bacterial communities on the leaf, root and rhizosphere over the ten week sampling period. Color represent bacterial classes and the growth stage of *B. napus* is indicated by the dotted line.

Table A3.1: Weekly diversity indices for the leaf, root and rhizosphere over the ten sampling weeks

		Leaf Development		Bolting	Flowering		Seed Development			Ripening	
	Week	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Leaf	Pielou's Evenness	0.9	0.9	0.9	0.9	0.5	0.7	0.7	0.8	0.8	0.9
	ACE	37.7	53.5	60.6	62.3	50.3	32.2	29.8	33.9	20.6	17.9
	Inverse Simpson	20.8	18.2	21.7	20.1	13.9	13.6	7.4	9.8	7.6	8.1
Root	Pielou's Evenness	0.9	0.8	0.8	0.7	0.7	0.7	0.7	0.8	0.8	0.8
	ACE	11.0	3.8	13.7	20.9	22.2	27.6	24.7	29.2	34.7	30.6
	Inverse Simpson	4.7	2.4	4.7	6.3	6.0	6.2	6.9	8.6	11.7	9.9
Rhizosphere	Pielou's Evenness	0.9	0.8	0.6	0.6	0.6	0.7	0.7	0.8	0.7	0.8
	ACE	426.7	461.2	299.9	281.2	252.7	315.8	364.4	389.6	491.6	466.1
	Inverse Simpson	127.0	64.3	25.1	11.8	14.6	18.4	24.3	29.3	42.1	45.7

Table A2.2: Weekly PERMANOVAs of the effect of NAM line on leaf bacterial communities.

Leaf

	Df	Sum of Sq	Mean Sq	F. Model	R2	P
Week 3						
NAM	7.00	0.14	0.02	1.05	0.34	0.39
Residuals	14.00	0.27	0.02		0.66	
Total	21.00	0.42			1.00	
Week 4						
NAM	7.00	2.35	0.34	0.98	0.26	0.48
Residuals	20.00	6.87	0.34		0.74	
Total	27.00	9.22			1.00	
Week 5						
NAM	7.00	1.38	0.20	0.69	0.17	0.98
Residuals	24.00	6.86	0.29		0.83	
Total	31.00	8.24			1.00	
Week 6						
NAM	7.00	1.94	0.28	0.71	0.12	0.99
Residuals	38.00	14.86	0.39		0.88	
Total	45.00	16.79			1.00	
Week 7						
NAM	7.00	1.75	0.25	1.18	0.25	0.22
Residuals	25.00	5.29	0.21		0.75	
Total	32.00	7.04			1.00	
Week 8						
NAM	7.00	1.12	0.16	0.86	0.22	0.69
Residuals	21.00	3.92	0.19		0.78	
Total	28.00	5.04			1.00	
Week 9						
NAM	7.00	1.14	0.16	0.53	0.12	0.99
Residuals	28.00	8.57	0.31		0.88	
Total	35.00	9.72			1.00	

Week 10						
NAM	7.00	1.52	0.22	0.61	0.10	0.98
Residuals	38.00	13.61	0.36		0.90	
Total	45.00	15.13			1.00	
Week 11						
NAM	7.00	0.65	0.09	1.34	0.34	0.15
Residuals	18.00	1.25	0.07		0.66	
Total	25.00	1.90			1.00	
Week 12						
NAM	6.00	1.64	0.27	1.01	0.28	0.43
Residuals	16.00	4.32	0.27		0.72	
Total	22.00	5.96			1.00	

Table A2.3: Weekly PERMANOVAs of the effect of NAM line on root bacterial communities.

Root	Df	Sum of Sq	Mean Sq	F. Model	R2	P
Week 3						
NAM	7.00	2.73	0.39	0.86	0.25	0.95
Residuals	18.00	8.15	0.45		0.75	
Total	25.00	10.88			1.00	
Week 4						
NAM	7.00	3.94	0.56	1.26	0.30	0.01
Residuals	21.00	9.41	0.45		0.70	
Total	28.00	13.35			1.00	
Week 5						
NAM	7.00	3.17	0.45	1.03	0.29	0.34
Residuals	18.00	7.89	0.44		0.71	
Total	25.00	11.06			1.00	

Week 6						
NAM	7.00	2.65	0.38	1.13	0.29	0.14
Residuals	19.00	6.34	0.33		0.71	
Total	26.00	8.98			1.00	
Week 7						
NAM	7.00	4.40	0.63	2.22	0.23	0.00
Residuals	51.00	14.46	0.28		0.77	
Total	58.00	18.86			1.00	
Week 8						
NAM	7.00	2.36	0.34	0.97	0.27	0.60
Residuals	18.00	6.24	0.35		0.73	
Total	25.00	8.59			1.00	
Week 9						
NAM	7.00	2.60	0.36	1.14	0.30	0.14
Residuals	19.00	6.01	0.32		0.70	
Total	26.00	8.54			1.00	
Week 10						
NAM	7.00	2.45	0.35	1.00	0.24	0.47
Residuals	22.00	7.71	0.35		0.76	
Total	29.00	10.16			1.00	
Week 11						
NAM	7.00	2.61	0.37	0.98	0.28	0.59
Residuals	18.00	6.83	0.38		0.72	
Total	25.00	9.44			1.00	
Week 12						
NAM	7.00	2.71	0.39	1.10	0.30	0.20
Residuals	18.00	6.37	0.35		0.70	
Total	25.00	9.08			1.00	

Table A2.4: Weekly PERMANOVAs of the effect of NAM line on soil bacterial communities.

Soil	Df	Sum of Sq	Mean Sq	F. Model	R2	P

Week 3						
NAM	7.00	2.55	0.36	1.08	0.27	0.20
Residuals	21.00	7.06	0.34		0.73	
Total	28.00	9.61			1.00	
Week 4						
NAM	7.00	2.84	0.41	1.18	0.27	0.04
Residuals	22.00	7.56	0.34		0.73	
Total	29.00	10.40			1.00	
Week 5						
NAM	7.00	2.54	0.36	1.10	0.29	0.23
Residuals	19.00	6.25	0.33		0.71	
Total	26.00	8.79			1.00	
Week 6						
NAM	7.00	2.39	0.34	1.25	0.32	0.05
Residuals	19.00	5.17	0.27		0.68	
Total	26.00	7.55			1.00	
Week 7						
NAM	7.00	4.10	0.59	2.61	0.28	0.00
Residuals	46.00	10.30	0.22		0.72	
Total	53.00	14.39			1.00	
Week 8						
NAM	7.00	2.15	0.31	1.09	0.29	0.20
Residuals	19.00	5.35	0.28		0.71	
Total	26.00	7.50			1.00	
Week 9						
NAM	7.00	2.09	0.30	1.14	0.27	0.09
Residuals	21.00	5.51	0.36		0.73	
Total	28.00	7.60			1.00	
Week 10						
NAM	7.00	2.26	0.32	1.26	0.29	0.02
Residuals	22.00	5.63	0.26		0.71	
Total	29.00	7.89			1.00	

Week 11						
NAM	7.00	2.01	0.29	0.93	0.25	0.71
Residuals	19.00	5.85	0.31		0.75	
Total	26.00	7.85			1.00	
Week 12						
NAM	7.00	1.98	0.28	1.15	0.30	0.07
Residuals	19.00	4.69	0.25		0.70	
Total	26.00	6.68			1.00	

Table A2.5: Two-way ANOVA for the effect of *B. napus* line (NAM) and growth stage (BBCH) on the BNTI values for the leaf, root and rhizosphere over the ten week sampling period. There were no significant interactions so they were not included in the final model.

