CHARACTERIZING CARROT MICROBIOMES AND THEIR POTENTIAL ROLE IN SOIL ORGANIC MATTER DECOMPOSITION

by

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ABSTRACT

Plant microbiomes are increasingly recognized for their potential to help plants with critical functions such as nutrient acquisition. Nitrogen is the most limiting nutrient in agriculture and growers apply substantial amounts to meet crop needs. Only 50% of N fertilizers are generally taken up by plants and the rest is subject to loss which negatively affects environmental quality. Organic fertilizers such as cover crops and animal manure can help reduce this loss, though these materials must mineralize via microbial mediated processes before they are available for plant uptake, which makes managing fertility using these sources difficult. Some plants can scavenge nutrients from organic materials by stimulating positive priming processes in soil. Carrot (*Daucus carota.* L) is known as an N scavenging crop, making it an ideal model crop to study these interactions. In a greenhouse trial, soils were amended with an isotopically labeled corn residue to track N movement, and planted with one of five carrot genotypes expected to differ in nitrogen use efficiency (NUE). Changes in soil β-glucosidase activity, ammonium (NH_4^+-N) and nitrate (NO_3^- -N) concentrations, soil bacterial community composition, weight and carbon and N concentrations, and total $\delta^{15}N$ of above and below ground carrot biomass were determined. Results indicate that there are genetic differences in the ability of carrots to promote priming under N limited conditions, which could be exploited to enhance NUE in carrots. Soil microbial communities differed between genotypes, indicating that some of these microbes could play a role in the differential N scavenging responses observed, and/or contribute to other important functions such as resistance to pests. Endophytic microbes residing inside carrot taproots also have potential to contribute to NUE and other benefits, but are notoriously difficult to isolate and culture. New next generation sequencing technologies have revolutionized the study of microbiomes, though using these tools to study bacterial endophytes in plants is still difficult due to co-amplification of plant organelles. Consequently, a second study was conducted to determine if subjecting carrot tissues to hollow fiber microfiltration followed by enzymatic digestion could enhance recovery and amplification of bacterial endophytes. Carrot taproot digests were subject to amplification using a standard V3-V4 16S primer set, as well as two alternative (blocking and mismatch) primer sets that have prevented amplification of plastids/mitochondria in other plant species. Results indicate that the microfiltration/digestion procedure can increase the number of bacterial endophyte OTUs assigned and could be further optimized for use in carrots. The blocking and

mismatch primer sets were not as effective in blocking co-amplification of plant products as they are in other studies, possibly due to the presence of a high number of chromoplasts in carrot tissues. Taxonomic assignment of bacterial endophytes differed significantly between the primer sets, indicating that multiple primer sets may be needed to fully characterize these communities in carrots. The enzymatic digestion procedure could artificially inflate certain taxa, which could be helpful if targeting specific taxa. These studies demonstrate that carrots are intimately connected with microbes residing in the soil and within their taproots, and further exploration of these plant-soil-microbial relationships could enhance the yield and sustainability of carrot production systems.

CHAPTER 1. INTRODUCTION

Carrot (*Daucus carota.* L) is one of the most consumed fresh vegetables. In the last 50 years, world carrot production has increased threefold in ha, and yield has risen twofold (FAO, 2018). Orange carrots play an important role in human health because they are rich in carotenoids and vitamin A (Simon, 2019). In recent years, growth in public demand for nutritious foods has increased the total size of the organic carrot industry, which now accounts for 14.3% of total carrot production in the U.S (Yiridoe et al., 2005). In terms of agronomic benefits for organic producers, carrots are considered a good nitrogen (N) scavenging crop, therefore they are a good crop to include in crop rotation strategies to prevent N losses from soil (Thorup-Kristensen, 2006).

Nitrogen (N) is a major limiting nutrient in agricultural systems. Nitrogen is intensively used for fertilization purposes to meet food production needs, but excessive N applications can cause harmful effects to the environment due to nitrate (NO_3^-) leaching, soil acidification and emissions of nitrogen oxide (NO, N_2O, and NO_2) gases (Wick et al., 2012). In soil N cycling, nitrate (NO_3^-) and ammonium (NH_4^+) are the most common forms of inorganic N available for plant uptake. Nitrate is very mobile, and the most readily available ion form for plants. Most plants preferentially uptake NH_4^+ because it requires less energy for plant uptake than NO_3^-, due to a reduction step needed before assimilation by plants (von Wirén et al., 2000). Optimal plant performance, however, depends on availability of both inorganic forms of N, and some organic forms such as amino acids, are also an important part of the soil N budget (Lammerts van Bueren & Struik, 2017).

The type of N fertilizer used can have a significant impact on N cycling processes in soil. For example, in conventional agricultural systems, N is supplied through mineral fertilizers, of which less than 50% is efficiently recovered by plants (Kant, 2017). Therefore, there is a need to identify strategies that regulate the N cycle towards better N use efficiency. Reduction of N pollution in agriculture must also be balanced with maintaining crop productivity (Coskun et al., 2017; Menneer et al. 2016; Subbarao et al., 2012). Organic farming has been proposed as one of the strategies to foster agricultural sustainability, maintain soil health and mitigate environmental impacts (Musyoka et al., 2019). In organic systems, N cycling processes are less predictable because nutrients are bound in organic residues and fertilizers (Van Bueren et al., 2002). Organic materials have to undergo N mineralization that converts organic N into NH_4^+ and NO_3^- to supply

plant needs (Hadas et al., 2004). Mineralization depends on difficult-to-control factors such as temperature, soil type, organic material, root architecture and rhizosphere microbial communities (Stein & Klotz, 2016; Strokal & Kroeze, 2014).

Some variation in N uptake can be attributed to the extent that different plants can access nutrients in soil organic matter (SOM) through decomposition (Kuzyakov, 2002). For example, during rhizosphere priming, energy-rich carbon (C) exudates from plant roots stimulate microbial activity, and influence whether these microbial communities mineralize or and immobilize nutrients (Haichar et al., 2014). One of the mechanisms that explains this phenomenon proposes that under low N availability, extracellular enzyme production by soil microorganisms is stimulated by a high amount of C inputs from plant roots (Yin et al., 2014). These microbial enzymes release nutrients locked in SOM, meeting microbial and plant nutrient requirements (positive priming) (Dijkstra et al., 2013). Alternatively, plants and microbes can compete for resources under low N conditions, which reduces microbial decomposition of SOM, resulting in negative priming and limiting plant and microbial growth (Dijkstra et al., 2010b; Pausch et al., 2013). Although both of these situations have been demonstrated, the mechanisms driving whether positive or negative priming will occur are still unclear. One factor that could affect these processes, is the composition of the soil microbial community. For example, microbes differ in their ability to decompose SOM and soils dominated by certain bacteria may be subject to negative or positive priming, depending on preferential substrate or dominance of one group of microbes over the other (Bell et al., 2015).

Another factor that could influence whether positive or negative priming processes occur is plant genotype. For example, plant genetics has been shown to play a role in the potential for *Pinus radiate* plants to scavenge N by altering soil microbial communities (Gallart et al., 2018). Moreover, the composition of rhizosphere microbial communities and N scavenging potential of the plants varied depending on the type of N source supplied. Recent studies conducted using barley (*Hordeum bulgare.* L) as a model crop found similar responses (Mwafulirwa et al., 2017; Terrazas et al., 2019). Most plant breeding programs are currently conducted under high-input conventional fertilization programs using mineral forms of N fertilizer, which could prevent the development of varieties that are best adapted to acquire N from organic fertility sources (Rakotoson et al., 2017). Therefore, alternative varietal development approaches could be needed.

Endophytes are important players in nitrogen availability in soil, affecting how plants mobilize, uptake, and assimilate N (Prieto et al., 2017). Endophytes are endosymbiotic microorganisms that colonize intercellular and intracellular spaces of plant organs, but generally do not cause any harm or significant morphological changes to the host plant (Malfanova et al., 2013). One of the most well studied examples of how endosymbionts can affect the N cycle, is the potential for specialized bacteria (*Rhizobia*) to form nodules on the roots of leguminous plants and fix atmospheric N (Pajares & Bohannan, 2016). Other so called 'free living diazotrophic bacteria' have also been shown to associate with plants both epiphytically and endophytically, and enhance plant growth by increasing N acquisition (Köberl et al., 2013). In addition to fixing atmospheric N, diazotrophic bacteria can influence nutrition in plants by secreting phytohormones that enhance root biomass and thereby increase soil N absorption (Hardoim et al., 2008). Plants can also interact with soil microbes to alter the N cycle via associations with rhizosphere bacterial communities using root C exudates. As mentioned before, factors affecting these relationships include plant genotype, growth stage, soil type, and soil health (Compant et al., 2010).

Crop management practices can have a major effect on the functional potential of soil microbial communities (Schulz & Boyle, 2007; Reeve et al., 2016). Soil health is also critical in shaping the composition of endophytes, because most endophytes are acquired by plants via horizontal transmission from the soil, versus vertical transmission via seeds (Frank, et al., 2017). As mentioned above, the composition of plant endophyte communities is important, because this can influence factors such as endophyte mediated increases in plant dry weight, root growth and tolerance to pathogens and abiotic stresses (Malfanova et al., 2013). For example, in carrots, taproots are colonized by a diverse assortment of microbial taxa, but the composition and potential for these microbes to help defend carrot plants against soil-borne pathogens depends on changes in soil health induced by different crop management practices (Abdelrazek, 2018; Abdelrazek et al., under review).

Because only around 1% of microorganisms can be isolated and cultured (Wang et al., 2019), knowledge of factors affecting the composition and functional capacity of microbiomes has lagged behind other fields. However, new next generation sequencing (NGS) technologies have revolutionized the field of microbial ecology, allowing researchers to begin to unravel how different components of a plants microbiome, such root bacterial endophytes, interact in agricultural and natural ecosystems. For example, recent studies have discovered core bacterial

communities associated with tomato (*Solanum lycopersicum.* L), barley and sugarcane (*Saccharum officinarum.* L), demonstrated how varietal difference and crop systems management can influence their composition and potential functional roles (Arruda et al., 2018; Bianco et al., 2018; Tian et al., 2017). However, there is much work left to be conducted, especially with respect to bacterial endophyte communities in plants. This is due the fact that it is difficult to amplify bacterial DNA from plant host tissue because plant DNA from mitochondria and plastids are also amplified using primer designed to target bacteria (Ghyselinck et al., 2013). These plant organelles were acquired as an endosymbiotic association with an proteobacterium–like ancestor by a eukaryotic cell, and evolved into mitochondria and plastids (Dyall et al., 2004; Sagan, 1967). This homology between organelles and bacteria leads to problems due to the co-amplification of mitochondrial 18S and chloroplast/plastids 16S rRNA region (Ghyselinck et al., 2013). For example, in previous attempts to amplify bacterial endophytes in carrot taproots using a standard bacterial V3-V4 primer set targeting the 16S rRNA region was unsuccessful due to co-amplification of plant products (Abdelrazek, 2018). Subsequent attempts using a set of so-called "blocking primers" which are designed to prevent amplification of plant organelles and have been successful in other plant species, was not effective in carrot taproots. Consequently, alternative strategies are needed to investigate bacterial endophytes in carrots.

Carrots are an interesting model crop to investigate how genetic differences could interact with soil microbial communities to facilitate N acquisition from organic sources in soil, because they are known as a N scavenging crop. The broad goals of the research described in this thesis study were: 1) determine whether carrot genotypes differ in their potential to facilitate organic matter decomposition to help scavenge N in soil; 2) identify microbial taxa that are stimulated by carrot roots and could play a role in nutrient acquisition as well as other important functions in carrot production systems; and, 3) identify the best approach to characterize bacterial endophyte communities in carrot taproots using next generation sequencing.

CHAPTER 2. DO CARROTS DIFFER IN THEIR ABILITY TO ALTER SOIL MICROBIAL COMMUNITIES AND SCAVENGE NITROGEN FROM ORGANIC MATERIALS IN SOIL?

2.1 Introduction

Carrots (*Daucus carota.* L) are among the top 10 vegetable crops in the world (Ahmad et al., 2017). More than 40 million tons of carrots are produced annually, with a total area harvested of 1.3 million ha worldwide (FAO, 2018). The U.S is the 2nd largest carrot producer after China, generating 1.4 million tons of carrots each year (FAO, 2018). Carrots play a major role in human health because they contain high levels of beta-carotene, a precursor of vitamin A, as well as vitamin C, which is involved in healthy vision and normal immune functions, and also provides additional antioxidant benefits (da Silva Dias, 2014). Carrots are also an important component of the rapidly growing organic vegetable industry (Kim et al., 2019). They now hold the greatest market share of vegetables in organic production, with 14.3% of the total U.S. carrot crop grown on organic farms (Lucier & Lin, 2011). While much of this demand is likely driven by the human health benefits provided by carrot crops, agronomic factors also play a role in the popularity of this crop within organic crop systems. Carrots are well known for their potential to act as a nitrogen-scavenging crop, and thus they are an excellent crop to include in a crop rotation or intercropping strategy to help prevent nitrogen loss from agricultural systems (Thorup-Kristensen, 2006).

Nitrogen (N) is an essential nutrient that is required by plants in large quantities to fulfill many critical processes such as photosynthesis. In response growers apply significant amounts of N fertilizers to meet plant needs, though only 50% of these fertilizers are generally captured by crop plants, and the rest is subject to loss via leaching and emissions of nitrous oxide (N_2O), a potent greenhouse gas, which negatively affect environmental health (Fageria & Baligar, 2005). Consequently, there is an urgent need to optimize N management in agricultural systems to reduce economic costs for producers, as well as protect the health of natural ecosystems (Hirel et al., 2011). Organic farming is a systems approach to agriculture that is aimed at creating a self-regulating system that avoids application of synthetic agricultural inputs while maintaining stable crop yields by relying on natural ecosystem functions such as nutrient cycling, biological pest control and soil health (Van Bueren et al., 2002). For example, rather than applying mineral

fertilizers that contain compounds such as ammonium (NH_4^+) and nitrate (NO_{3-}) that are immediately available for plant uptake, organic growers rely on nutrients derived from plant (i.e. leguminous cover crops) and animal waste products instead to meet plant N needs (Hartz & Johnstone, 2006).

One of the most commonly used N fertilizers in organic vegetable production systems is feather meal. Feather meal is a byproduct of the poultry industry that contains between 12 and 16% total N, though most of this is not in forms that are available for plant uptake (Mikkelsen & Hartz, 2008). For example, 70% to 90% of the N in feather meals tends to be in the form of proteins, which must be mineralized by soil microorganisms into other forms before it is available for plant uptake. There is evidence to suggest that plants can directly uptake dissolved low molecular weight organic compounds like urea, amino acids and oligopeptides to help fulfill plant N needs (Moreau et al., 2019). However, plants generally prefer NH_4^+ and NO_3^- (Laberge et al., 2006), so N compounds in organic fertilizers must go through several microbial driven processes before they can be taken up to meet plant needs. Factors that can affect the release of these soluble N compounds from organic fertility sources include the composition of the organic material, management practices such as soil tillage, and environmental factors such as rainfall (Mikkelsen & Hartz, 2008). Individual soil microbial taxa also vary in their potential to decompose organic materials and cycle nitrogen. However, the composition and activity of soil microbes with a role in soil carbon (C) and N cycles are controlled by many factors that are still not well understood. Consequently, synchronizing available N with critical periods of plant uptake to optimize crop productivity when using organic fertility sources is challenging (Baresel et al., 2008; Graaff et al., 2009; Hirel et al., 2011; Hoagland et al., 2008 Mikkelsen & Hartz, 2008).

In classical models of plant nutrient acquisition, researchers theorized that there is an evolutionary trade-off between slow and fast plant growth strategies that are driven by spatial and temporal variation in soil nutrient availability (Grime, 1977). However, in recent years researchers have discovered that this is much more complex, and plants can play a fundamental role in mediating nutrient availability in soil to help meet their needs (Moreau et al., 2019). For example, roots traits that can influence plant N acquisition include root growth rate, root architecture, amount and duration of root hairs, and hosting specific microbes in roots that can help plants acquire nutrients (e.g. mycorrhizal fungi) (Bardgett et al., 2014; Blagodatskaya et al., 2014; Poirier et al., 2018). Evidence is also accumulating that plants can interact with broad groups of microbes

in bulk soil to alter microbial transformations and obtain nutrients. In a study using ^{15}N isotope tracing in a mustard (*Brassica napa*) system, researchers were able to demonstrate that the potential for plants to obtain N from high C:N ratio organic amendments, was directly correlated with soil microbial activity and N mineralization rates (Heijboer et al., 2016). Therefore, soils with more active soil microbes and fauna in close interaction with plant roots, could support more efficient nutrient cycling and enhance crop health (de Vries & Wallenstein, 2017). Consequently, determining how plants interact with soil microbial communities to facilitate C and N cycling could increase crop yield and reduce further N losses to the environment (Dijkstra et al., 2013; Meier et al., 2017).