		Df	Sum of Sq	Mean Sq	F value	P
Leaf	NAM	7.00	25.40	3.63	1.56	0.15
	BBCH	31.00	531.60	17.15	7.35	<0.0001
	Residuals	246.00	573.80	2.33		
Root	NAM	7.00	7.80	1.11	1.02	0.42
	BBCH	32.00	159.18	4.97	5.69	<0.0001
	Residuals	267.00	418.00	1.57		
Rhizopshere	NAM	7.00	27.53	3.93	3.94	<0.0001
	BBCH	32.00	209.30	6.54	6.56	<0.0001
	Residuals	266.00	265.42	1.00		

APPENDIX 3

Supplemental information for Chapter 5

Table A3.1: PERMANOVA results of the influence of Level of Invasion (Treatment) and Season on the total bacterial and fungal communities and the influence of Invasion Level (Treatment) on seasonal communities.

	Df	Sums of Sqs	Mean Sqs	F. Model	R2	P
		Bacterial Community Composition				
Season	2	3.10	1.55	6.45	0.02	0.001
Treatment	2	0.74	0.37	1.53	0.01	0.008
Season*Treatment	4	1.15	0.29	1.20	0.01	0.072
Residuals	518	124.44	0.24		0.96	
Total	526	129.43			1.00	
		Fungal Community Composition				
Season	2	2.91	1.46	3.10	0.01	0.001
Treatment	2	1.11	0.55	1.18	0.00	0.024
Season*Treatment	4	2.04	0.51	1.09	0.01	0.059
Residuals	514	241.34	0.05		0.98	
Total	522	247.40			1.00	
		Seasonal Bacterial Community Composition				
Green-up						
Treatment	2	0.62	0.31	0.31	0.01	0.065
Residuals	173	40.88	0.24		0.99	
Total	175	41.49			1.00	
Peak						
Treatment	2	0.62	0.31	1.29	0.01	0.087
Residuals	211	50.48	0.24		0.99	
Total	213	51.10			1.00	

Senescence						
Treatment	2	0.65	0.33	1.32	0.02	0.043
Residuals	134	33.09	0.25		0.98	
Total	136	33.74			1.00	
		Seasonal Fungal Community Composition				
Green-up						
Treatment	2	1.01	0.51	1.09	0.01	0.014
Residuals	171	79.14	0.46		0.99	
Total	173	80.15			1.00	
Peak						
Treatment	2	1.13	0.57	1.23	0.01	0.021
Residuals	208	95.87	0.46		0.99	
Total	210	97.00			1.00	
Senescence						
Treatment	2	1.00	0.50	1.02	0.01	0.318
Residuals	135	66.33	0.49		0.99	
Total	137	67.33			1.00	

Table A3.2: The percentage of each ecological assembly processes for bacterial and fungal communities in Native, Invaded and Mid plots during green-up, peak biomass and senescence. This table accompanies Figure 3 in the main text.

Organism	Treatment	Season	Process	Percent
Bacteria	Invaded	Green-up	Dispersal Limitation	22.59
Bacteria	Invaded	Green-up	Drift	19.16
Bacteria	Invaded	Green-up	Heterogenous Selection	0.11
Bacteria	Invaded	Green-up	Homogenizing Dispersal	0.55
Bacteria	Invaded	Green-up	Homogenous Selection	57.59
Bacteria	Invaded	Peak	Dispersal Limitation	10.83
Bacteria	Invaded	Peak	Drift	12.72
Bacteria	Invaded	Peak	Heterogenous Selection	0.07
Bacteria	Invaded	Peak	Homogenizing Dispersal	1.47
Bacteria	Invaded	Peak	Homogenous Selection	74.91
Bacteria	Invaded	Senescence	Dispersal Limitation	19.38
Bacteria	Invaded	Senescence	Drift	9.77
Bacteria	Invaded	Senescence	Heterogenous Selection	1.36
Bacteria	Invaded	Senescence	Homogenizing Dispersal	2.54
Bacteria	Invaded	Senescence	Homogenous Selection	66.95
Bacteria	Mid	Green-up	Dispersal Limitation	14.43
Bacteria	Mid	Green-up	Drift	15.36
Bacteria	Mid	Green-up	Heterogenous Selection	2.22
Bacteria	Mid	Green-up	Homogenizing Dispersal	0.93
Bacteria	Mid	Green-up	Homogenous Selection	67.07
Bacteria	Mid	Peak	Dispersal Limitation	8.07
Bacteria	Mid	Peak	Drift	9.22
Bacteria	Mid	Peak	Heterogenous Selection	2.12
Bacteria	Mid	Peak	Homogenizing Dispersal	1.63
Bacteria	Mid	Peak	Homogenous Selection	78.93

Bacteria	Mid	Senescence	Dispersal Limitation	20.67
Bacteria	Mid	Senescence	Drift	11.61
Bacteria	Mid	Senescence	Heterogenous Selection	0.12
Bacteria	Mid	Senescence	Homogenizing Dispersal	2.56
Bacteria	Mid	Senescence	Homogenous Selection	65.04
Bacteria	Native	Green-up	Dispersal Limitation	14.01
Bacteria	Native	Green-up	Drift	13.35
Bacteria	Native	Green-up	Heterogenous Selection	0.55
Bacteria	Native	Green-up	Homogenizing Dispersal	0.38
Bacteria	Native	Green-up	Homogenous Selection	71.71
Bacteria	Native	Peak	Dispersal Limitation	5.96
Bacteria	Native	Peak	Drift	10.54
Bacteria	Native	Peak	Heterogenous Selection	0.04
Bacteria	Native	Peak	Homogenizing Dispersal	3.06
Bacteria	Native	Peak	Homogenous Selection	80.4
Bacteria	Native	Senescence	Dispersal Limitation	13.28
Bacteria	Native	Senescence	Drift	16.81
Bacteria	Native	Senescence	Heterogenous Selection	5.55
Bacteria	Native	Senescence	Homogenizing Dispersal	5.88
Bacteria	Native	Senescence	Homogenous Selection	58.49
Fungi	Invaded	Green-up	Dispersal Limitation	8.64
Fungi	Invaded	Green-up	Drift	36.7
Fungi	Invaded	Green-up	Heterogenous Selection	37.73
Fungi	Invaded	Green-up	Homogenizing Dispersal	0.68
Fungi	Invaded	Green-up	Homogenous Selection	16.25
Fungi	Invaded	Peak	Dispersal Limitation	4.14
Fungi	Invaded	Peak	Drift	52.32
Fungi	Invaded	Peak	Heterogenous Selection	31.86