Plants release up to 40% of their photosynthetically derived C from their roots through both active and passive processes. These rhizodeposits can influence decomposition of soil organic matter (SOM) in a phenomenon known as priming. While this strategy has been extensively studied for its effects on C fluxes from soil (Finzi et al., 2015), Blagodatsky et al. (2010), Murphy et al. (2015), and Mwafulirwa et al. (2017) suggest that this phenomenon could also be important for understanding and managing soil N cycling as well. Factors that seem to affect priming include plant species, plant reproductive stage, soil type and soil nutrient availability (Dijkstra et al., 2013; Sasse et al., 2018). For example, positive priming often takes place in N-limited soils, where plant roots stimulate microbial activity by providing microbes with energy-rich C compounds. This leads to greater abundance and activity of soil microbes, and thus results in greater rates of SOM mineralization and more plant available N compounds for plant uptake (Yin et al., 2018). In contrast, negative priming occurs when the presence of organic substrates such as those provided by plant root exudates, reduces the rate of SOM mineralization. This can occur in both N-limited and N-rich soils. For example, in N-rich soils, microbes may use the rhizodeposits to meet their C and energy needs, and then become satiated which prevents further mineralization of SOM (Huo et al., 2017). Alternatively, in N-limited soils where plants and microbes are competing for nutrients, plants could compete more effectively, causing microbes to starve and thereby cease further mineralization activity (Moreau et al., 2019). The exact dynamics regulating these processes are still not well understood, which currently prevents agronomists from being able to leverage theses processes to enhance N use efficiency in agricultural systems.

Recent studies have demonstrated that plant genetics can influence how plants perform under different N scenarios (Terrazas et al., 2019), which could be mediated, at least in part, by

priming processes. For example, wheat (*Triticum aestivum*) genotypes with high root growth rates and high C investment in roots responded positively to compost additions, and were able to use N from the compost to achieve high growth rates (Junaidi et al., 2018). Moreover, others have demonstrated that the total amount of C released to soil through root exudates varies among plant species as well as genotypes (Kuzyakov, 2002; Schenk, 2006; L. Yin et al., 2018). Consequently, it may be possible to identify plant genotypes that are able to stimulate SOM priming and thereby enhance N use efficiency in systems where plants rely on organic fertility sources to obtain nutrients. However, finding these genotypes could be difficult. For example, while mineral forms of N fertilizer are relatively inexpensive and can rapidly increase crop yield, there is evidence that the availability of large amounts of plant available N disrupts relationships between plants and soil microbes with a role in organic matter decomposition (Heijboer et al., 2016). This is because it can be energetically expensive for plants to provide C resources to support soil microbes. Currently, most plant breeding programs are conducted under conventional farming systems, where plants are selected for high yield in the presence of mineral fertilization regimes, and these varieties may not have the traits needed to interact with soil microbes to obtain nutrients from organic sources (Mwafulirwa et al., 2016; Van Bueren et al., 2002). To overcome this challenge, some researchers have looked to wild accessions of crop ancestors to identify sources of germplasm that are more likely to interact with beneficial microbes because they evolved together in low input systems. For example, when comparing the wild ancestors of maize (*Zea mays*. L), wheat, and barley with modern varieties, the wild accessions often stimulate greater microbial activity and foster the development of distinct rhizosphere microbial communities, which are expected to be correlated with greater plant health and productivity (Mwafulirwa et al., 2016; Peiffer et al., 2013; Terrazas et al., 2019). Other researchers have looked to varieties that differ in phenotypic characteristics expected to be related to differences in nitrogen use efficiency to determine if there are root traits involved that could be exploited to enhance nutrient scavenging in soil (Garnett et al., 2009).

Carrots are an interesting model crop to investigate how genetic differences could interact with soil microbial communities to facilitate N acquisition from organic sources in soil. Our lab has previously demonstrated that different carrot genotypes host distinct microbial assemblages (Abdelrazek, 2018; Abdelrazek et al., in review) and as mentioned above, carrots are known to be a nitrogen scavenging crop (Westerveld et al., 2006). Standard recommendations for N fertilizer in carrots range from 50 to 220 kg/ha depending on soil type, the previous crop planted, the type

of fertilizer being applied and the time of year when fertilizers are being applied (Boskovic-Rakocevic, 2012; Veitch et al., 2014). However, N uptake from mineral fertilizers is very low in carrots, with only 8 to 33% of the N in fertilizers actually making it into carrot crops (Reid et al., 2017). Moreover, carrots do not tend to respond to increased rates of mineral fertilizers N to enhance carrot yield, which suggests that these plants may be obtaining N from other sources such as decomposing organic matter, or N sources found deeper in the soil profile rather than from the soluble N available in mineral fertilizers (Veitch et al., 2014). Westerveld et al. (2006) also determined that N uptake in carrot roots and foliage increased linearly regardless of soil type, cultivar and N rate in tropical and subtropical regions, providing evidence that carrots are broadly adapted to scavenge N regardless of their environment. Interestingly, Reid et al. (2017) found that less N was taken up by carrots when grown on mineral soils compared with organic soils, and that specific carrot varieties were able to accumulate much more N in roots than required for optimal growth. Organic or muck soils, generally have much greater abundance and activity of soil microbes than mineral soils (Ros et al., 2003), thus the results of this study imply that carrots can interact with microbes when present to obtain nitrogen. If carrot genotypes differ in C exudates, then genotype may be relevant in driving soil C and N cycles, and thus these genotypes could be potentially exploited to obtain N from organic fertility sources (Navazio, 2014).

The goals of this study were to: 1) determine whether carrot genotypes differ in their potential to facilitate organic matter decomposition to help scavenge N in soil, and, 2) identify microbial taxa that are stimulated by carrot roots and could play a role in nutrient acquisition as well as other important functions in carrot production systems. To accomplish these goals, we grew five carrot genotypes that were expected to differ in N use efficiency based on previously observed differences in top size, in soil amended with a ^{15}N labeled corn residue. We measured soil β-glucosidase activity and NH_4^+-N and NO_3^--N at three time points to estimate decomposition and N mineralization rates, and characterized the composition of soil bacterial communities at harvest using Illumina sequencing. Finally, we determined the total weight and %C, %N and $\delta^{15}N$ isotopic composition of carrot roots and shoots at harvest to determine the extent to which each carrot genotype could access and benefit from decomposition of the corn residues.

2.2 Materials and Methods

2.2.1 Experimental design

A greenhouse experiment was conducted using an artificial soil constructed using a 50:50 mix of agricultural field soil and sand. The initial characteristics of this soil mixture were determined using standard procedures of S2N analysis at the Midwest Soil Testing Lab (www.midwestlabs.com). The soil mixture contained 1% total organic matter (OM), a pH of 7.2, and 110, 359, 327, and 1598 mg/kg soil plant available phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca), respectively. Soil cation exchange capacity (CEC) of the soil mixture was 11.6 meq/100g soil, and the % base saturation on CEC sites was 7.9, 23.5 and 61 for K, Mg, and Ca respectively. Initial NO_3^--N and NH_4^+-N values were 6.48 and 1.2 mg N/kg, respectively, and determined using techniques described in section 2.2.3. Percent total nitrogen, carbon and of $\delta^{15}N$ isotopic composition in the initial soil mixture and soil amendments (Table 2.1) was determined using techniques described in section 2.2.5.

Before planting carrot seeds, each pot was amended with 1g of 603 ‰ $\delta^{15}N$ enriched corn material, and 1.2 g of an organic feather meal fertilizer (Down to Earth, Eugene, Oregon), which contained the equivalent of 144 mg of N. The organic feather meal organic fertilizer also contained 12% total N.

Table 2.1: Material, total %C, %N $\delta^{15}N$ isotopic composition and total N contribution from the soil and amendments used in the greenhouse study

Material	Material added per pot (g)	%C	%N	$\delta^{15}N$	Total N contribution (g)
Feather meal	1.2	45.64	13.76	3.9	0.165
^{15}N enriched corn residue	1	42.5	0.69	603	0.008
Initial soil	8000	0.7	0.1	8.3	8

Five carrot genotypes were selected for use in the greenhouse trial, which included two experimental breeding lines, E3999 and E8503, and three commercial carrot varieties, Karotan, Sun255 and Uppercut, which are all currently being evaluated for their potential application in organic management systems (Table 2.2). These genotypes were chosen because they have similar

taproot morphological characteristics, yet vary significantly in top-size, which is suspected to play a role in their nitrogen use efficiency. They also vary in susceptibility to nematodes, which we have previously determined can alter composition of root microbial communities and potentially play a role in this resistance as well as other factors such as nutrient acquisition (Abdelrazek et al., under review). Carrot seeds were obtained from Dr. Phil Simon, USDA-ARS Vegetable Crops Research Unit, Madison, WI.

Seven carrot seeds of each genotype were planted in 7.7 L pots (15.2 x 40.6 cm) filled with 8.0 kg of the 1:1 sand:soil mixture described above, and placed in the Purdue Greenhouse Horticulture facilities during the first week of June. The average day and nighttime temperatures were 22 °C and 18° C ± 1° C, respectively. Relative humidity was maintained between 50 and 70%. After seed germination, the pots were thinned to one carrot seedling. The experiment was set up as a complete randomized block design with five carrot genotypes and an unplanted control treatment, each with six replicates, for a total of 36 pots.

Carrots were harvested in September, 90 days after seeding. The total wet weight of above and below ground biomass was determined at harvest, and then plant materials were oven dried at 70°C for 4 days to quantify dry weight of each fraction. Then each plant fraction was ground using a UDY cyclone sample mill (UDY Corp., Boulder, Col) to obtain fragments of approximately 1 mm for analyses described in section 2.2.5.

Table 2.2: Carrots genotype description from the Carrot Improvement for Organic Agriculture (CIOA) project.

Carrot genotypes description						
Entry	Color	Shape	Length	Top Size	Nematode resistance	Origin
Exp Nb3999	Orange	Cylindrical	Long	Small	Yes	Brazil/Europe
Exp Nb8503	Orange	Thin Cylindrical	Long	Medium	Yes	Brazil/Europe
Karotan	Orange	Conical	Medium-Long	Large	No	Europe
Sun255	Orange	Conical	Long	Medium	No	Europe
Uppercut	Orange	Conical	Long	Large	No	Europe

2.2.2 Soil sample collection during the greenhouse experiment

At 6 and 10 weeks after seeding and at harvest, three soil cores were collected to a depth of 0-10 cm in each pot with a 1.27cm probe to minimize any potential damage to the carrot roots. The three soil cores from each pot were mixed and homogenized in a plastic bag and stored at 4° C until analysis for soil ß-glucosidase enzyme activity and DNA extractions. A subsample from each bag was air-dried for two days and sieved (2 mm) in preparation for extractions to quantify ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations, and total element C and N.

2.2.3 Quantification of plant available nitrogen in soils

A modified protocol by Weaver et al. (1994) was used to quantify plant available N in each soil sample. Briefly, 12.5 ml of KCl (1M) was added to 5g soil samples and the soil slurries were incubated at room temperature for 30 minutes while shaking at 250 rpm. Samples were then filtered through a 42 Waltman filter paper for 10-15 minutes and stored at -20° C until analysis using an AQ2 Discrete SEAL Analyzer (Seal Analytical, Southampton, Hampshire, United Kingdom) to quantify nitrate (NO_3^-) and ammonium (NH_4^+) according to EPA methods: NO_3^--N (AGR-231-A-Rev. 0) and NH_4^+-N (AGR-210-A Rev. 0), All samples were conducted in duplicate. Soil mg N per kg of dry soil for both NH_4^+-N and NO_3^--N were calculated as follows:

$$Cs = \frac{Ce\ x\ V}{W} \tag{1}$$

Where *Ce* is the concentration of N as NH_4^+-N and NO_3^--N in the KCl extract in mg N/L, *V* the volume of the KCl extract and *W* the dry kg of soil.

N made available from organic sources during the experiment was calculated as follows:

$$NRO = ((IN(harvest) - IN(\text{ initial}))\ x\ kg\ soil\ ) + total\ plant\ N\ uptake \tag{2}$$

Where $IN(harvest)$ is the soil inorganic N (NH_4^+-N + NO_3^--N) at harvest, $IN(\text{ initial})$ is the initial soil inorganic N (NH_4^+-N + NO_3^- -N), $kg\ soil$ the kg of soil in each pot and $total\ plant\ N\ uptake$ (total % N x total plant dry weight).

2.2.4 Soil ß -glucosidase enzyme assay

Soil ß-glucosidase activity was quantified collected at three time points (6 and 10 weeks after seeding and at harvest) in triplicate in each soil sample. The assay is based on the determination of p-nitrophenol released after incubating soil with p-nitrophenyl (Eivazi & Tabatabai, 1988). Briefly, 1 g of moist soil per sample was incubated for 1 hour with a solution containing 0.25ml of toluene, 4 ml of modified universal buffer (MUB) and 1 ml of p-nitrophenyl b-d-glucoside (PNG). After the incubation, a solution containing 1 ml of $CaCl_2$ and 4 ml of tris buffer at pH12, were added to stop the enzymatic reaction. The soil suspensions were then filtered immediately through 42 Whatman filter paper. There technical replicates of each sample were then half diluted, and color intensity at 400nm was quantified. During each measurement, an additional blank suspension with no soil was included as a control. Total p-nitrophenol per ml of the filtrates was calculated using the following formula:

p-nitrophenol (ug g^{-1} dwt h^{-1}) =

$$\frac{C\ x\ v}{dwt\ x\ SW\ x\ t} \tag{3}$$

Where *C* is the concentration of p nitrophenol, *dwt* is the dry weight of 1 g of soil, *v* is the total volume of the soil suspension in ml, and *SW* is the weight of the soil sample and *t* is the incubation time.

2.2.5 Soil and plant carbon and nitrogen elemental composition

30 mg of the initial soil sample was analyzed to quantify total C and N elemental composition using a Thermo Scientific FlashEA 1112 Nitrogen and Carbon Analyzer for Soils, Sediments, and Filters (CE Elantech, Lakewood, NJ). In addition, 0.5 mg of the organic feather meal fertilizer and 3.8 mg of the ^{15}N labeled corn residue were analyzed using the same procedure to quantity %C and %N content. C and N elemental isotope composition of carrot root and shoot biomass, total $\delta^{15}N$ in ^{15}N labeled corn residue, soil and the organic feather meal fertilizer was quantified using the PDZ Europa Elemental Analyzer interfaced to a Sercon Ltd. 20-22 isotope ratio mass spectrometer (EA-IRMS) at the Purdue Stable Isotope facility, using established protocols .

2.2.6 Nitrogen isotope ratio calculations

Values of delta ^{15}N ($\delta^{15}N$ ‰) and N content in samples were determined using the PDZ Europa Elemental Analyzer interfaced to a Sercon Ltd. 20-22 isotope ratio mass spectrometer (EA-IRMS) at the Purdue Stable Isotope facility. N content is represented by the amount of $^{14}N + ^{15}N$ in the sample. $\delta\ ^{15}N$ ‰ was the proportional amount of ^{15}N in parts per mil in the sample relative to an international isotope standard of air (0.00366 atom ‰^{15}N) according to the following equation:

$$\delta^{15}N \text{ sample}(‰) = \left(\frac{R\ sample}{R\ standard} - 1\right) \text{ x } 1000 \quad (4)$$

Where *R sample* is the isotope ratio$^{15}N/^{14}N$ of the sample and the *R standard* is the atmospheric nitrogen with a $^{15}N/^{14}N$ ratio of 0.00368 (Van Cleemput, Zapata, & Vanlauwe, 2008).