Fungi	Invaded	Peak	Homogenizing Dispersal	0.36
Fungi	Invaded	Peak	Homogenous Selection	11.32
Fungi	Invaded	Senescence	Dispersal Limitation	2.57
Fungi	Invaded	Senescence	Drift	55.41
Fungi	Invaded	Senescence	Heterogenous Selection	39.8
Fungi	Invaded	Senescence	Homogenizing Dispersal	0.18
Fungi	Invaded	Senescence	Homogenous Selection	2.05
Fungi	Mid	Green-up	Dispersal Limitation	9.53
Fungi	Mid	Green-up	Drift	47.83
Fungi	Mid	Green-up	Heterogenous Selection	27.84
Fungi	Mid	Green-up	Homogenizing Dispersal	0.56
Fungi	Mid	Green-up	Homogenous Selection	14.25
Fungi	Mid	Peak	Dispersal Limitation	16.47
Fungi	Mid	Peak	Drift	51.56
Fungi	Mid	Peak	Heterogenous Selection	7.33
Fungi	Mid	Peak	Homogenizing Dispersal	0.46
Fungi	Mid	Peak	Homogenous Selection	24.18
Fungi	Mid	Senescence	Dispersal Limitation	3.46
Fungi	Mid	Senescence	Drift	65.13
Fungi	Mid	Senescence	Heterogenous Selection	27.82
Fungi	Mid	Senescence	Homogenizing Dispersal	0.26
Fungi	Mid	Senescence	Homogenous Selection	3.33
Fungi	Native	Green-up	Dispersal Limitation	10.53
Fungi	Native	Green-up	Drift	53.04
Fungi	Native	Green-up	Heterogenous Selection	11.3
Fungi	Native	Green-up	Homogenizing Dispersal	0.37
Fungi	Native	Green-up	Homogenous Selection	24.76
Fungi	Native	Peak	Dispersal Limitation	9.76

Fungi	Native	Peak	Drift	44.12
Fungi	Native	Peak	Heterogenous Selection	33.59
Fungi	Native	Peak	Homogenizing Dispersal	1.28
Fungi	Native	Peak	Homogenous Selection	11.25
Fungi	Native	Senescence	Dispersal Limitation	6.72
Fungi	Native	Senescence	Drift	74.96
Fungi	Native	Senescence	Heterogenous Selection	15.8
Fungi	Native	Senescence	Homogenizing Dispersal	0
Fungi	Native	Senescence	Homogenous Selection	2.52

Table A3.3: The results of the ANCOVA on bacterial and fungal mean βNTI values together with the basic model consisting of lm(Service~ bacterial βNTI* fungal βNTI*Season *Treatment) where Treatment is Level of Invasion.

Forage

	Df	Sum Sq	Mean Sq	F	P
Bac BNTI	1	70660	70660	18.74	< 0.05
Fun BNTI	1	73993	73993	19.62	< 0.05
Season	2	524307	262154	69.52	< 0.05
Treatment	2	157404	78702	20.87	< 0.05
Bac*Fun	1	4588	4588	1.21	0.27
Bac*Season	2	7290	3645	0.97	0.38
Fun*Season	2	4104	2052	0.54	0.58
Bac*Treatment	2	14398	7199	1.91	0.15
Fun*Treatment	2	2650	1325	0.35	0.7
Season*Treatment	4	26299	6575	1.74	0.14
Bac*Fun*Season	2	4466	2233	0.59	0.55
Bac*Fun*Treatment	2	11253	5627	1.49	0.23
Bac*Treat*Season	4	7957	1989	0.53	0.72
Fun*Season*Treatment	4	2388	597	0.16	0.96
4x	4	5555	1389	0.37	0.83

Residuals	474	1787402	3771		

Glyphosate

Bac BNTI	1	3.2	3.2	0.45	0.5
Fun BNTI	1	187.6	187.6	25.99	< 0.05
Season	2	1178.8	589.41	81.66	< 0.05
Treatment	2	14.2	7.09	0.98	0.38
Bac*Fun	1	0.2	0.22	0.03	0.86
Bac*Season	2	27.4	13.69	1.89	0.15
Fun*Season	2	10.2	5.09	0.71	0.49
Bac*Treatment	2	18	8.99	1.25	0.29
Fun*Treatment	2	28.9	14.47	2	0.14
Season*Treatment	4	6.7	1.66	0.23	0.92
Bac*Fun*Season	2	20.6	10.29	1.43	0.24
Bac*Fun*Treatment	2	17.6	8.8	1.22	0.3
Bac*Treat*Season	4	19.8	4.95	0.69	0.6
Fun*Season*Treatment	4	34.9	8.71	1.21	0.31
4x	4	19.4	4.85	0.67	0.61
Residuals		3392.3	7.22		

Climate

Bac BNTI	1	125.81	125.81	55.47	< 0.05
Fun BNTI	1	18.73	18.73	8.26	< 0.05
Season	2	1769.22	884.61	390.06	< 0.05
Treatment	2	16.59	8.3	3.66	< 0.05
Bac*Fun	1	5.88	5.88	2.59	0.11
Bac*Season	2	14.2	7.1	3.13	< 0.05
Fun*Season	2	23.24	11.62	5.12	< 0.05
Bac*Treatment	2	26.29	13.15	5.79	< 0.05
Fun*Treatment	2	21.11	10.55	4.66	< 0.05
Season*Treatment	4	44.73	11.18	4.93	< 0.05
Bac*Fun*Season	2	0.16	0.08	0.04	0.97
Bac*Fun*Treatment	2	16.53	8.26	3.64	< 0.05
Bac*Treat*Season	4	25.76	6.44	2.84	< 0.05
Fun*Season*Treatment	4	35.14	8.79	3.87	< 0.05

4x	4	7.41	1.85	0.81	0.51
Residuals		1079.5	2.27		

Soil

Bac BNTI	1	902.8	902.8	83.9	< 0.05
Fun BNTI	1	2.9	2.9	0.27	0.6
Season	2	1157	578.5	53.76	< 0.05
Treatment	2	21	10.52	0.98	0.38
Bac*Fun	1	30.3	30.32	2.81	0.09
Bac*Season	2	1180.9	590.47	54.87	< 0.05
Fun*Season	2	33.4	16.71	1.55	0.21
Bac*Treatment	2	461	230.51	21.42	< 0.05
Fun*Treatment	2	7.7	3.83	0.36	0.7
Season*Treatment	4	504.2	126.04	11.71	< 0.05
Bac*Fun*Season	2	88.9	44.46	4.13	< 0.05
Bac*Fun*Treatment	2	37.3	18.67	1.73	0.18
Bac*Treat*Season	4	415.7	130.94	9.66	< 0.05
Fun*Season*Treatment	4	41.8	10.45	0.97	0.42
4x	4	47.7	11.92	1.11	0.35
Residuals		5122	10.76		