Using the $\delta^{15}N$ value obtained for carrot roots and shoots, we determined the %N in each plant that was obtained from the ^{15}N enriched corn residue using the following calculation :

$$\%N\ FR = 100\ x\ \frac{\delta^{15}NT(r,s)}{\delta^{15}NR} \quad (5)$$

Eq. (5) calculates %N obtained from ^{15}N labeled corn residue based on the $\delta\ ^{15}N$ values from carrot roots and shoots *NT (r,s)* ,and $\delta\ ^{15}N$ values of corn residue *NR*.

2.2.7 Bacterial soil microbiome analysis

Soil DNA extraction, 16s rRNA bacterial DNA amplification and illumina sequencing

DNA was extracted from 0.25 g soil samples collected at harvest using DNeasy PowerSoil Kits (Qiagen, Venlo, Netherlands) in triplicate for each pot, for a total of 109 extractions. The quantity and quality of each extraction was quantified using a Nanodrop 2000 microvolume spectrophotometer (Thermo Fischer Scientific, Waltman, MA, U.S.A) and then the three technical replicates of DNA from each pot were pooled in preparation for PCR using primers targeting the V3-V4 16s rRNA bacterial region: V3_341F: CCTACGGGAGGCAGCAG and

V4_806R: GGACTACHVGGGTWTCTAAT. Each primer also contained a so-called "CS" linker sequence, which is necessary to run the 2nd PCR with Illumina adapters and barcodes.

All samples were diluted to 1 ng/µl , and then 1 µl of DNA was used as a template for 25 µl PCR reactions consisting of 10.5 µl H2O, 12.5 µl Q5® High-fidelity Master Mix (NEB), 0.5 µl primer V3 (10 µM), and 0.5 µl primer V4 (10 µM), in triplicate for each sample. PCR thermocycler conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 28 cycles of: 95°C for 30 sec, 50°C for 30 sec, 72°C for 1 min and a final elongation at 72°C for 10 min. After PCR, 4 µl of each amplification product (~ 460 pb) was visualized on a 1% agarose gel to verify that the PCR reaction was successful. Finally, a total of 36 samples (25 µl each) of final PCR products were sent to the Genome Research Core Facility at the University of Illinois in Chicago, U.S.A., for sequencing on the MiSeq Illumina platform.

Bioinformatic analysis

Sequences obtained from the Illumina platform were demultiplexed based on the individual barcodes assigned to each sample, and were trimmed according to 25 phred quality value using the TrimGalore tool (Krueger, 2015). Then reads from forward and reverse primers were merged using the join_parired_ends.py script found in QIIME v2. (Caporaso et al., 2010) documentation (http://qiime.org/scripts/join_paired_ends.html) and quality checked using the FastQC tool (Andrews, 2010). QIIME v2. (Caporaso et al., 2010) was used for picking, identifying chimeric sequences, assigning bacterial taxonomy, and aligning final operational taxonomic units (OTUs) with the SILVA bacterial data base (Pruesse et al., 2007), then OTUs were filtered for chloroplasts and mitochondria sequences. Statistical analyses were also conducted to calculate α diversity (Shannon indexes) using the alpha_rarefaction.py script that uses a nonparametric index to measure total number of OTUs and evenness (relative abundance of OTUs), found in QIIME v2. documentation (http://qiime.org/scripts/alpha_diversity.html). A post-hoc Tukey pairwise comparison was performed to test for differences in α diversity for each pair of means. To analyze bacterial ß-diversity, a Bray-Curtis dissimilarity distance matrix was created and visualized using a PcoA ordination plot, and an ADONIS test was conducted to determine statistical differences between the soil genotypes using the R package "vegan" (Oksanen, 2007), in R version 3.6.2 (R Core Team, 2019)

2.2.8 Statistical analyses

Differences in soil ß-glucosidase activity, NH_4^+-N, and NO_3^--N collected at the three time points were evaluated using a linear mixed model with time and genotype as fixed factors, and pot as a random factor. NH_4^+-N and NO_3^- -N values were ln-transformed to meet normality assumptions. Then a two-way ANOVA analysis was conducted to determine if the effect of time, genotype and the genotype x time interaction were significantly different. Inorganic N made available during the experiment was evaluated using a linear model and a one-way ANOVA was conducted to determine if genotype was significant. Data from root and shoot dry weights were fitted to a linear regression model and a non-parametric Kruskal Wallis test was conducted to determine if there were differences among the carrot genotypes. Then the R:S ratio, %C, %N, C:N ratio and total δ^{15}N abundance values were each fitted to a linear regression model and subject to a one way ANOVA. A post-hoc Tukey pairwise comparison was performed for each of these values to identify differences among the carrot genotypes. Finally, data was fitted to a linear regression and performed ANOVA analyses to determine differences in $\delta^{15}N\ T(r,s)$ together and separating roots and shoots to determine differences among the genotypes.

2.3 Results

2.3.1 Soil ß-glucosidase enzyme assay

Results of the two-way ANOVA analysis indicated that there were significant differences in soil ß-glucosidase activity among the three time points (6 and 10 weeks after seeding and at harvest), and there was an interaction between carrot genotype and time (Table 2.3).

Soil ß-glucosidase activity decreased over time in all carrot genotype treatments, with the exception of Karotan, which increased between 10 weeks after seeding and harvest (Figure 2.1). At 6 weeks after planting, only genotypes 3999 and Sun255 had significant differences in soil ß-glucosidase activity, however, at 10 weeks after seeding, ß-glucosidase activity began to diverge more dramatically between the carrot genotypes. Specifically, carrot genotype 8503 had significantly greater soil ß-glucosidase activity than Karotan, Sun255 and the unplanted control. At harvest, there was not significant differences between genotypes (Table 2.4).

Table 2.3: Two-way ANOVA model evaluating differences in soil ß-glucosidase activity of five carrot genotypes and an unplanted control at 6 weeks, 10 weeks and harvest.

	chisq	df	pr(>chisq)
(intercept)	1172.892	1	<0.0001 ***
genotype	5.066	5	0.41
time	11.008	2	0.004 **
genotype x time	26.298	10	0.003 **

Significant p-values correspond to *** p <0.001 and ** p<0.01 (alpha value = 0.05).

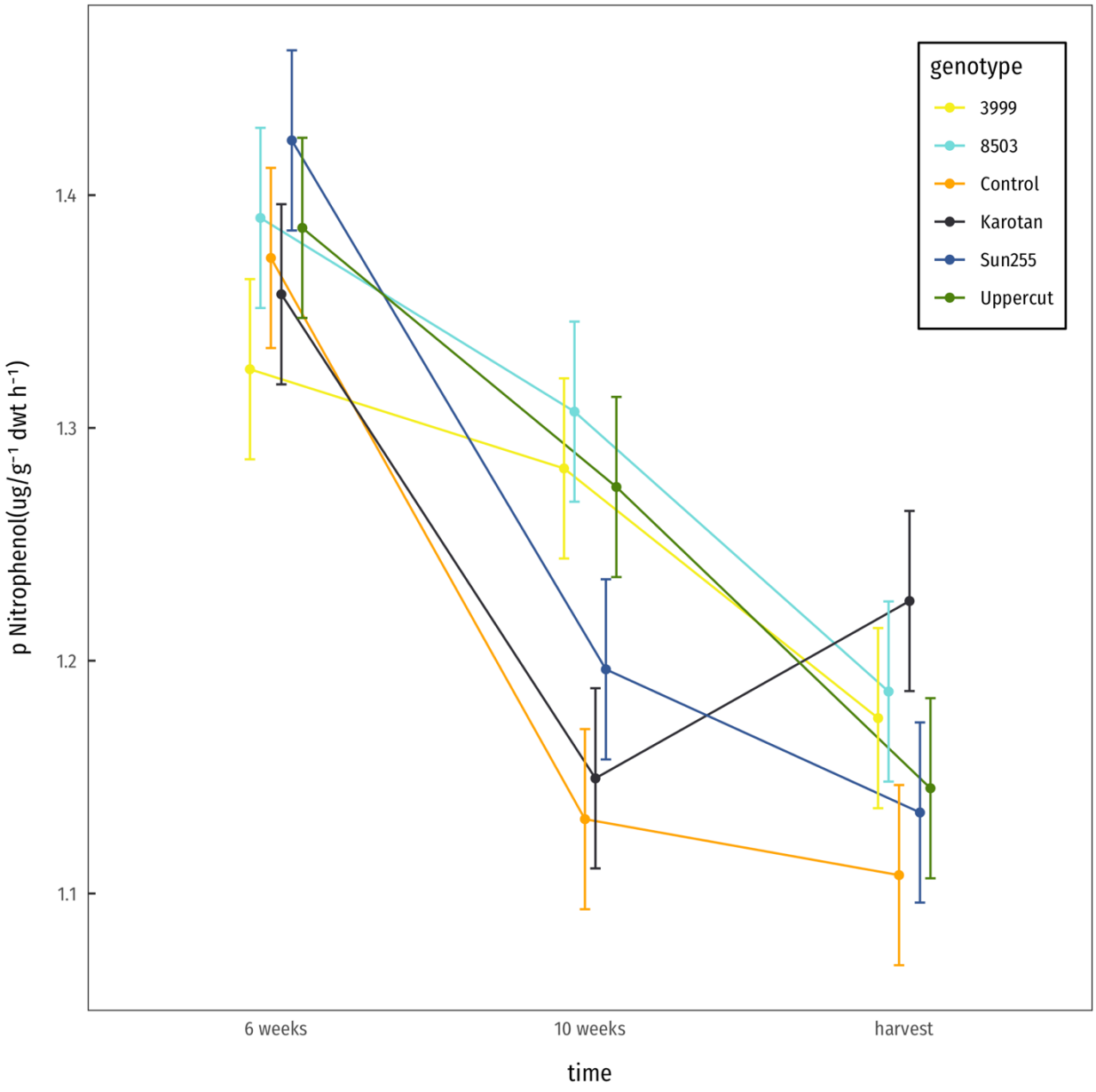

Figure 2.1: Soil ß-glucosidase activity 6 and 10 weeks after seeding and at harvest.

Table 2.4: Tukey pairwise comparison of ß-glucosidase activity at 6 weeks, 10 weeks and harvest between genotypes.

time	genotype	mean	se	df	lower CL	upper CL	group
6 weeks							
	3999	1.33	0.0369	10.3	1.24	1.41	b
	8503	1.39	0.0369	10.3	1.31	1.47	ab
	Control	1.37	0.0369	10.3	1.29	1.45	ab
	Karotan	1.36	0.0369	10.3	1.28	1.44	ab
	Sun255	1.42	0.0369	10.3	1.34	1.51	a
	uppercut	1.39	0.0369	10.3	1.3	1.47	ab
10 weeks							
	3999	1.28	0.0411	42.8	1.2	1.37	ab
	8503	1.31	0.0411	42.8	1.22	1.39	a
	Control	1.13	0.0411	42.8	1.05	1.21	c
	Karotan	1.15	0.0411	42.8	1.07	1.23	c
	Sun255	1.2	0.0411	42.8	1.11	1.28	bc
	Uppercut	1.27	0.0411	42.8	1.19	1.36	ab
harvest							
	3999	1.18	0.0392	44.3	1.1	1.25	a
	8503	1.19	0.0392	44.3	1.11	1.27	a
	Control	1.11	0.0392	44.3	1.03	1.19	a
	Karotan	1.23	0.0392	44.3	1.15	1.3	a
	Sun2255	1.13	0.0392	44.3	1.06	1.21	a
	Uppercut	1.15	0.0392	44.3	1.07	1.22	a

* Different letters indicate significant differences between genotypes in the Tukey test pairwise comparison (alpha value = 0.05).

2.3.2 Plant available N in soil (NH_4^+-N and NO_3^--N)

Results of the two-way ANOVA analysis indicated that values for soil NH_4^+-N were significantly different between the three time points (6 and 10 weeks after seeding and at harvest), and there was also an interaction between carrot genotype and time (Table 2.5).

Soil NH_4^+-N levels were similar among carrot genotypes at 6 weeks after seeding, but differed significantly at 10 weeks after seeding and harvest (Figure 2.2). Specifically, at 10 weeks after seeding, soil NH_4^+-N levels in pots planted with the carrot genotypes all decreased and appeared to diverge into two groups, with Karotan and 3999 having the highest levels of soil, while genotypes Sun255, 8503 Uppercut having the lowest levels of soil NH_4^+-N. Between 10 weeks after seeding and harvest, soil NH_4^+-N values in carrot genotype Karotan remained the same, while 3999 and Sun255 increased. Carrot genotypes 8503, Sun255 and Uppercut appear to have an increment between 10 weeks after planting and harvest while the unplanted control continued to drop after 10 week of planting.

Table 2.5: Two-way ANOVA model evaluating differences in soil NH_4^+-N of five carrot genotypes and an unplanted control at 6 weeks, 10 weeks and harvest.

	chisq	df	pr(>chisq)
(intercept)	179.953	1	<0.0001 ***
genotype	2.624	5	0.757
time	9.111	2	0.010*
genotype x time	42.271	10	<0.0001 ***

Significant p-values correspond to *** $p<0.001$ and *$p<0.05$ (alpha value=0.05).

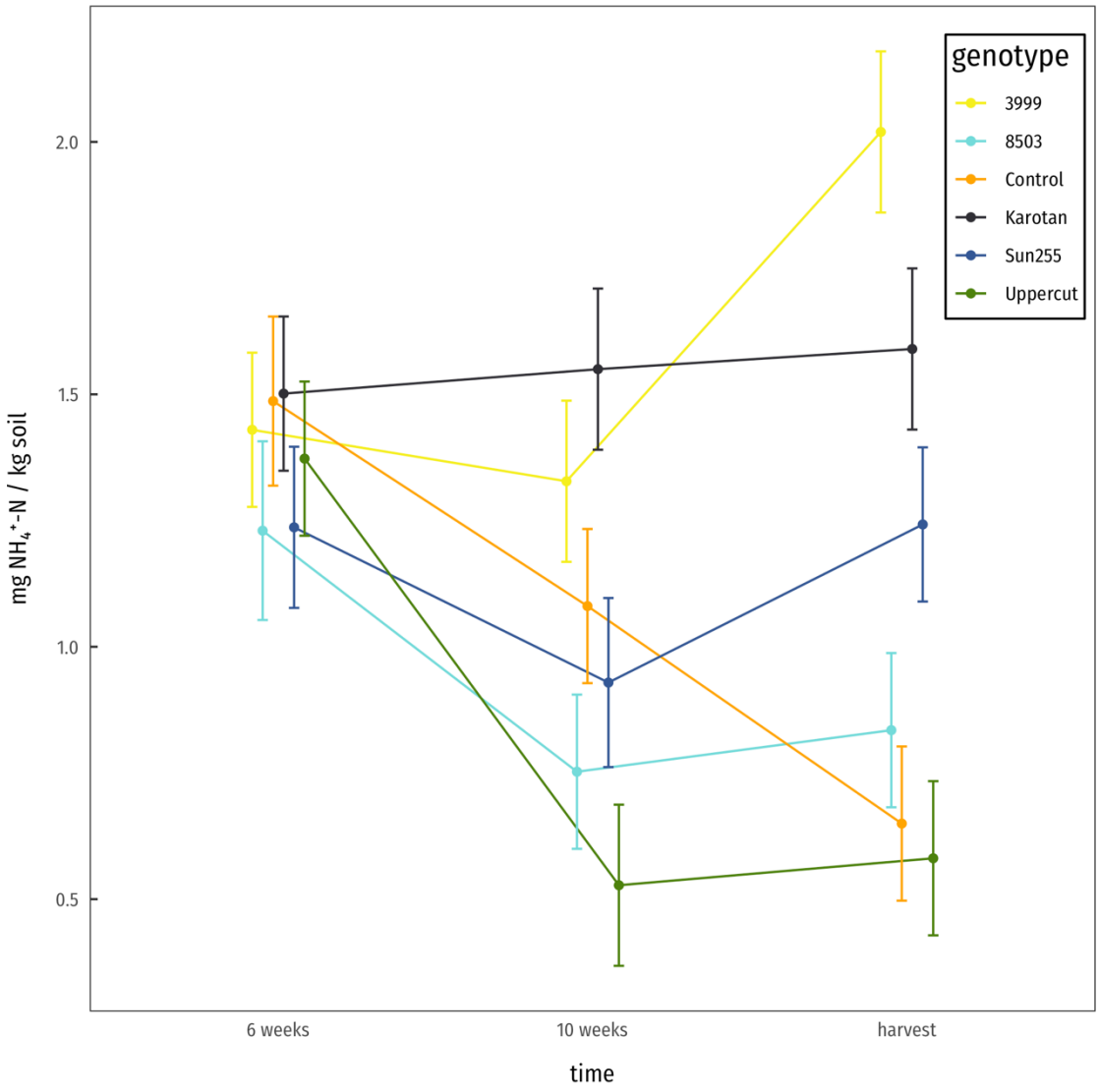

Figure 2.2: Soil NH_4^+-N at 6 and 10 weeks after seeding and at harvest

At six weeks after seeding, there were no significant differences in soil NH_4^+-N levels among the carrot genotypes, however, there were many differences at 10 weeks after seeding and at harvest (Table 2.6). Specifically, carrot genotypes Karotan had significantly greater levels of soil NH_4^+-N than genotypes 8503 and Uppercut, and genotype 3999 had significantly higher levels than Uppercut as well. At harvest, carrot genotypes Karotan and 3999, had significantly greater levels of soil NH_4^+-N than 8503 and Uppercut, as well as the unplanted control.

Table 2.6: Tukey pairwise comparison of soil NH_4^+-N at 6 and 10 weeks after seeding and at harvest between genotypes

time	genotype	mean	se	df	lower CL	upper CL	group
6 weeks							
	3999	0.88	0.0409	31.9	0.797	0.963	a
	8503	0.787	0.0467	39.9	0.692	0.881	a
	Control	0.901	0.0443	37.4	0.812	0.991	a
	Karotan	0.906	0.0409	31.9	0.823	0.989	a
	Sun255	0.803	0.0425	34.5	0.716	0.889	a
	uppercut	0.852	0.0409	31.9	0.768	0.935	a
10 weeks							
	3999	0.762	0.0749	44.2	0.611	0.913	ab
	8503	0.552	0.0718	41.8	0.407	0.697	bc
	Control	0.699	0.0718	41.8	0.554	0.844	ab
	Karotan	0.921	0.0749	44.2	0.77	1.072	a
	Sun255	0.653	0.0784	46.8	0.495	0.811	abc
	Uppercut	0.407	0.0749	44.2	0.256	0.558	c
harvest							
	3999	1.048	0.0821	58.1	0.884	1.213	a
	8503	0.582	0.0784	57	0.425	0.739	bc
	Control	0.493	0.0784	57	0.336	0.65	bc
	Karotan	0.935	0.0821	58.1	0.771	1.099	a
	Sun255	0.758	0.0784	57	0.601	0.915	ab
	Uppercut	0.409	0.0784	57	0.252	0.566	c

* Different letters indicate significant differences between genotypes in the Tukey test pairwise comparison (alpha value = 0.05).

Results of the two-way ANOVA analysis indicated that only carrot genotype was a significant factor in soil NO_3^--N values in soil (Table 2.7). At 6 and 10 weeks after seeding there were significant differences in soil NO_3^--N levels between the carrot genotypes (Figure 2.3; Table 2.8). At 6 weeks, carrot genotypes 3999 and Sun255 had significantly lower levels than genotype Karotan and the unplanted control. At 10 weeks, all soil NO_3^--N levels decreased and carrot genotypes Sun255 and Uppercut were significantly lower than unplanted control. At harvest, there was not significant differences in soil NO_3^--N levels between the carrot genotypes.

Table 2.7: Two-way ANOVA model evaluating differences in soil NO_3^--N levels of five carrot genotypes and an unplanted control at 6 weeks, 10 weeks and harvest.

	chisq	df	pr (>chisq)
(intercept)	104.436	1	< 0.001 ***
genotype	20.767	5	0.0008 ***
time	4.753	2	0.092
genotype x time	11.436	10	0.324

Significant p-values correspond to *** $p<0.001$ (alpha value = 0.05).

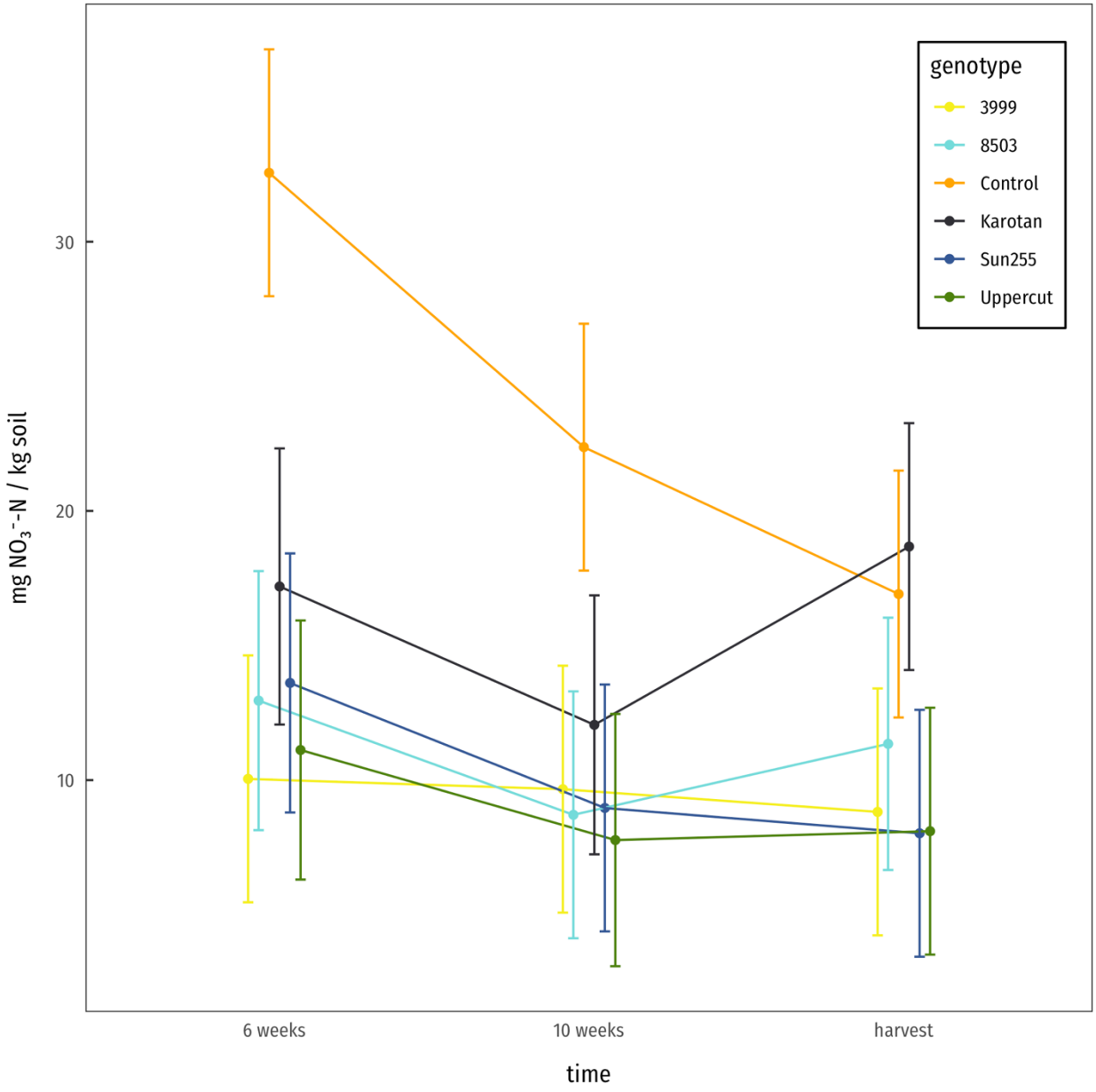

Figure 2.3: Soil NO_3^--N levels at 6 and 10 weeks after seeding and at harvest

Table 2.8: Tukey pairwise comparison of soil NO_3^--N between genotypes at 6 weeks, 10 weeks and harvest

time	genotype	mean	se	df	lower CL	upper CL	group
6 weeks							
	3999	2.22	0.353	7.02	1.39	3.06	b
	8503	2.53	0.353	7.02	1.69	3.36	ab
	Control	3.1	0.353	7.02	2.26	3.93	a
	Karotan	3.13	0.353	7.02	2.3	3.97	a
	Sun255	2.44	0.353	7.02	1.61	3.28	b
	Uppercut	2.52	0.353	7.02	1.68	3.35	ab
10 weeks							
	3999	2.15	0.371	8.89	1.311	2.99	ab
	8503	1.99	0.371	8.89	1.152	2.84	ab
	Control	2.64	0.371	8.89	1.803	3.49	a
	Karotan	2.41	0.371	8.89	1.57	3.25	ab
	Sun255	1.76	0.371	8.89	0.921	2.6	b
	Uppercut	1.79	0.371	8.89	0.947	2.63	b
harvest							
	3999	2	0.284	12.5	1.38	2.61	a
	8503	2.39	0.284	12.5	1.77	3	a
	Control	2.58	0.284	12.5	1.96	3.2	a
	Karotan	2.03	0.284	12.5	1.41	2.65	a
	Sun255	1.88	0.284	12.5	1.26	2.5	a
	Uppercut	1.82	0.284	12.5	1.21	2.44	a

* Different letters indicate significant differences between genotypes in the Tukey test pairwise comparison (alpha value = 0.05).

2.3.3 Soil bacterial microbiome at harvest

Twenty-two unique bacterial families were identified in the soils collected at harvest, and between 18 and 28% of bacterial relative abundance was classified as “other” and 11 to 16 % as “uncultured”, and not all families were present in all treatments (Figure 2.4). *Oxalobacteriaceae, Opitutaceae, Micrococcaceae* families were only present in Karotan, Sun255 and Uppercut genotypes. Bacteria within the *Chromatiacea* family was only present in 3999, 8503 and Sun255 genotypes, the *Intrasporangiaciae* family was only present in Karotan and Sun255 genotypes, the *Familyl* family was only present in 3999, Sun255 and Uppercut, the *Rhodospirillaceae* family was only found in 3999 and unplanted control, and the *Sphingomonadaceaea* family was only found in Sun255 and Uppercut.

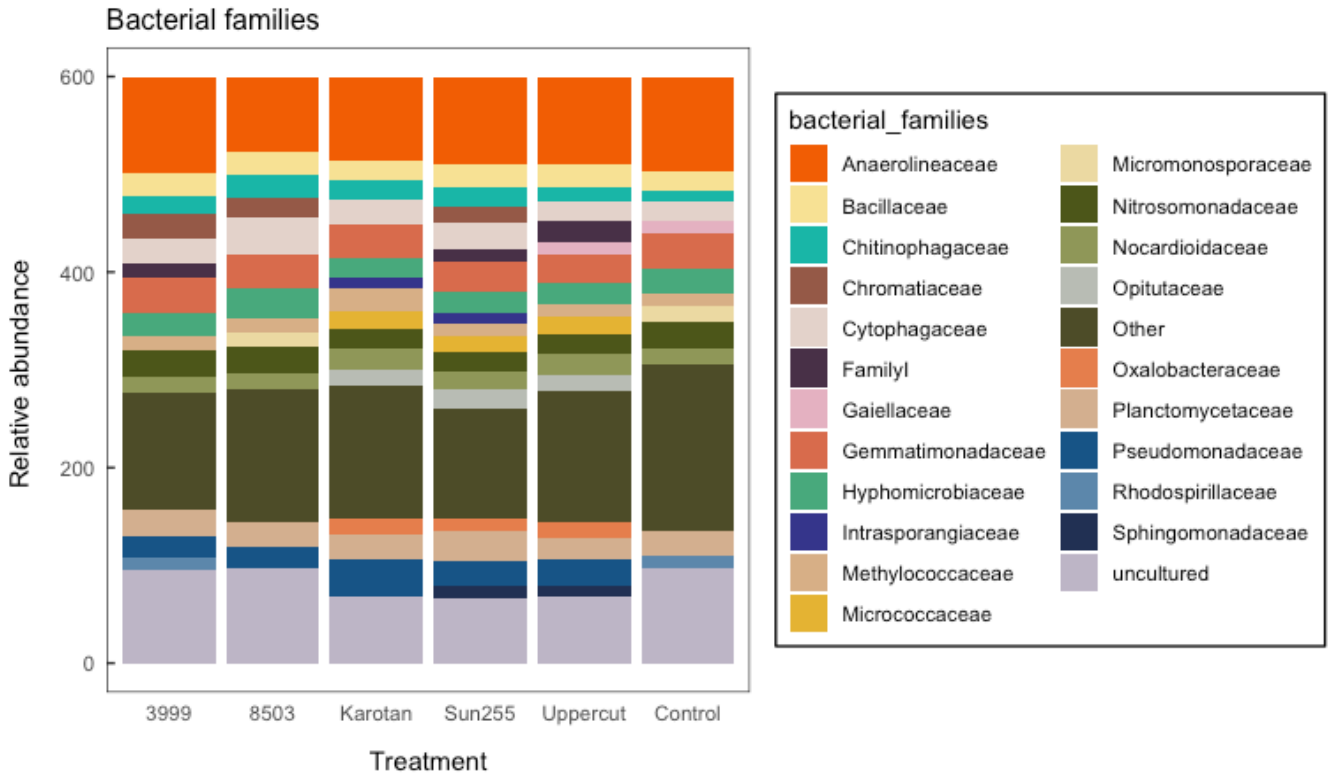

Figure 2.4: Taxonomic families of bacteria in the soil of five carrot genotypes and an unplanted control at harvest

2.3.4 Soil bacterial alpha (α) diversity

When comparing alpha diversity among the bacterial communities, the one-way ANOVA analysis indicated there were significant difference among the carrot genotypes and the unplanted control (Table 2.9). Specifically, there were significant differences in alpha diversity of soil bacterial communities between genotypes 3999, 8503 and Sun255 and the unplanted control treatment (Figure 2.5).

Table 2.9: One-way ANOVA model evaluating differences in alpha diversity of soil bacterial communities in the soils of five carrot genotypes and an unplanted control using the shannon index.

	sum sq	df	F value	pr(>F)
intercept	815.33	1	7610.995	< 0.001***
genotypes	2.51	5	4.685	0.002 **
residuals	3.21	30		

Significant p-values correspond to *** p <0.001 and ** p<0.01 (alpha value = 0.05).

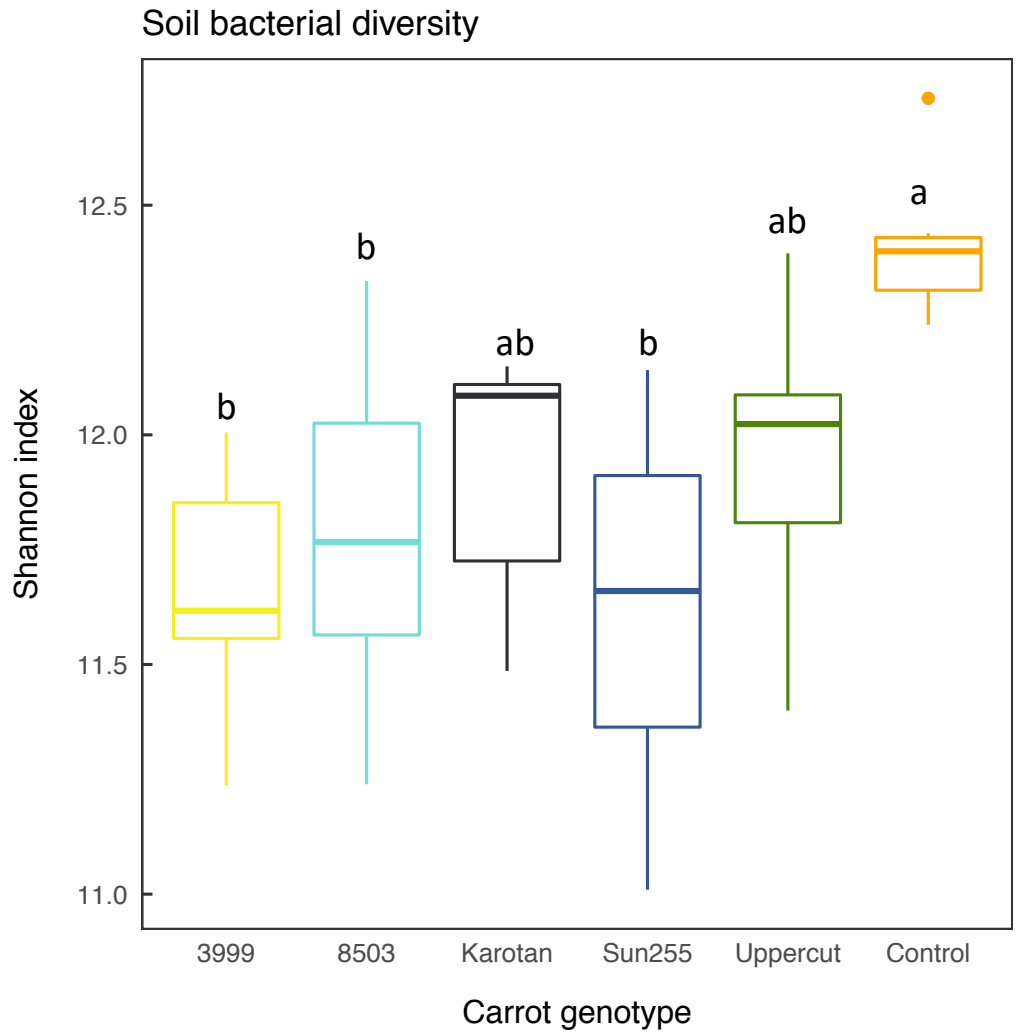

Figure 2.5: Alpha diversity of soil communities between genotypes and soil control. Points indicate outliers for each group. *Different letters indicate significant differences between genotypes in the Tukey test pairwise comparison (alpha value = 0.05).