Nutrient

Bac BNTI	1	293.53	293.53	57.49	< 0.05
Fun BNTI	1	8.26	8.26	1.62	0.2
Season	2	1996.53	998.27	195.51	< 0.05
Treatment	2	17.72	8.86	1.73	0.18
Bac*Fun	1	17.97	17.97	3.52	0.06
Bac*Season	2	9.33	4.66	0.91	0.40
Fun*Season	2	18.64	9.32	1.83	0.16
Bac*Treatment	2	87.99	43.99	8.62	< 0.05
Fun*Treatment	2	0.37	0.18	0.04	0.96
Season*Treatment	4	56.4	14.1	2.76	< 0.05
Bac*Fun*Season	2	0.67	0.33	0.07	0.94
Bac*Fun*Treatment	2	47.3	23.65	4.63	< 0.05
Bac*Treat*Season	4	200.69	50.17	9.82	< 0.05
Fun*Season*Treatment	4	51.66	12.92	2.53	< 0.05

4x	4	5.4	1.35	0.26	0.9
Residuals		2430.47	5.11		

Table A3.4: The results of the ANCOVA on bacterial mean βNTI values with the basic model consisting of lm(Service~ bacterial βNTI *Season *Treatment) where Treatment is Level of Invasion.

Bacteria

Forage

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	70097	70097	18.89	< 0.05
Treatment	2	253832	126916	34.21	< 0.05
Season	2	496587	248293	66.92	< 0.05
BNTI*Treatment	2	16267	8133	2.19	0.11
BNTI*Season	2	10028	5014	1.35	0.26
Treatment*Season	4	27367	6842	1.84	0.12
3x Interaction	4	10235	2559	0.69	0.6
Residuals	494	1832829	3710		

Water

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	3.2	3.2	0.44	0.51
Treatment	2	167.6	83.79	11.55	< 0.05
Season	2	1190.3	595.13	82.01	< 0.05
BNTI*Treatment	2	5.9	2.96	0.41	0.67
BNTI*Season	2	36.3	18.17	2.5	0.08
Treatment*Season	4	4.1	1.03	0.14	0.97
3x Interaction	4	23.9	5.97	0.82	0.51
Residuals	494	3548.6	7.26		

CO2

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	128.09	128.09	51.11	< 0.05

Treatment	2	106.24	53.12	21.2	< 0.05
Season	2	1674.5	837.25	334.08	< 0.05
BNTI*Treatment	2	20.99	10.49	4.19	< 0.05
BNTI*Season	2	25.74	12.87	5.14	< 0.05
Treatment*Season	4	12.94	3.24	1.29	0.27
3x Interaction	4	25.56	6.39	2.55	< 0.05
Residuals	494	1243.03	2.51		

Soil

BNTI	1	913.3	913.3	84.11	< 0.05
Treatment	2	43.3	21.66	1.99	0.14
Season	2	1136.9	568.44	53.35	< 0.05
BNTI*Treatment	2	368.9	184.43	16.99	< 0.05
BNTI*Season	2	1227.5	613.75	56.53	< 0.05
Treatment*Season	4	558.3	139.59	12.86	< 0.05
3x Interaction	4	442.5	110.62	10.19	< 0.05
Residuals	494	5385.6	10.86		

Nutrients

BNTI	1	300.28	300.28	56.09	< 0.05
Treatment	2	66.1	33.05	6.17	< 0.05
Season	2	1959.44	979.72	183	< 0.05
BNTI*Treatment	2	40.17	20.08	3.75	< 0.05
BNTI*Season	2	7.8	3.9	0.73	0.48
Treatment*Season	4	67.32	16.83	3.14	< 0.05
3x Interaction	4	170.09	42.52	7.94	< 0.05
Residuals	494	2655.38	5.35		

Table A3.5: The results of the ANCOVA on fungal mean βNTI values with the basic model consisting of lm(Service~ fungal βNTI *Season *Treatment) where Treatment is Level of Invasion

Fungi

Forage

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	95829	95829	25.66	< 0.05
Treatment	2	172250	86125	23.06	< 0.05
Season	2	557452	278726	74.63	< 0.05
BNTI*Treatment	2	6491	3246	0.87	0.42
BNTI*Season	2	4687	2343	0.63	0.53
Treatment*Season	4	25196	6299	1.69	0.15
3x Interaction	4	5182	1295	0.35	0.85
Residuals	492	1387629	3735		

Water

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	175.9	175.9	24.21	< 0.05
Treatment	2	74.7	37.36	5.16	< 0.05
Season	2	1124	562.02	77.67	< 0.05
BNTI*Treatment	2	37.5	18.76	2.59	0.07
BNTI*Season	2	4.6	2.31	0.32	0.73
Treatment*Season	4	9.1	2.28	0.32	0.87
3x Interaction	4	22.6	5.66	0.78	0.54
Residuals	492	3531.1	7.24		

CO2

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	6.45	6.45	0.27	0.1
Treatment	2	54.73	27.36	11.46	< 0.05
Season	2	1867.06	933.53	391.03	< 0.05
BNTI*Treatment	2	38.1	19.05	7.98	< 0.05
BNTI*Season	2	21.78	10.89	4.56	< 0.05
Treatment*Season	4	32.92	8.23	3.45	< 0.05

3x Interaction	4	29.9	7.47	3.13	< 0.05
Residuals	492	1179.36	2.39		

Soil

BNTI	1	40.3	40.3	2.46	0.12
Treatment	2	2.6	1.32	0.08	0.92
Season	2	1820.1	910.05	55.7	< 0.05
BNTI*Treatment	2	11.1	5.57	0.34	0.71
BNTI*Season	2	27.1	13.54	0.83	0.44
Treatment*Season	4	37	9.25	0.57	0.69
3x Interaction	4	45.9	11.48	0.7	0.59
Residuals	492	8070.7	16.34		

Nutrients

BNTI	1	30.16	30.16	5.23	< 0.05
Treatment	2	31.41	15.7	2.72	0.06
Season	2	2253.53	1126.77	195.53	< 0.05
BNTI*Treatment	2	8.83	4.42	0.77	0.46
BNTI*Season	2	19.23	9.61	1.67	0.19
Treatment*Season	4	8.98	2.25	0.39	0.81
3x Interaction	4	44.09	11.02	1.91	0.11
Residuals	492	2846.68	5.76		

Table A3.6: The results of the seasonal ANCOVA on bacterial and fungal mean βNTI values together with the basic model consisting of lm(Service~ bacterial βNTI* fungal βNTI*Treatment) where Treatment is Level of Invasion.