2.3.5 Soil bacterial beta (ß) diversity

Results of the principal components analysis (PCoA) and ADONIS test indicated that there were significant differences in beta diversity among the soil bacteria communities. Based on the R^2 value in the ADONIS test, 50 % of variation in distances was explained by carrot genotypes, and two of PCoA axes explained 21% of the total variability (Figure 2.6; Table 2.10). Specifically, carrot genotypes 3999 and 8503 clustered together in group A, while Karotan, Uppercut and Sun255 clustered in group B, and the untreated control treatment clustered into a third group classified as group C (Figure 2.6).

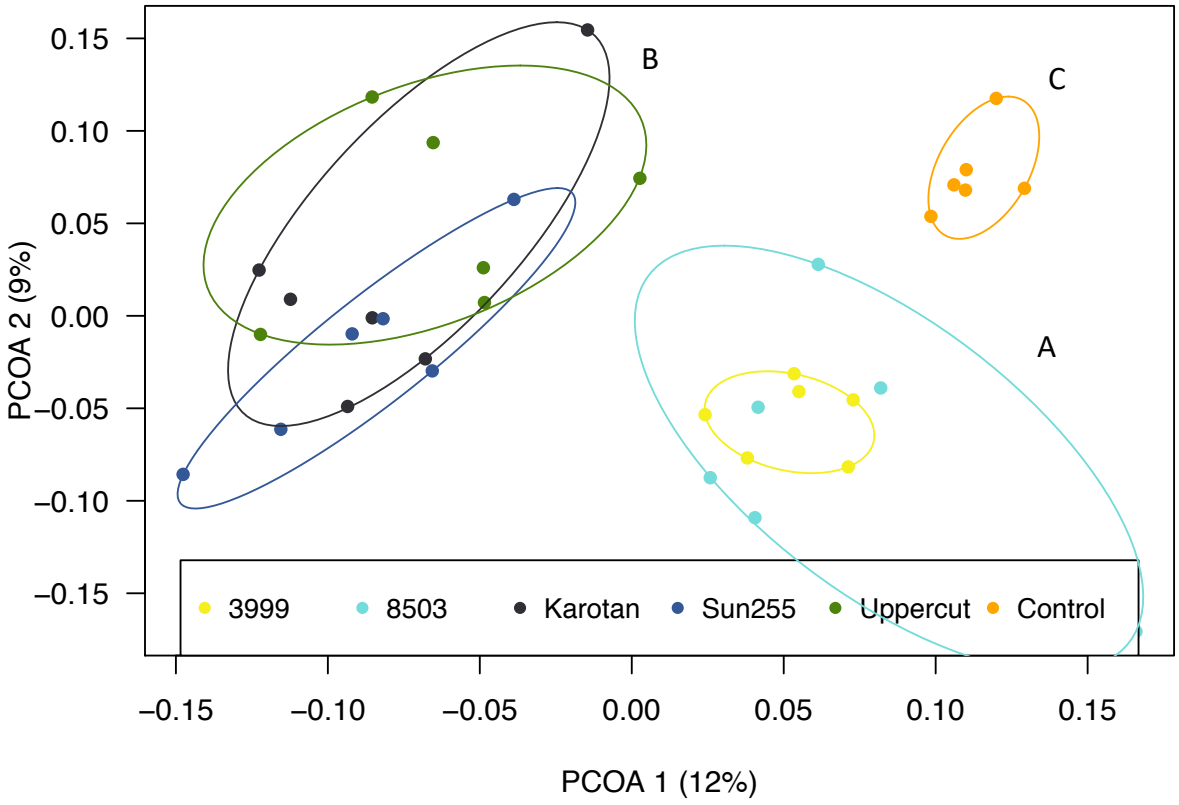

Figure 2.6: Principal components analysis (PcoA) explaining 21% of the variability in beta diversity of soil bacterial communities in the soil of five carrot genotypes and an untreated control based on Bray-Curtis distances.

Table 2.10: Permutational analysis of variance ADONIS of soil bacterial communities in the soil of five carrot genotypes and an untreated control based on Bray-Curtis distances.

Number of permutations:	999					
	df	sums of sqs	mean sqs	F model	R^2	Pr(>F)
genotype	5	0.432	0.086	6.09	0.503	>0.001 ***
residuals	30	0.426	0.014		0.496	
total	35	0.859			1	

Significant p-values correspond to *** p <0.001 (alpha value = 0.05).

2.3.6 Root and shoot dry weight and root:shoot ratio

Based on the non-parametric Kruskal Wallis test, there were no statistically significant differences in total root or shoot dry weight between the five carrot genotypes (Table 2.11; Figure 2.7). In contrast, there were statistical differences in the Tukey test for root:shoot ratios (R:S) between some of the carrot genotypes (Figure 2.8; Table 2.12). Specifically, carrot genotype 8503 had a greater root:shoot ratio than Karotan or Uppercut.

Table 2.11: Non-parametric Kruskal Wallis test summary of shoot and root dry weight

dry weight	chi-squared	df	p-value
roots	1.243	4	0.871
shoots	5.727	4	0.220

alpha value = 0.05

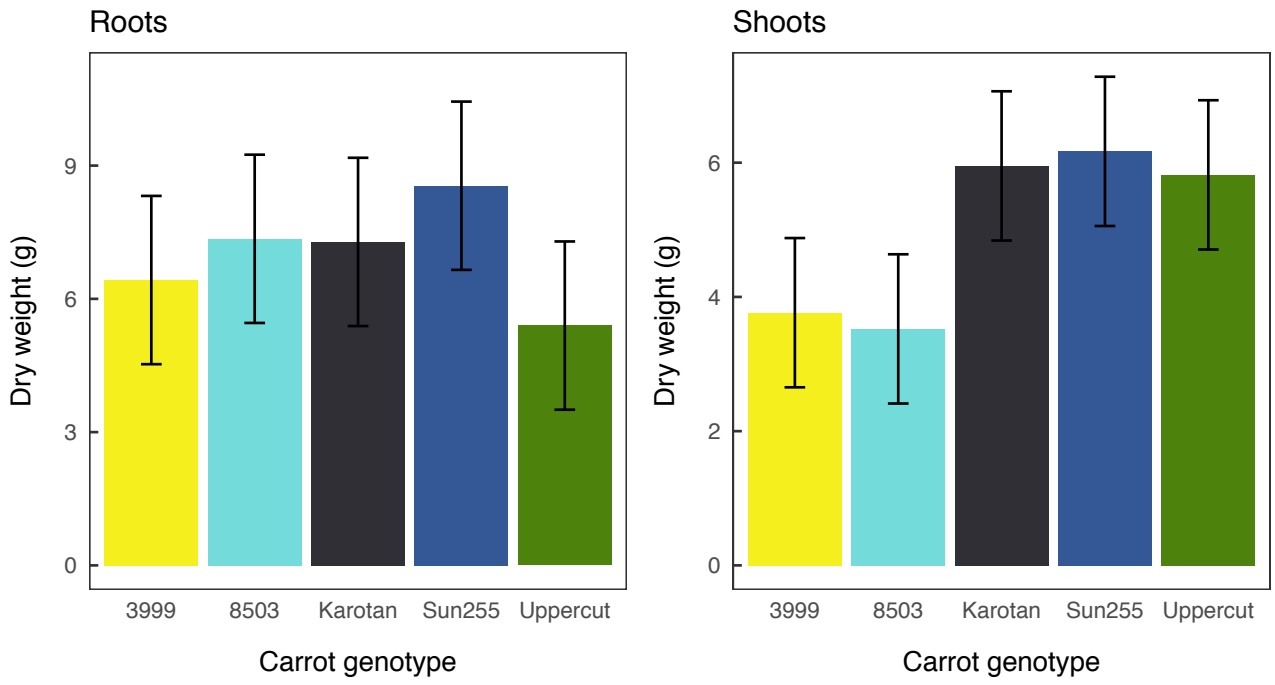

Figure 2.7: Root and shoot dry weight (g) of five carrot genotypes grown in a greenhouse trial

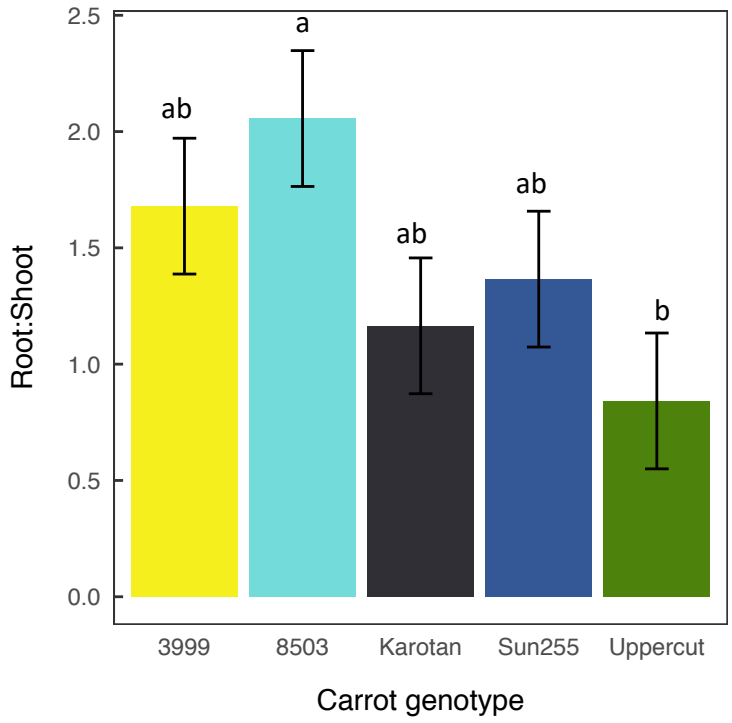

Figure 2.8: Root: shoot ratio of five carrot genotypes grown in the greenhouse.*Letters indicate significant differences between genotypes in the Tukey test pairwise comparison (alpha value = 0.05).

Table 2.12: One-way ANOVA root:shoot ratio of five carrot genotypes grown in a greenhouse trial

	sum sq	df	F	pr(>F) value
(intercept)	16.9211	1	33.0778	>0.001 ***
genotype	5.2451	4	2.5633	0.06315.
residuals	12.7889	25		

Significant p-values correspond to *** p <0.001 and (.) marginal differences correspond to p < 0.10 (alpha value = 0.05).

2.3.7 Total C and N, and δ ^{15}N elemental composition in carrot roots and shoots

Results of the two-way ANOVA analysis indicated that the total %C in carrot root and shoot tissues was not significant between the carrot genotypes (Table 2.13). Total %N in carrot roots was also not significantly different between the carrot genotypes, though there were differences among shoots (Table 2.13). Specifically, there was greater total %N in the shoots of carrot genotype 3999 Karotan, Sun255 and Uppercut, and genotype 8503 had great total %N than Uppercut. According to the one-way ANOVA analysis, total δ^{15}N in carrot roots and shoots were not significantly different between the carrot genotypes (Table 2.13).

Table 2.13: Average total %C, %N and $\delta^{15}N$ (‰), in five carrot genotypes based on a one-way ANOVA

	genotype	C %	±	F	p-value	N %	±	F	p-value	$\delta^{15}N$	±	F	p-value
	3999	40.5	0.26			1.44	0.14			13.23	0.43		
	8503	39.8	0.15			1.17	0.09			15.08	0.56		
roots	Karotan	39.7	0.47	1.33	0.29	1.24	0.2	1.59	0.21	13.26	0.24	1.744	0.171
	Sun255	40.5	0.28			1.18	0.1			13.96	0.87		
	Uppercut	39.7	0.5			0.97	0.12			13.42	0.6		
	3999	38	0.38			2.32 a	0.2			14.66	0.32		
	8503	37.3	0.39			2.03 ab	0.13			14.47	0.72		
shoots	Karotan	38.2	0.38	1.29	0.299	1.51 bc	0.14	8.24	0.0002 ***	13.68	0.14	1.422	0.255
	Sun255	38.6	0.55			1.45 bc	0.11			13.19	0.25		
	Uppercut	37.5	0.5			1.36 c	0.14			14.3	0.77		

*Letters indicate significant differences between genotypes in the Tukey test pairwise comparison. Significant p-values correspond to ***$p<0.001$ (alpha value = 0.05).

2.3.8 C:N ratio in carrot roots and shoots

Based on the one-way ANOVA analysis, there were no statistically significant differences in the C:N ratio among the roots of the five carrot genotypes, however there were differences in shoots (Table 2.14; Figure 2.9). Specifically, results of the Tukey pairwise comparison indicated that carrot genotypes Karotan, Sun255 and Uppercut all had a greater C:N ratio in shoots than 3999 and 8503.

Table 2.14: One-way ANOVA model evaluating differences in C:N ratio of the five carrot genotypes of roots and shoots.

tissue		sum sq	ff	F value	pr(>F)
roots	genotype	617.8	4	2.054	0.117
shoots		680.62	4	9.775	<0.001 ***

Significant p-values correspond to ***p<0.001 (alpha value = 0.05).

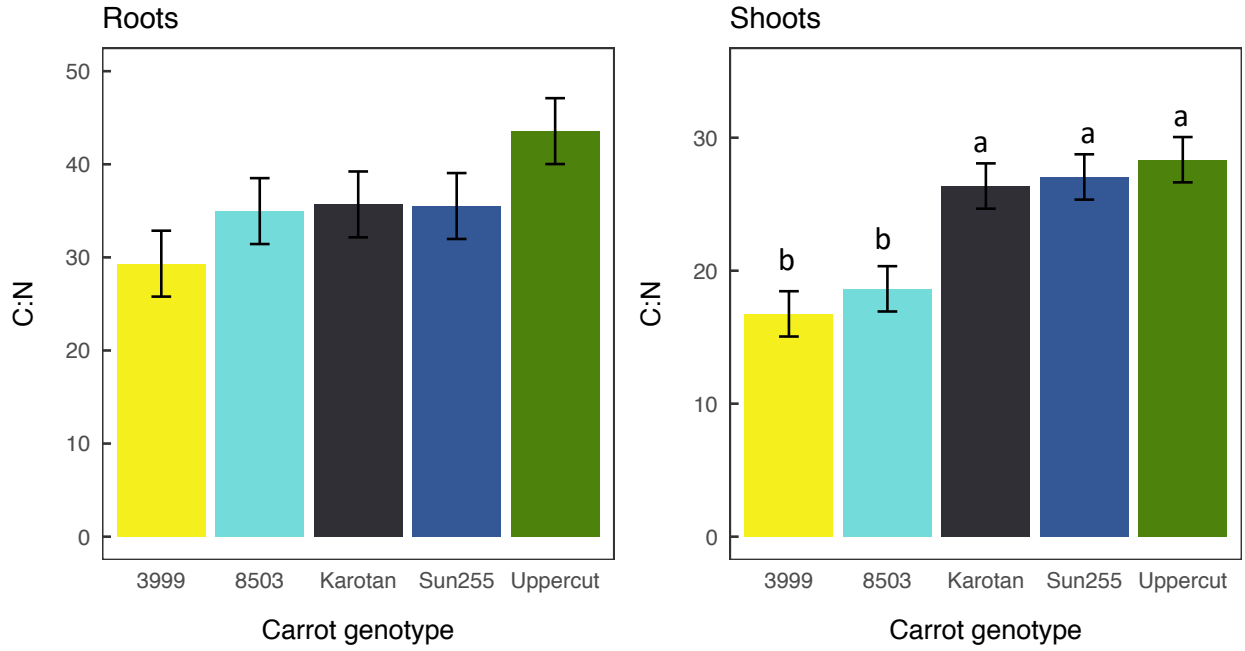

Figure 2.9: Differences in the C:N ratio in the roots and shoots of five carrot genotypes grown in a greenhouse trial evaluating difference in soil organic matter priming. *Letters indicate significant differences between genotypes in the Tukey test pairwise comparison (alpha value = 0.05).

2.3.9 $\delta^{15}N$ from labeled corn material in carrot roots and shoots

There were no statistically significant differences in total $\%N\ FR$ in total carrot biomass (*roots* + *shoots*) derived from the labeled corn material between the genotypes when the roots and shoots were combined (Figure 2.10; Table 2.15) and carrot shoots and roots separately (Figure 2.11; Table 2.15). There were no statistically significant differences in inorganic N made available from organic sources during the experiment (Figure 2.12, Table 2.16).

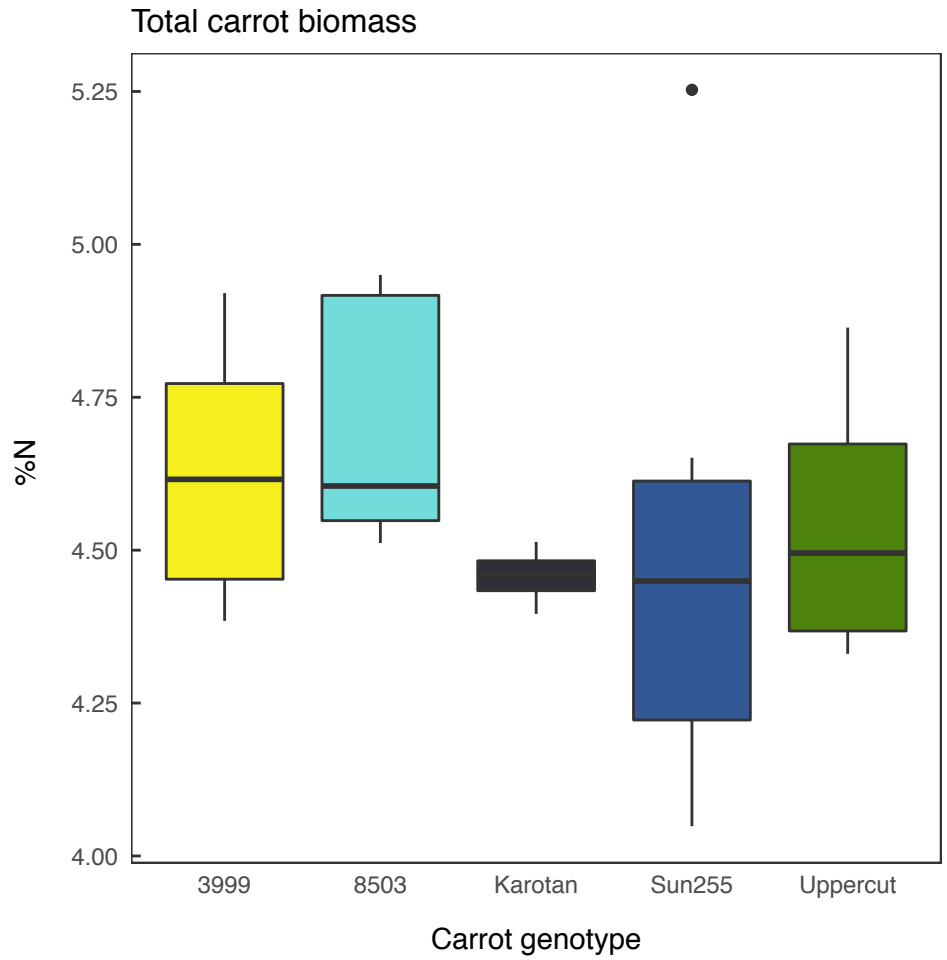

Figure 2.10: Total %N derived from residue in the plant %N FR (roots + shoots). Points indicate outliers for each of the five genotypes.

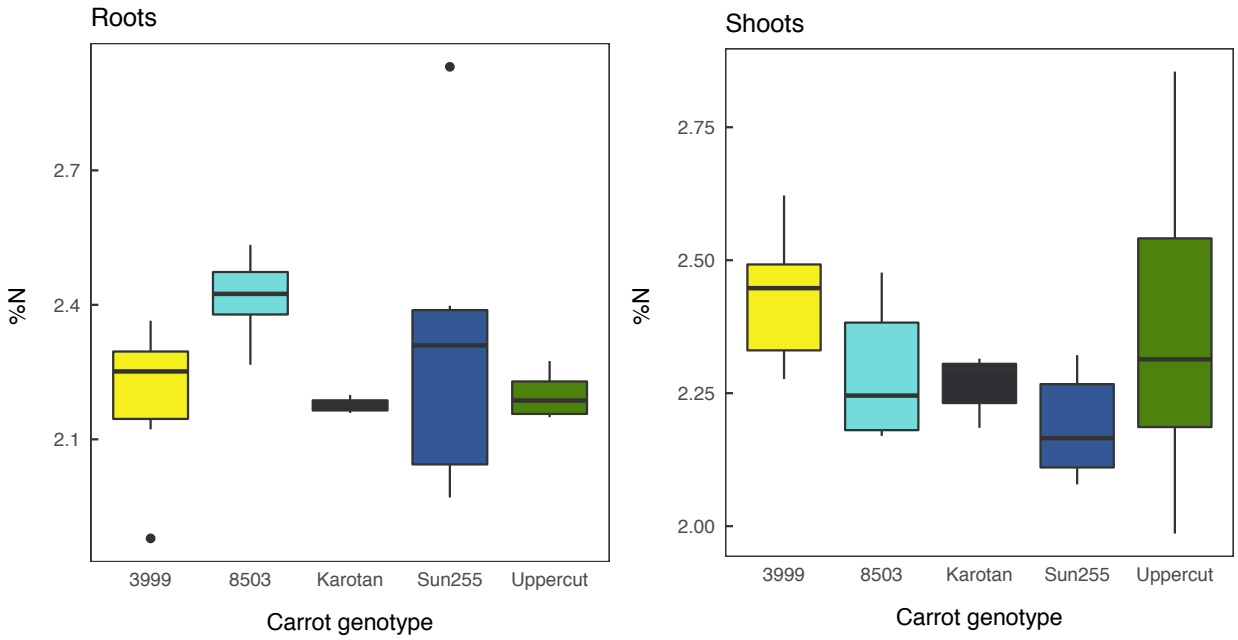

Figure 2.11: Roots and shoots %N derived from corn residue (%N FR) Points indicate outliers for each of the five genotype

Table 2.15: One-way ANOVA results for %N FR in total carrot biomass and roots and shoots separately

tissue		sum sq	df	F value	pr(>F)
total	genotype	0.698	4	1.076	0.389
	residuals	4.052	25		
roots	genotype	0.279	4	1.694	0.187
	residuals	0.907	22		
shoots	genotype	0.215	4	1.805	0.160
	residuals	0.713	24		

alpha value = 0.05

Table 2.16: One-way ANOVA results for inorganic N made available during the experiment in soils of the five genotypes evaluated.

	sum sq	df	F value	pr(>F)
genotype	29630	4	0.48	0.750
residuals	370403	24		

alpha value = 0.05

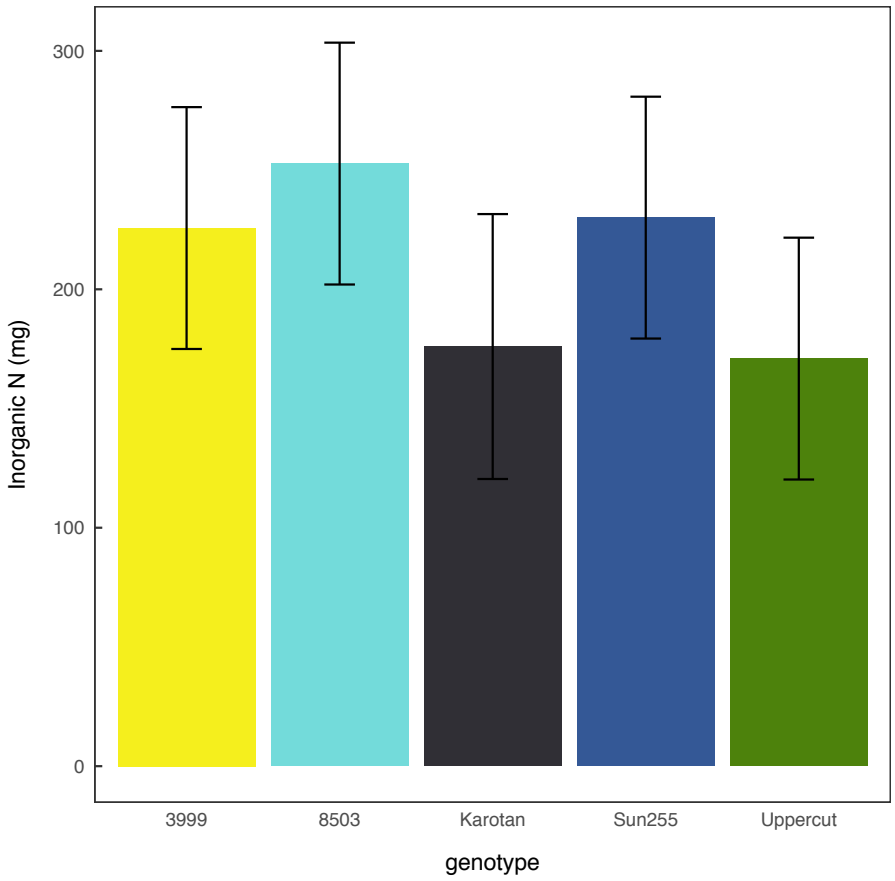

Figure 2.12: Inorganic N made available during the in soils of the five genotypes evaluated

2.4 Discussion

Identifying crop varieties with potential to promote positive priming of organic materials in soil is critical for optimizing productivity in organic farming systems. This also has potential to reduce N losses in conventional farming systems, as it could help growers rely more on alternative fertility sources such as cover crops to help supplement N needs. As an N scavenging crop, carrots are an ideal model crop to investigate genetic differences in priming of soil organic materials. Top size is a phenotypic trait that is expected to be related to nitrogen use efficiency in carrots (Simon, 2019). Consequently, we grew five carrot genotypes previously found to differ it top size, in pots amended with ^{15}N enriched corn residue. During the course of the greenhouse experiment, we monitored changes in soil ß-glucosidase activity and concentrations of NH_4^+-N and NO_3^--N at three time points to quantify differences in the potential for the carrot genotypes to stimulate positive priming of organic materials in soil. Soil β-glucosidase is an extracellular enzyme assay

that has previoulsy been used to quantify the effects of rhizosphere priming activity (Chang et al., 2007). For example, Zhu et al., (2014) found that soil enzyme oxidative activity increased by 19-56% in the presence of living roots, and they found a positive relationships between gross N mineralization rates and oxidative enzyme activity, indicating that rhizosphere priming can play a role in N acquisition.

Not surprisingly in our study at 6 weeks after seeding, when carrot plants were still very small, there were few differences in ß-glucosidase activity and no differences in NH_4^+-N among the five carrot genotypes (Figs. 2.1 and 2.2; Table 2.4 and 2.6). However, genotype Sun255 did have greater ß-glucosidase activity than 3999, indicating that Sun255 could have been releasing greater concentrations of root exudates, or stimulating the soil microbial community in some other way to enhance oxidative enzymatic activity. Since most genotypes had significantly lower NO_3^--N concentrations than the unplanted control treatment (Figure 2.3; Table 2.8), we conclude that carrots prefer NO_3^- during early stages of development, which is consistent with studies conducted using other plant species (Dechorgnat et al., 2011). There were differences in NO_3^--N concentrations at six weeks after planting (Figure 2.3; Table 2.8). In particular, Karotan had significantly greater NO_3^--N concentrations than 3999, indicating that this genotype is very slow to establish. Consequently, early applications of mineral fertilizers with Karotan could be subject to greater environmental loss.

As the experiment progressed, differences in ß-glucosidase activity and plant available N among the five carrot genotypes become much more dramatic (Figs. 2.1-2.3; Tables 2.4, 2.6 and 2.8), providing evidence that there are varietal differences in potential to scavenge N via priming processes. For example, at 10 weeks after seeding, carrot genotypes 3999, 8503 and Uppercut all had greater ß-glucosidase activity than the other genotypes, indicating that they were better at enhancing SOM decomposition. These soils also had lower levels of both NH_4^+-N and NO_3^--N indicating that they were actively scavenging this plant available N as it was being released. Since plants also have potential to stimulate nitrification activity (Rudisill et al., 2016), it is also possible that 3999, 8503 and Uppercut were also promoting this process to obtain greater concentrations of NO_3^--N, as this form of N is generally preferred by plants. Because ß-glucosidase activity in Karotan and Sun255 was low and Karotan had high NH_4^+-N at 10 weeks after seeding, we suspect that this genotypes are slower growing, and are not as capable of stimulating priming processes to

obtain N from organic materials. Karotan also had higher NO_3^--N concentration than the other genotypes at this time, indicating that this genotype was still growing very slowly.

By harvest, enzymatic activity and N concentrations among the five carrot genotypes had changed. Karotan, which had been very slow to establish, had the highest level of ß-glucosidase activity (Figure 2.1) and high levels of NH_4^+-N and NO_3^--N (Figure 2.2) indicating that this genotype was now promoting priming processes. Genotypes 8503. 3999, Sun255 and Uppercut all had low levels of NO_3^--N, indicating that they were also still actively taking up nutrients.

To determine the extent to which the five carrot genotypes could indeed obtain N from decomposing organic materials in soil, we quantified total plant biomass, concentration of total C and N in plant biomass, and calculated the C:N ratio and $\delta^{15}N$ isotopic composition in the plant biomass. Plant C and N content have previously been shown to be good predictors of how adequate the N supply is in terms of N uptake efficiency by roots, and CO_2 assimilation by leaves during photosynthesis (Lawlor, 2002). The C:N ratio in plant tissue also tends to vary given the amount of nutrients available in soil, making it a good predictor of N acquisition capability as well (Theobald et al., 1998). Finally, isotopic labeling is one of the most accurate ways to determine where plants obtained N from. In this study, soils were amended with a ^{15}N enriched corn material that had an initial $\delta^{15}N$ composition of 603‰. Because corn residues have a very high C:N ratio, we expected that this residue would be difficult to decompose and thus would serve as a good material to quantify priming by the carrot genotypes. Because the soils used in this experiment were very low in SOM and fertility, we also added a small amount of feather meal organic fertilizer, with had an initial $\delta^{15}N$ composition of 3.9‰. Because feather meal has a low C:N, we expected this material would mineralize relatively quickly providing plants with some N to survive, but would still allow us to quantify decomposition and uptake of the corn residue. Previous studies in carrots have demonstrated that the $\delta^{15}N$ isotope composition of the crop can be influenced by whether organic or inorganic fertilizers are applied. For example, Bateman et al. (2007) found than conventional grown carrots (−1.3‰) were isotopically lighter than organic carrots (+5.4‰) In our study, isotopic values in carrot biomass ranged from between 13.19 and 15.08 ‰, indicating that the carrot plants did obtain N from the corn residue used to quantify priming responses in this study.