Green-up
Forage

	Df	Sum Sq	Mean Sq	F	P
Bac BNTI	1.00	514.00	514.00	0.45	0.50
Fun BNTI	1.00	264.00	263.70	0.23	0.63
Treatment	2.00	38702.00	193351.20	16.94	< 0.05
Bac*Fun	1.00	24.00	24.40	0.02	0.88
Bac*Treatment	2.00	7675.00	3837.70	3.36	< 0.05
Fun*Treatment	2.00	3515.00	1757.70	1.54	0.22
3x	2.00	1263.00	631.30	0.55	0.58
Residuals	157.00	179337.00	1142.30		

Water

Bac BNTI	1.00	2.61	2.61	0.30	0.58
Fun BNTI	1.00	17.12	17.12	1.99	0.16
Treatment	2.00	8.23	4.12	0.48	0.62
Bac*Fun	1.00	1.10	1.09	0.13	0.72
Bac*Treatment	2.00	8.86	4.43	0.51	0.60
Fun*Treatment	2.00	18.27	9.13	1.06	0.35
3x	2.00	29.47	17.74	1.71	0.18
Residuals	157.00	1327.14	8.62		

CO2

Bac BNTI	1.00	2.45	2.45	0.77	0.39
Fun BNTI	1.00	14.96	14.96	0.47	< 0.05
Treatment	2.00	58.82	29.41	9.22	< 0.05
Bac*Fun	1.00	1.33	1.33	0.42	0.52
Bac*Treatment	2.00	39.37	19.68	6.17	< 0.05
Fun*Treatment	2.00	26.50	13.25	4.15	< 0.05
3x	2.00	2.32	1.16	0.36	0.69
Residuals	157.00	504.10	3.19		

Soil

Bac BNTI	1.00	0.07	0.07	0.01	0.94
Fun BNTI	1.00	0.40	0.40	0.04	0.86
Treatment	2.00	15.70	7.85	0.74	0.48
Bac*Fun	1.00	15.70	15.68	1.48	0.23
Bac*Treatment	2.00	311.04	155.52	14.63	< 0.05
Fun*Treatment	2.00	43.12	21.56	2.03	0.13
3x	2.00	3.02	1.51	0.14	0.87
Residuals	157.00	1679.31	10.63		

Nutrient

Bac BNTI	1.00	0.07	0.07	0.01	0.94
Fun BNTI	1.00	0.43	0.43	0.04	0.85
Treatment	2.00	16.74	8.37	0.74	0.48
Bac*Fun	1.00	16.74	16.73	1.48	0.22
Bac*Treatment	2.00	331.70	165.85	14.63	< 0.05
Fun*Treatment	2.00	45.99	22.99	2.03	0.14
3x	2.00	3.23	1.62	0.14	0.86
Residuals	157.00	1791.33	11.34		

Peak Forage

Bac BNTI	1.00	5969.00	5969.00	1.71	0.19
Fun BNTI	1.00	3261.00	3260.60	0.93	0.34
Treatment	2.00	36583.00	18291.30	5.23	< 0.05
Bac*Fun	1.00	1135.00	1134.60	0.32	0.57
Bac*Treatment	2.00	10594.00	5297.20	1.52	0.22
Fun*Treatment	2.00	90.00	45.00	0.01	0.99
3x	2.00	7722.00	3861.00	1.10	0.33
Residuals	195.00	681422.00	3494.50		

Water

Bac BNTI	1.00	26.03	26.03	3.11	0.07
Fun BNTI	1.00	34.39	34.39	4.11	< 0.05
Treatment	2.00	2.83	1.42	0.17	0.84
Bac*Fun	1.00	0.32	0.32	0.04	0.84
Bac*Treatment	2.00	22.03	11.01	1.31	0.27
Fun*Treatment	2.00	25.02	12.51	1.50	0.23
3x	2.00	17.51	8.75	1.05	0.35
Residuals	195.00	1631.49	8.37		

CO2

Bac BNTI	1.00	0.03	0.03	0.17	0.68
Fun BNTI	1.00	0.01	0.01	0.06	0.81
Treatment	2.00	0.01	0.01	0.04	0.96
Bac*Fun	1.00	0.02	0.02	0.09	0.76
Bac*Treatment	2.00	0.12	0.06	0.29	0.75
Fun*Treatment	2.00	1.98	0.99	0.47	< 0.05
3x	2.00	0.27	0.13	0.63	0.54
Residuals	195.00	41.69	0.21		

Soil

Bac BNTI	1.00	1403.86	1403.86	87.63	< 0.05
Fun BNTI	1.00	10.21	10.21	0.63	0.43
Treatment	2.00	92.10	46.05	2.87	0.06
Bac*Fun	1.00	167.83	167.83	10.48	< 0.05
Bac*Treatment	2.00	933.04	466.52	29.12	< 0.05
Fun*Treatment	2.00	8.01	4.00	0.25	0.78
3x	2.00	46.96	23.48	1.47	0.23
Residuals	195.00	3140.09	16.02		

Nutrient

Bac BNTI	1.00	2.74	2.74	1.70	0.19
Fun BNTI	1.00	5.10	5.10	3.16	0.08
Treatment	2.00	10.66	5.33	3.30	< 0.05
Bac*Fun	1.00	0.07	0.07	0.05	0.83
Bac*Treatment	2.00	1.93	0.96	0.60	0.55
Fun*Treatment	2.00	1.22	0.61	0.38	0.68
3x	2.00	2.24	1.12	0.69	0.50
Residuals	195.00	316.32	1.61		

Senescence
Forage

Bac BNTI	1.00	451.00	451.00	0.06	0.81
Fun BNTI	1.00	7791.00	7791.00	1.03	0.31
Treatment	2.00	122905.00	61452.00	8.09	< 0.05
Bac*Fun	1.00	2884.00	2884.00	0.38	0.54
Bac*Treatment	2.00	3360.00	1680.00	0.22	0.80
Fun*Treatment	2.00	4033.00	2016.00	0.27	0.77
3x	2.00	938.00	469.00	0.06	0.94
Residuals	122.00	926643.00	7595.00		

Water

Bac BNTI	1.00	7.02	7.02	1.96	0.16
Fun BNTI	1.00	0.42	0.42	0.12	0.73
Treatment	2.00	10.99	5.50	1.53	0.22
Bac*Fun	1.00	12.36	12.36	3.45	0.07
Bac*Treatment	2.00	0.77	0.38	0.11	0.90
Fun*Treatment	2.00	20.80	10.40	2.90	0.06
3x	2.00	1.77	0.89	0.25	0.78
Residuals	122.00	433.68	3.58		

CO2

Bac BNTI	1.00	9.18	9.18	2.10	0.15
Fun BNTI	1.00	22.95	22.95	5.25	< 0.05
Treatment	2.00	13.45	6.72	1.54	0.22
Bac*Fun	1.00	0.13	0.13	0.03	0.87
Bac*Treatment	2.00	15.34	7.67	1.75	0.18
Fun*Treatment	2.00	46.42	23.21	5.31	< 0.05
3x	2.00	7.54	3.77	0.86	0.42
Residuals	122.00	533.71	4.37		