Results of the plant assays were consistent with the soil assays, providing further support that there are genetic differences in the potential for carrot plants to stimulate organic matter

priming to obtain N. The five carrot genotypes had similar %C contents with a maximum difference of only 1% in roots and shoots (Table 2.14). However, there were significant differences in %N in carrot shoots as well as C:N ratio among the genotypes (Table 2.13; Figure 2.9), indicating that some genotypes were more efficient and acquiring N. Moreover, some genotypes had greater %N derived from corn residue in carrot biomass (Figs. 2.10 and 2.11), providing evidence that there were differences in the potential for these carrot genotypes to stimulate the decomposition of organic materials in soil to obtain N.

Interestingly, the experimental carrot genotypes 8503 and 3999 appeared to have the greatest potential to acquire N by enhancing decomposition of organic materials in soil. In addition to having greater levels of soil ß-glucosidase activity after 10 weeks of planting (Figure 2.1; Table 2.4), these genotypes also both had higher %N and lower C:N ratios in their shoot biomass (Table 2.13; Figure 2.9). Moreover, 8503 also had higher relative values of $\delta^{15}N$ in its roots than the other genotypes, and 3999 had greater relative values of $\delta^{15}N$ in its shoots (Table 2.13), providing evidence that they were able to scavenge more of the ^{15}N tied up in the organic materials in soil. The reason that 8503 had the greatest values of $\delta^{15}N$ in its roots is unclear, though it is possible that this genotype matured earlier than the other genotypes, and it had begun to translocate resources into its roots in preparation for senescence. The significance and implications for greater acquisition and storage of N compounds in 8503 and 3999 is also unclear, though it is possible that this could help these genotypes have greater productivity, particularly during times of stress. For example, greater concentrations of N can lead to greater chlorophyll activity, which allows plants to have a higher investment in photosynthetic machinery for light reactions, cell growth and division in leaves, and production of proteins such as RuBisCo (Bassi et al., 2018). When inorganic forms of N such as NO_3^- in soil are plentiful, more N tends to be stored in plant tissues as amino acids or proteins that are "metabolically inactive" (Funk et al., 2013). Stored N can be a strategy plants use to face asynchrony in N supply and demand during later life stages, allowing them to have more efficient utilization of N when it becomes limited (Liu et al., 2018).

The experimental carrot genotypes 8503 and 3999 also had lower shoot dry weight and a higher R:S ratio than the other genotypes (Figure 2.8; Table 2.13), and specifically 8503 had the highest inorganic N made available during the experiment (Figure 2.12), which is consistent with the results of previous studies quantifying differences in top size between these genotypes (Simon, 2019). This indicates that carrots with a greater investment in belowground structures could be

better at scavenging N from organic materials in soil. The same effect was detected in wheat (*Triticum aestivum.* L), were genotypes with greater investment in belowground structures responded more positively to compost additions, with greater N uptake and plasticity in root morphology (Junaidi et al., 2018). A higher investment in root biomass could also allow for greater exudation rates of low molecular weight substrates which are critical for positive rhizosphere priming in soils (Pausch et al., 2016). However, the carrot genotype Uppercut, also had higher soil ß-glucosidase activity (Figure 2.1) and high values of $\delta^{15}N$ in its shoot biomass (Table 2.14), but made lower inorganic N available than 3999 and 8503 (Figure 2.12), so it is unclear how much investment in above vs. below ground structures plays a role in positive priming of soil organic matter in carrot.

Genetic variation in the potential for plants to respond to N deficiencies in soil by promoting priming of organic materials has been observed in other crops. For example, Tiemens-Hulscher et al. (2014) observed differences in the potential for potato (*Solanum tuberosum*) cultivars to cope with low quantities of plant available N in organic farming systems. Again, the reason for this is unclear, though it is possible that some plant genotypes might promote negative priming, which prevents them from being able to scavenge N from organic materials. During, negative priming, plants and microbes compete for soil resources such as N, which decreases microbial growth and residue mineralization (Cheng, 2009; Huo et al., 2017). Consequently, the carrot genotypes Sun255 and Karotan may have been less competitive for the limited amount of soil N that was available in this experiment.

The second objective of this trial was to identify microbial taxa that are stimulated by carrot roots and could play a role in nutrient scavenging as well as other important functions in carrot production systems. Not surprisingly, soil microbial diversity was higher in the unplanted control soil than soils planted with the five carrot genotypes (Figure 2.5). The composition of rhizosphere microbial communities often represents a subset of those in bulk soil, as only a subset of the soil microbial community can respond to and effectively compete for the labile carbon compounds released in root exudates. For example, Hargreaves et al. (2015) observed distinct differences in microbial communities inhabiting bulk soil and the rhizosphere of switchgrass (*Panicum virgatum*). Moreover, they observed greater abundances of bacteria that are known to be active as cellulose and chitin degraders in the switchgrass rhizosphere, indicating that the plants could have been promoting positive priming of organic materials to obtain nutrients by enhancing the population

of these taxa. Results of a study conducted by Turner et al. (2013) provide support for this hypothesis by demonstrating distinct differences in rhizosphere community structure between pea (*Pisum sativum*) and wheat plants, have greater concentrations of bacteria with cellulolytic activity in the rhizospheres of wheat plants which cannot fix atmospheric N like pea .

Interestingly, there were distinct differences in bacterial community structure among the carrot genotypes in this trial, with 3999 and 8503 clustering apart from Uppercut, Sun255 and Karotan (Figure 2.6; Table 2.10). Consequently, it is possible that some of the distinct microbial assemblages in 3999 and 8503 played a role in helping these plants acquire N. For example, many isolates of *Rhodospirillaceae*, which were enriched in 3999, can fix atmospheric N (Madigan et al., 1984). At the same time, unique microbial community assemblages observed among the different carrot genotypes could have been due to their potential to withstand nutrient shortages, and played a role in negative priming of the corn residue in this trial. For example, in a previous study investigating relationships between rhizosphere bacterial community structure and N availability, only a subset of microbial taxa were able to tolerate low soil nutrients and persist in the rhizosphere until harvest (Bell et al., 2015). In this study *Opitutaceae*, were enriched in Karotan, Sun255 and Uppercut. These bacteria belong to the phylum Verrucomicrobia, which have been noted to be negatively correlated with soil fertility (Navarrete et al., 2015).

Another factor that could be responsible for the distinct microbial community assemblages observed among the carrot genotypes in this trial is their resistance and susceptibility against pathogenic nematodes. Several studies have identified distinct microbiomes associated with resistant and susceptible cucumber and tomato genotypes (Kwak et al., 2018; Upreti & Thomas, 2015; Yao & Wu, 2010), and we recently discovered this phenomenon in carrots (Abdelrazek et al., in review). The exact cause of the resistance in 3999 and 8503 is not yet clear, but it is possible that these genotypes are able to sense the presence of the nematode at the root surface and strengthen their cell walls to resist invasion. In contrast, Sun255, Karotan and Uppercut may need to mount alternative strategies to resist attack by pathogenic nematodes such as by recruiting antagonistic microbes. Interestingly, taxa belonging to the *Oxalobacteriaceae,* (Cretoiu et al., 2013) and *Micrococcaceae* (van der Voort et al., 2016) which were enriched in these three genotypes, have been observed to be present in elevated concentrations in disease suppressive soils. Consequently, it is possible that these microbes could play a role in pathogen suppression, and this should be explored in future studies

2.5 Conclusions

Carrot genotypes differ in their potential to stimulate enzymatic activity in soil and scavenge nitrogen from organic materials indicating evidence for possible positive and negative priming effects. The experimental genotypes 3999 and 8503 can potentially perform better than other carrot genotypes when supplied with organic forms of fertilizers, and thus may be better suited for organic and low-input production systems. In contrast, Karotan is very slow to develop, and planting this variety could result in early season N loss.

Planting carrots does alter soil microbial communities and carrot genotypes differ in the types of microbial assemblages they promote in soil. The consequences of these differences are not yet clear, though this could play a role in nutrient acquisition and/or pathogen suppression and should be further explored.

2.6 References

Abdelrazek, S. (n.d.). Crop management system and carrot genotype affect endophyte composition and Alternaria dauci suppression. *PLoS ONE*.

Abdelrazek, S. (2018). *Carrot endophytes: Diversity, ecology and function*. Purdue University.

Ahmad, T., Cawood, M., Iqbal, Q., & Batool, A. (2017). Phytochemicals in Daucus carota and their importance in nutrition – Review article, 1–22. https://doi.org/10.7287/peerj.preprints.3187v1

Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data.

Arenz, B. E., Schlatter, D. C., Bradeen, J. M., & Kinkel, L. L. (2015). Blocking primers reduce co-amplification of plant DNA when studying bacterial endophyte communities. *Journal of Microbiological Methods*, *117*, 1–3. https://doi.org/10.1016/j.mimet.2015.07.003

Arruda, P., Imperial, J., de Araújo, L. M., de Souza, R. S. C., Armanhi, J. S. L., & Damasceno, N. de B. (2018). A Community-Based Culture Collection for Targeting Novel Plant Growth-Promoting Bacteria from the Sugarcane Microbiome. *Frontiers in Plant Science*, *8*(January), 1–17. https://doi.org/10.3389/fpls.2017.02191

Bardgett, R. D., Mommer, L., & De Vries, F. T. (2014). Going underground: Root traits as drivers of ecosystem processes. *Trends in Ecology and Evolution*, *29*(12), 692–699. https://doi.org/10.1016/j.tree.2014.10.006

Baresel, J. P., Zimmermann, G., & Reents, H. J. (2008). Effects of genotype and environment on N uptake and N partition in organically grown winter wheat (Triticum aestivum L.) in Germany. *Euphytica*, *163*(3), 347–354. https://doi.org/10.1007/s10681-008-9718-1

Bassi, D., Menossi, M., & Mattiello, L. (2018). Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. *Scientific Reports*, *8*(1), 1–13. https://doi.org/10.1038/s41598-018-20653-1

Bateman, A. S., Kelly, S. D., & Woolfe, M. (2007). Nitrogen Isotope Composition of Organically and Conventionally Grown Crops. https://doi.org/10.1021/jf0627726

Beckers, B., Op De Beeck, M., Thijs, S., Truyens, S., Weyens, N., Boerjan, W., & Vangronsveld, J. (2016). Performance of 16s rDNA primer pairs in the study of rhizosphere and endosphere bacterial microbiomes in metabarcoding studies. *Frontiers in Microbiology*, *7*(MAY). https://doi.org/10.3389/fmicb.2016.00650

Bell, C. W., Asao, S., Calderon, F., Wolk, B., & Wallenstein, M. D. (2015). Plant nitrogen uptake drives rhizosphere bacterial community assembly during plant growth. *Soil Biology and Biochemistry*, *85*, 170–182. https://doi.org/10.1016/j.soilbio.2015.03.006

Bianco, A., Fancello, F., Balmas, V., Zara, G., Dettori, M., & Budroni, M. (2018). The microbiome of Sardinian barley and malt. *Journal of the Institute of Brewing*, *124*(4), 344–351. https://doi.org/10.1002/jib.522

Blagodatskaya, E., Littschwager, J., Lauerer, M., & Kuzyakov, Y. (2014). Plant traits regulating N capture define microbial competition in the rhizosphere. *European Journal of Soil Biology*, *61*, 41–48. https://doi.org/10.1016/j.ejsobi.2014.01.002

Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., & Kuzyakov, Y. (2010). Model of apparent and real priming effects: Linking microbial activity with soil organic matter decomposition. *Soil Biology and Biochemistry*, *42*(8), 1275–1283. https://doi.org/10.1016/j.soilbio.2010.04.005

Bodenhausen, N., Horton, M. W., & Bergelson, J. (2013). Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. *PLoS ONE*, *8*(2), e56329. https://doi.org/10.1371/journal.pone.0056329

Bowman, J. S., Rasmussen, S., Blom, N., Deming, J. W., Rysgaard, S., & Sicheritz-Ponten, T. (2012). Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. *ISME Journal*, *6*(1), 11–20. https://doi.org/10.1038/ismej.2011.76

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., … Schulze-Lefert, P. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, *488*(7409), 91–95. https://doi.org/10.1038/nature11336

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., … Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, *108*(Supplement_1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

Caporaso, J. Gregory, Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., … Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(SUPPL. 1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

Caporaso, J Gregory, Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). correspondence QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. *Nature Publishing Group*, *7*(5), 335–336. https://doi.org/10.1038/nmeth0510-335

Card, S., Johnson, L., Teasdale, S., & Caradus, J. (2016). Deciphering endophyte behaviour: The link between endophyte biology and efficacious biological control agents. *FEMS Microbiology Ecology*, *92*(8), 1–19. https://doi.org/10.1093/femsec/fiw114

Chang, E.-H., Chung, R.-S., & Tsai, Y.-H. (2007). Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. *Soil Science and Plant Nutrition*, *53*(2), 132–140. https://doi.org/10.1111/j.1747-0765.2007.00122.x

Chelius, M. K., & Triplett, E. W. (2001). The diversity of archaea and bacteria in association with the roots of Zea mays L. *Microbial Ecology*, *41*(3), 252–263. https://doi.org/10.1007/s002480000087

Cheng, W. (2009). Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. *Soil Biology and Biochemistry*, *41*(9), 1795–1801. https://doi.org/10.1016/j.soilbio.2008.04.018

Cheng, Z., Park, E., & Glick, B. R. (2007). 1-Aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Canadian Journal of Microbiology*, *53*(7), 912–918. https://doi.org/10.1139/W07-050

Compant, S., Clément, C., & Sessitsch, A. (2010, May 1). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*. Pergamon. https://doi.org/10.1016/j.soilbio.2009.11.024

Coskun, D., Britto, D. T., Shi, W., & Kronzucker, H. J. (2017). How Plant Root Exudates Shape the Nitrogen Cycle. *Trends in Plant Science*, *22*(8), 661–673. https://doi.org/10.1016/j.tplants.2017.05.004

Cretoiu, M. S., Korthals, G. W., Visser, J. H. M., & Van Elsas, J. D. (2013). Chitin amendment increases soil suppressiveness toward plant pathogens and modulates the actinobacterial and oxalobacteraceal communities in an experimental agricultural field. *Applied and Environmental Microbiology*, *79*(17), 5291–5301. https://doi.org/10.1128/AEM.01361-13

Curtis, M. A., Zenobia, C., & Darveau, R. P. (2011, October 4). The relationship of the oral microbiotia to periodontal health and disease. *Cell Host and Microbe*. NIH Public Access. https://doi.org/10.1016/j.chom.2011.09.008

da Silva Dias, J. C. (2014). Nutritional and Health Benefits of Carrots and Their Seed Extracts. *Food and Nutrition Sciences*, *05*(22), 2147–2156. https://doi.org/10.4236/fns.2014.522227

de Vries, F. T., & Wallenstein, M. D. (2017, July 1). Below-ground connections underlying above-ground food production: a framework for optimising ecological connections in the rhizosphere. (R. Bardgett, Ed.), *Journal of Ecology*. Blackwell Publishing Ltd. https://doi.org/10.1111/1365-2745.12783

Dechorgnat, J., Nguyen, C. T., Armengaud, P., Jossier, M., Diatloff, E., Filleur, S., & Daniel-Vedele, F. (2011). From the soil to the seeds: The long journey of nitrate in plants. *Journal of Experimental Botany*, *62*(4), 1349–1359. https://doi.org/10.1093/jxb/erq409

Dijkstra, F. A., Carrillo, Y., Pendall, E., & Morgan, J. A. (2013). Rhizosphere priming: A nutrient perspective. *Frontiers in Microbiology*, *4*(JUL), 1–8. https://doi.org/10.3389/fmicb.2013.00216

Dijkstra, F. A., Morgan, J. A., Blumenthal, D., & Follett, R. F. (2010). Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. *Soil Biology and Biochemistry*, *42*(7), 1073–1082. https://doi.org/10.1016/j.soilbio.2010.02.026

Dyall, S. D., Brown, M. T., & Johnson, P. J. (2004). Ancient Invasions: From Endosymbionts to Organelles. *Science*, *304*(5668), 253–257. https://doi.org/10.1126/science.1094884

Egea, I., Barsan, C., Bian, W., Purgatto, E., Latché, A., Chervin, C., … Pech, J. C. (2010). Chromoplast differentiation: Current status and perspectives. *Plant and Cell Physiology*, *51*(10), 1601–1611. https://doi.org/10.1093/pcp/pcq136