Soil

Bac BNTI	1.00	3.53	3.53	1.42	0.24
Fun BNTI	1.00	13.74	13.74	5.54	< 0.05
Treatment	2.00	4.32	2.16	0.87	0.42
Bac*Fun	1.00	0.09	0.09	0.04	0.84
Bac*Treatment	2.00	2.44	1.22	0.49	0.61
Fun*Treatment	2.00	39.12	19.56	7.88	< 0.05
3x	2.00	7.46	3.73	1.50	0.23
Residuals	122.00	302.64	2.48		

Nutrient

Bac BNTI	1.00	3.76	3.76	1.42	0.24
Fun BNTI	1.00	14.66	14.66	5.54	< 0.05
Treatment	2.00	4.60	2.30	0.87	0.42
Bac*Fun	1.00	0.10	0.10	0.04	0.85
Bac*Treatment	2.00	2.60	1.30	0.49	0.61
Fun*Treatment	2.00	41.72	20.86	7.88	< 0.05
3x	2.00	7.96	3.98	1.50	0.23
Residuals	122.00	322.82	2.65		

Table A3.7: The results of the seasonal ANCOVA on bacterial mean βNTI values together with the basic model consisting of lm(Service~ bacterial βNTI *Treatment) where Treatment is Level of Invasion.

Bacteria

Forage

Green

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	527.00	527.00	0.45	0.50
Treatment	2	30337.00	15168.50	12.91	< 0.05
Interaction	2	7978.00	3989.10	3.40	< 0.05
Residuals	164	192629.00	1174.60		

Peak

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	5477.00	5477.30	1.57	0.21
Treatment	2	40924.00	20461.80	5.87	< 0.05
Interaction	2	7407.00	3703.50	1.06	0.35
Residuals	202	70627.00	3488.30		

Senescence

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	451	451	0.06	0.8
Treatment	2	126952	63476	0.68	< 0.05
Interaction	2	6028	3014	0.41	0.66
Residuals	128	935573	7309		

Water

Green

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	2.61	2.61	0.30	0.58
Treatment	2	21.23	10.61	1.23	0.29
Interaction	2	10.50	5.25	0.61	0.54
Residuals	164	1978.50	8.61		

Peak

BNTI	1	25.69	25.69	3.03	0.08
Treatment	2	7.09	3.54	0.42	0.66
Interaction	2	19.70	9.84	1.16	0.31
Residuals	202	1708.21	8.46		

Senescence					
BNTI	1	7.02	7.02	1.93	0.16
Treatment	2	11.23	5.61	1.54	0.21
Interaction	2	7.62	3.80	1.05	0.35
Residuals	128	461.94	3.64		

CO2

Green					
BNTI	1	2.44	2.44	0.7	0.4
Treatment	2	32.1	16.05	0.459	< 0.05
Interaction	2	39.44	19.72	5.65	< 0.05
Residuals	164	575.92	3.49		

Peak					
BNTI	1	0.038	0.038	0.17	0.67
Treatment	2	0.03	0.01	0.07	0.93
Interaction	2	0.14	0.07	0.32	0.73
Residuals	202	43.98	0.22		

Senescence					
BNTI	1	23.03	23.03	5.58	< 0.05
Treatment	2	9.84	4.92	1.09	0.34
Interaction	2	39.23	19.61	4.37	< 0.05
Residuals	128	574.6	4.5		

Soil

Green					
BNTI	1	0.04	0.04	0.01	0.95
Treatment	2	10.98	5.45	0.5	0.61
Interaction	2	246.11	123.05	11.16	< 0.05
Residuals	164	1819.5	11.03		
Peak					
BNTI	1	1401.1	1401.1	88.91	< 0.05
Treatment	2	101.5	50.77	3.22	< 0.05
Interaction	2	1101	550.52	34.94	< 0.05
Residuals	202	31.98.8	15.76		
Senescence					
BNTI	1	3.53	3.53	1.23	0.27
Treatment	2	0.59	0.29	0.1	0.90
Interaction	2	1.98	0.99	0.35	0.71
Residuals	128	367.24	2.87		

Nutrients

Green					
BNTI	1	0.04	0.04	0.01	0.95
Treatment	2	11.71	5.85	0.5	0.61
Interaction	2	262.46	131.23	11.16	< 0.05
Residuals	164	1940.85	11.76		
Peak					
BNTI	1	0.56	0.56	0.35	0.55
Treatment	2	13.69	6.85	4.29	< 0.05
Interaction	2	0.98	0.49	0.31	0.74
Residuals	202	322.3	1.59		

Senescence					
BNTI	1	3.76	3.76	1.23	0.27
Treatment	2	0.63	0.32	0.1	0.9
Interaction	2	2.12	1.06	0.35	0.71
Residuals	128	391.72	3.06		

Table 3.8: The results of the seasonal ANCOVA on fungal mean βNTI values together with the basic model consisting of lm(Service~ fungal βNTI *Treatment) where Treatment is Level of Invasion.

Fungi

Forage

Green

	Df	Sum Sq	Mean Sq	F	P
BNTI	1	353.00	353.00	0.30	0.59
Treatment	2	35103.00	17551.60	14.73	< 0.05
Interaction	2	1665.00	832.70	0.70	0.50
Residuals	163	194173.00	1191.20		

Peak					
BNTI	1	3617.00	3617.00	1.03	0.31
Treatment	2	34015.00	17007.30	4.82	< 0.05
Interaction	2	90.00	44.80	0.01	0.99
Residuals	201	709053.00	3527.60		

Senescence					
BNTI	1	8029	8029	1.1	0.29
Treatment	2	123026	61513	8.43	< 0.05
Interaction	2	3547	1774	0.24	0.78
Residuals	128	934403	7300		

Water

Green					
BNTI	1.00	15.45	15.45	1.79	0.18
Treatment	2.00	6.46	3.23	0.38	0.69
Interaction	2.00	14.22	7.11	0.83	0.44
Residuals	164.00	1376.67	8.60		

Peak					
BNTI	1.00	32.01	32.01	3.79	0.05
Treatment	2.00	4.80	2.40	0.28	0.75
Interaction	2.00	25.92	12.96	1.54	0.22
Residuals	202.00	1696.88	8.44		

Senescence					
BNTI	1.00	0.75	0.75	0.21	0.65
Treatment	2.00	10.61	5.30	1.47	0.23
Interaction	2.00	18.86	9.43	2.62	0.07
Residuals	128.00	457.59	3.60		

CO2

Green					
BNTI	1	16.3	16.3	4.75	< 0.05
Treatment	2	50.74	25.37	7.36	< 0.05
Interaction	2	20.26	10.13	2.95	< 0.05
Residuals	164	562.56	3.43		

Peak					
BNTI	1	0.01	0.01	0.07	0.79
Treatment	2	0.01	0.01	0.04	0.96
Interaction	2	1.91	0.96	4.57	< 0.05
Residuals	202	42.2	0.21		

Senescence					
BNTI	1	9.18	9.18	1.88	0.17
Treatment	2	2.51	1.26	0.26	0.77
Interaction	2	13.88	6.94	1.42	0.24
Residuals	128	623.13	4.87		
Soil					
Green					
BNTI	1	0.35	0.35	0.03	0.89
Treatment	2	15.5	7.75	0.62	0.54
Interaction	2	7.16	3.59	0.29	0.75
Residuals	164	2045.33	12.47		
Peak					
BNTI	1	21.8	21.8	0.77	0.38
Treatment	2	71.5	35.23	1.25	0.29
Interaction	2	4.8	2.4	0.08	0.92
Residuals	202	5705.1	28.24		
Senescence					
BNTI	1	14.72	14.72	5.88	< 0.05
Treatment	2	4.08	2.03	0.81	0.45
Interaction	2	34.24	17.12	6.8	< 0.05
Residuals	128	320.29	2.5		
Nutrients					
Green					
BNTI	1	0.38	0.38	0.03	0.87
Treatment	2	16.52	8.26	0.62	0.54

Interaction	2	7.63	3.82	0.29	0.75
Residuals	164	2181.69	13.3		
Peak					
BNTI	1	5.39	5.39	3.37	0.06
Treatment	2	10.34	5.17	3.23	< 0.05
Interaction	2	1.21	0.61	0.38	0.69
Residuals	202	323.34	1.6		
Senescence					
BNTI	1	15.7	15.7	5.88	< 0.05
Treatment	2	4.35	2.17	0.81	0.45
Interaction	2	36.53	18.26	6.84	< 0.05
Residuals	128	341.65	2.67		

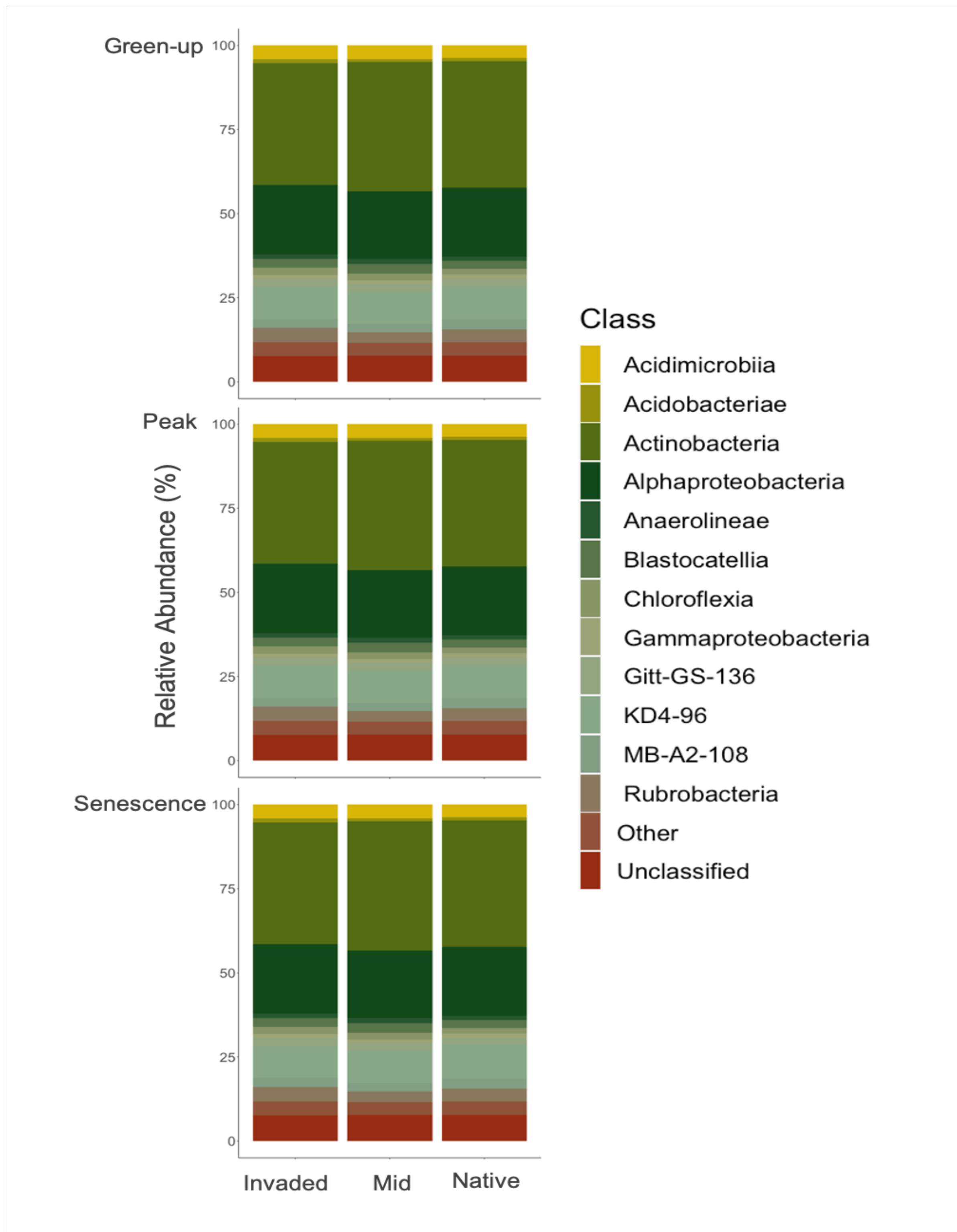


Figure A3.1: Relative abundance of bacterial communities at the class level for Native, Mid and Invaded plots during Green-up (top), Peak Biomass (middle) and Senescence (bottom).

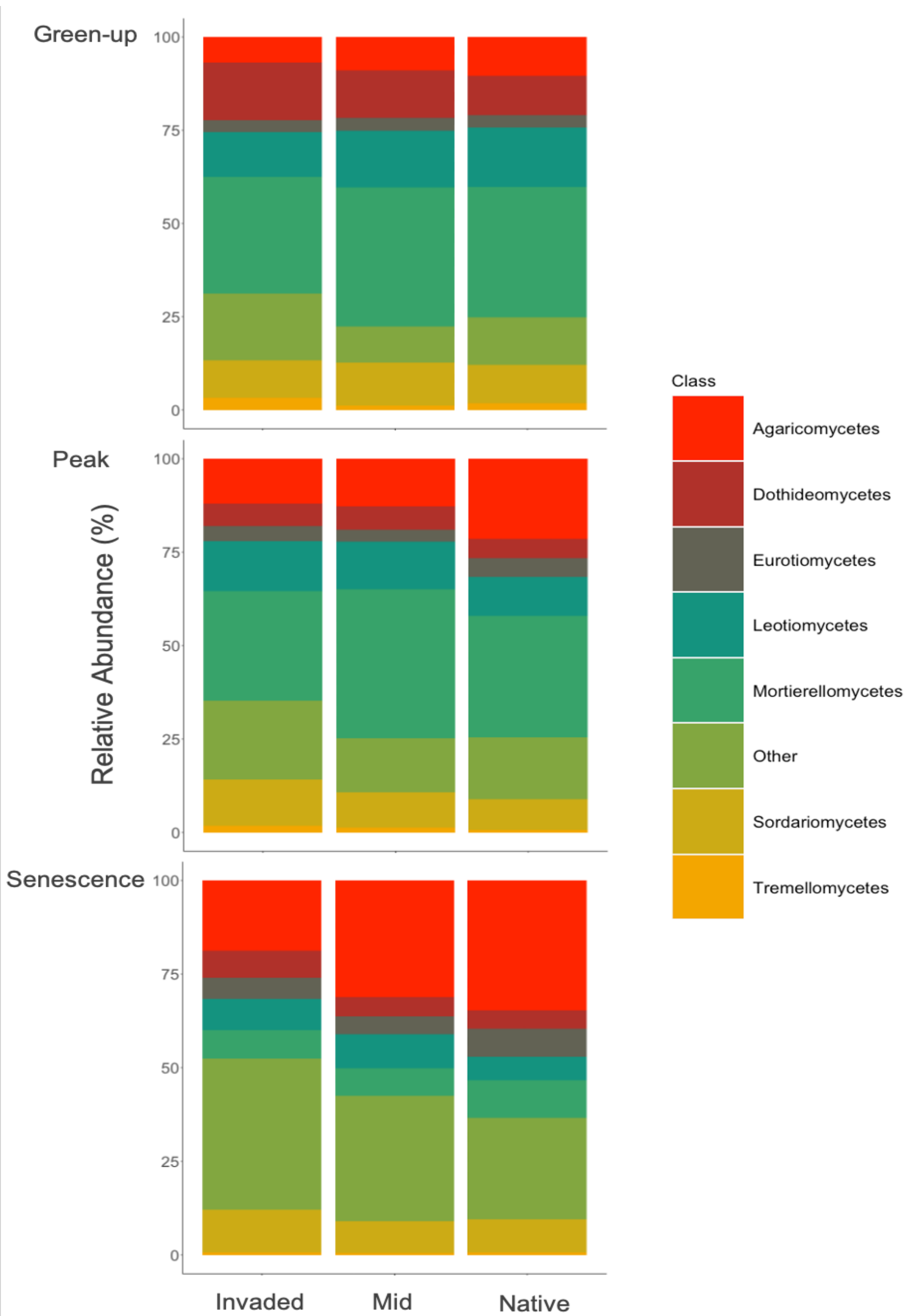


Figure A3.2: Relative abundance of fungal communities at the class level for Native, Mid and Invaded plots during Green-up (top), Peak Biomass (middle) and Senescence (bottom).

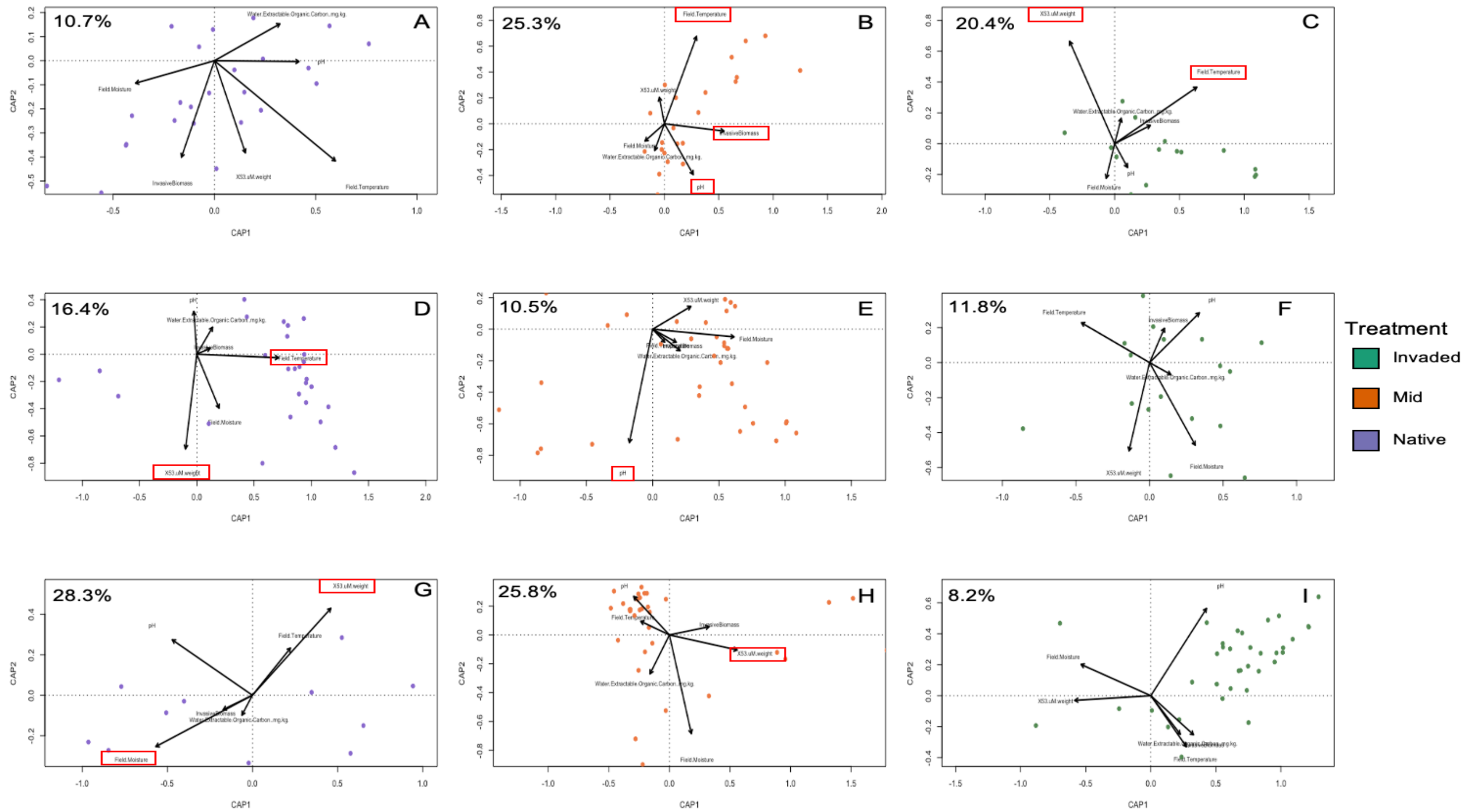


Figure A3.3: Fungal distance-based redundancy analysis (dbRDA) of the weighted UniFrac distances during Green-up (A-C), Peak Biomass (D-F), and Senescence (G-I) for Native plots (A, D, G), Mid plots (B, E, H), and Invaded plots (C, F, I). All dbRDAs were constrained by were constrained by 53 µm aggregate weight, soil pH, field moisture, invasive biomass, field temperature and water extractable organic carbon content. Significant terms are indicated by a red box around the term.