Eida, A. A., Ziegler, M., Lafi, F. F., Michell, C. T., Voolstra, C. R., Hirt, H., & Saad, M. M. (2018). Desert plant bacteria reveal host influence and beneficial plant growth properties. *Plos One*, *13*(12), e0208223. https://doi.org/10.1371/journal.pone.0208223

Eivazi, F., & Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, *20*(5), 601–606. https://doi.org/10.1016/0038-0717(88)90141-1

Fageria, N. K., & Baligar, V. C. (2005, January 1). Enhancing Nitrogen Use Efficiency in Crop Plants. *Advances in Agronomy*. Academic Press. https://doi.org/10.1016/S0065-2113(05)88004-6

FAO. (2018). Food and Agriculture Organization of the United Nations Statistics database. Retrieved February 10, 2020, from http://www.fao.org/faostat/en/#data/QC/visualize

Finzi, A. C., Abramoff, R. Z., Spiller, K. S., Brzostek, E. R., Darby, B. A., Kramer, M. A., & Phillips, R. P. (2015). Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Global Change Biology*, *21*(5), 2082–2094. https://doi.org/10.1111/gcb.12816

Fitzpatrick, C. R., Copeland, J., Wang, P. W., Guttman, D. S., Kotanen, P. M., & Johnson, M. T. J. (2018). Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences*, 201717617. https://doi.org/10.1073/pnas.1717617115

Frank, A., Saldierna Guzmán, J., & Shay, J. (2017). Transmission of Bacterial Endophytes. *Microorganisms*, *5*(4), 70. https://doi.org/10.3390/microorganisms5040070

Funk, J. L., Glenwinkel, L. A., & Sack, L. (2013). Differential Allocation to Photosynthetic and Non-Photosynthetic Nitrogen Fractions among Native and Invasive Species. *PLoS ONE*, *8*(5). https://doi.org/10.1371/journal.pone.0064502

Gallart, M., Adair, K. L., Love, J., Meason, D. F., Clinton, P. W., Xue, J., & Turnbull, M. H. (2018). Host Genotype and Nitrogen Form Shape the Root Microbiome of Pinus radiata. *Microbial Ecology*, *75*(2), 419–433. https://doi.org/10.1007/s00248-017-1055-2

Garnett, T., Conn, v., & Kaiser, b. n. (2009). Root based approaches to improving nitrogen use efficiency in plants. *Plant, Cell & Environment*, *32*(9), 1272–1283. https://doi.org/10.1111/j.1365-3040.2009.02011.x

Ghyselinck, J., Pfeiffer, S., Heylen, K., Sessitsch, A., & De Vos, P. (2013). The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies. *PloS One*, *8*(8), 1–14. https://doi.org/10.1371/journal.pone.0071360

Giagnoni, L., Pastorelli, R., Mocali, S., Arenella, M., Nannipieri, P., & Renella, G. (2016). Availability of different nitrogen forms changes the microbial communities and enzyme activities in the rhizosphere of maize lines with different nitrogen use efficiency. *Applied Soil Ecology*, *98*, 30–38. https://doi.org/10.1016/j.apsoil.2015.09.004

Gilbert, J. A., Jansson, J. K., & Knight, R. (2014, August 22). The Earth Microbiome project: Successes and aspirations. *BMC Biology*. BioMed Central Ltd. https://doi.org/10.1186/s12915-014-0069-1

Grime, J. P. (1977). Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. *The American Naturalist*, *111*(982), 1169–1194. https://doi.org/10.1086/283244

Hadas, A., Kautsky, L., Goek, M., & Kara, E. E. (2004). Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. *Soil Biology and Biochemistry*, *36*(2), 255–266. https://doi.org/10.1016/j.soilbio.2003.09.012

Haichar, F. el Z., Santaella, C., Heulin, T., & Achouak, W. (2014). Root exudates mediated interactions belowground. *Soil Biology and Biochemistry*, *77*, 69–80. https://doi.org/10.1016/j.soilbio.2014.06.017

Hanshew, A. S., Mason, C. J., Raffa, K. F., & Currie, C. R. (2013). Minimization of chloroplast contamination in 16S rRNA gene pyrosequencing of insect herbivore bacterial communities. *Journal of Microbiological Methods*, *95*(2), 149–155. https://doi.org/10.1016/j.mimet.2013.08.007

Hansmann, P. (1987). Daffodil Chromoplast DNA : Comparison with Chloroplast DNA , Physical Map and Gene Localization, *122*(1987), 118–122.

Hardoim, P. R., van Overbeek, L. S., & Elsas, J. D. van. (2008). Properties of bacterial endophytes and their proposed role in plant growth. *Trends in Microbiology*, *16*(10), 463–471. https://doi.org/10.1016/j.tim.2008.07.008

Hargreaves, S. K., Williams, R. J., & Hofmockel, K. S. (2015). Environmental filtering of microbial communities in agricultural soil shifts with crop growth. *PLoS ONE*, *10*(7), e0134345. https://doi.org/10.1371/journal.pone.0134345

Hartz, T. K., & Johnstone, P. R. (2006). Nitrogen availability from high-nitrogen-containing organic fertilizers. *HortTechnology*, *16*(1), 39–42. https://doi.org/10.21273/horttech.16.1.0039

Heijboer, A., ten Berge, H. F. M., de Ruiter, P. C., Jørgensen, H. B., Kowalchuk, G. A., & Bloem, J. (2016). Plant biomass, soil microbial community structure and nitrogen cycling under different organic amendment regimes; a 15N tracer-based approach. *Applied Soil Ecology*, *107*, 251–260. https://doi.org/10.1016/j.apsoil.2016.06.009

Hirel, B., Tétu, T., Lea, P. J., & Dubois, F. (2011). Improving nitrogen use efficiency in crops for sustainable agriculture. *Sustainability*, *3*(9), 1452–1485. https://doi.org/10.3390/su3091452

Huo, C., Luo, Y., & Cheng, W. (2017). Rhizosphere priming effect: A meta-analysis. *Soil Biology and Biochemistry*, *111*, 78–84. https://doi.org/10.1016/j.soilbio.2017.04.003

Junaidi, J., Kallenbach, C. M., Byrne, P. F., & Fonte, S. J. (2018). Root traits and root biomass allocation impact how wheat genotypes respond to organic amendments and earthworms. *PLoS ONE*, *13*(7), 1–24. https://doi.org/10.1371/journal.pone.0200646

Kant, S. (2017). Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. *Seminars in Cell and Developmental Biology*, *74*, 89–96. https://doi.org/10.1016/j.semcdb.2017.08.034

Kim, G. S., Seok, J. H., Mark, T. B., & Reed, M. R. (2019). The price relationship between organic and non-organic vegetables in the U.S.: evidence from Nielsen scanner data. *Applied Economics*, *51*(10), 1025–1039. https://doi.org/10.1080/00036846.2018.1524570

Köberl, M., Schmidt, R., Ramadan, E. M., Bauer, R., & Berg, G. (2013). The microbiome of medicinal plants: Diversity and importance for plant growth, quality, and health. *Frontiers in Microbiology*, *4*(DEC), 1–9. https://doi.org/10.3389/fmicb.2013.00400

Kõiv, V., Arbo, K., Maiväli, Ü., Kisand, V., Roosaare, M., Remm, M., & Tenson, T. (2019). Endophytic bacterial communities in peels and pulp of five root vegetables. *PLoS ONE*, *14*(1), 1–17. https://doi.org/10.1371/journal.pone.0210542

Krueger, F. (2015). Wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files. *The Babraham Institute, Bioinformatics Group*.

Ku, S., Ximenes, E., Kreke, T., Foster, K., Deering, A. J., & Ladisch, M. R. (2016). Microfiltration of enzyme treated egg whites for accelerated detection of viable Salmonella. *Biotechnology Progress*, *32*(6), 1464–1471. https://doi.org/10.1002/btpr.2343

Kuzyakov, Y. (2002). Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition & Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, *165*(4), 382–396. https://doi.org/10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2-#

Kwak, M.-J., Kong, H. G., Choi, K., Kwon, S.-K., Song, J. Y., Lee, J., … Kim, J. F. (2018). Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nature Biotechnology*, *36*(11). https://doi.org/10.1038/nbt.4232

Laberge, G., Ambus, P., Hauggaard-Nielsen, H., & Jensen, E. S. (2006). Stabilization and plant uptake of N from 15N-labelled pea residue 16.5 years after incorporation in soil. *Soil Biology and Biochemistry*, *38*(7), 1998–2000. https://doi.org/10.1016/j.soilbio.2005.11.023

Lammerts van Bueren, E. T., & Struik, P. C. (2017). Diverse concepts of breeding for nitrogen use efficiency. A review. *Agronomy for Sustainable Development*, *37*(5). https://doi.org/10.1007/s13593-017-0457-3

Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: Mechanisms are the key to understanding production systems. In *Journal of Experimental Botany* (Vol. 53, pp. 773–787). Oxford University Press. https://doi.org/10.1093/jxb/53.370.773

Liu, L., Liang, M., Li, L., Sun, L., Xu, Y., Gao, J., … Huang, S. (2018). Synergistic effects of the combined application of Bacillus subtilis H158 and strobilurins for rice sheath blight control. *Biological Control*, *117*(August 2017), 182–187. https://doi.org/10.1016/j.biocontrol.2017.11.011

Liu, T., Ren, T., White, P. J., Cong, R., & Lu, J. (2018). Storage nitrogen co-ordinates leaf expansion and photosynthetic capacity in winter oilseed rape. *Journal of Experimental Botany*, *69*(12), 2995–3007. https://doi.org/10.1093/jxb/ery134

Liu, X., Vibbert, H. B., Foster, K., Ladisch, M. R., Amalaradjou, M. A. R., Jones, J., … Li, X. (2013). Rapid Sample Processing for Detection of Food-Borne Pathogens via Cross-Flow Microfiltration. *Applied and Environmental Microbiology*, *79*(22), 7048–7054. https://doi.org/10.1128/aem.02587-13

Ljiljana Boskovic-Rakocevic. (2012). Effect of nitrogen fertilization on carrot quality. *African Journal of Agricultural Reseearch*, *7*(18), 2884–2890. https://doi.org/10.5897/ajar11.1652

Louarn, S., Nawrocki, A., Thorup-Kristensen, K., Lund, O. S., Jensen, O. N., Collinge, D. B., & Jensen, B. (2013). Proteomic changes and endophytic micromycota during storage of organically and conventionally grown carrots. *Postharvest Biology and Technology*, *76*, 26–33. https://doi.org/10.1016/j.postharvbio.2012.08.011

Lucier, G., & Lin, B. H. (2011). Factors affecting carrot consumption in the United States. *Eating Right: The Consumption of Fruits and Vegetables*, 199–220.

Lundberg, D. S., Yourstone, S., Mieczkowski, P., Jones, C. D., & Dangl, J. L. (2013). Practical innovations for high-throughput amplicon sequencing. *Nature Methods*, *10*(10), 999–1002. https://doi.org/10.1038/nmeth.2634

Madigan, M., Cox, S. S., & Stegeman, R. A. (1984). Nitrogen fixation and nitrogenase activities in members of the family Rhodospirillaceae. *Journal of Bacteriology*, *157*(1), 73–78. https://doi.org/10.1128/jb.157.1.73-78.1984

Malfanova, N., Lugtenberg, B. J. J., & Berg, G. (2013). Bacterial Endophytes: Who and Where, and What are they doing there? *Molecular Microbial Ecology of the Rhizosphere*, *1*, 391–403. https://doi.org/10.1002/9781118297674.ch36

Meena, V. S., Meena, S. K., Verma, J. P., Kumar, A., Aeron, A., Mishra, P. K., … Dotaniya, M. L. (2017). Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: A review. *Ecological Engineering*, *107*, 8–32. https://doi.org/10.1016/j.ecoleng.2017.06.058

Meier, I. C., Finzi, A. C., & Phillips, R. P. (2017). Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. *Soil Biology and Biochemistry*, *106*, 119–128. https://doi.org/10.1016/j.soilbio.2016.12.004

Menneer, J. C., Sprosen, M. S., & Ledgard, S. F. (2016). Effect of timing and formulation of dicyandiamide (DCD) application on nitrate leaching and pasture production in a Bay of Plenty pastoral soil, *8233*(October). https://doi.org/10.1080/00288230809510468

Mikkelsen, R., & Hartz, T. K. (2008). Nitrogen Sources for Organic Crop Production. Better Crops. Vol. 92, No. 4. *Better Crops*, *92*(4), 16–19.

Moreau, D., Bardgett, R. D., Finlay, R. D., Jones, D. L., & Philippot, L. (2019). A plant perspective on nitrogen cycling in the rhizosphere. *Functional Ecology*, *33*(4), 540–552. https://doi.org/10.1111/1365-2435.13303

Murphy, C. J., Baggs, E. M., Morley, N., Wall, D. P., & Paterson, E. (2015). Rhizosphere priming can promote mobilisation of N-rich compounds from soil organic matter. *Soil Biology and Biochemistry*, *81*, 236–243. https://doi.org/10.1016/j.soilbio.2014.11.027

Musyoka, M. W., Adamtey, N., Muriuki, A. W., Bautze, D., Karanja, E. N., Mucheru-Muna, M., … Cadisch, G. (2019). Nitrogen leaching losses and balances in conventional and organic farming systems in Kenya. *Nutrient Cycling in Agroecosystems*, *114*(3), 237–260. https://doi.org/10.1007/s10705-019-10002-7

Muyzer, G., De Waal,' And, E. C., & Uitierlinden2, A. G. (1993). *Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16S rRNA Downloaded from*. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*. Retrieved from http://aem.asm.org/

Mwafulirwa, L., Baggs, E. M., Russell, J., George, T., Morley, N., Sim, A., … Paterson, E. (2016). Barley genotype influences stabilization of rhizodeposition-derived C and soil organic matter mineralization. *Soil Biology and Biochemistry*, *95*(April), 60–69. https://doi.org/10.1016/j.soilbio.2015.12.011

Mwafulirwa, L. D., Baggs, E. M., Russell, J., Morley, N., Sim, A., & Paterson, E. (2017). Combined effects of rhizodeposit C and crop residues on SOM priming, residue mineralization and N supply in soil. *Soil Biology and Biochemistry*, *113*, 35–44. https://doi.org/10.1016/j.soilbio.2017.05.026

Navarrete, A. A., Soares, T., Rossetto, R., van Veen, J. A., Tsai, S. M., & Kuramae, E. E. (2015). Verrucomicrobial community structure and abundance as indicators for changes in chemical factors linked to soil fertility. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *108*(3), 741–752. https://doi.org/10.1007/s10482-015-0530-3

Navazio, J. (2014). How to Breed Carrots for Organic Agriculture. *Organic Seed Alliance*.

Nithya, A., & Babu, S. (2017). Prevalence of plant beneficial and human pathogenic bacteria isolated from salad vegetables in India. *BMC Microbiology*, *17*(1), 1–16. https://doi.org/10.1186/s12866-017-0974-x

Oksanen, J. (2007). vegan : Community Ecology Package. R package version 1.8-5.

Pajares, S., & Bohannan, B. J. M. (2016). Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. *Frontiers in Microbiology*, *7*(JUL), 1–20. https://doi.org/10.3389/fmicb.2016.01045

Pausch, J., Loeppmann, S., Kï¿½hnel, A., Forbush, K., Kuzyakov, Y., & Cheng, W. (2016). Rhizosphere priming of barley with and without root hairs. *Soil Biology and Biochemistry*, *100*, 74–82. https://doi.org/10.1016/j.soilbio.2016.05.009

Pausch, J., Zhu, B., Kuzyakov, Y., & Cheng, W. (2013). Plant inter-species effects on rhizosphere priming of soil organic matter decomposition. *Soil Biology and Biochemistry*, *57*, 91–99. https://doi.org/10.1016/j.soilbio.2012.08.029

Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., … Ley, R. E. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(16), 6548–6553. https://doi.org/10.1073/pnas.1302837110

Poirier, V., Roumet, C., & Munson, A. D. (2018). The root of the matter: Linking root traits and soil organic matter stabilization processes. *Soil Biology and Biochemistry*, *120*(August 2017), 246–259. https://doi.org/10.1016/j.soilbio.2018.02.016

Prieto, K. R., Echaide-Aquino, F., Huerta-Robles, A., Valério, H. P., Macedo-Raygoza, G., Prado, F. M., … Beltran-García, M. J. (2017). Endophytic bacteria and rare earth elements; promising candidates for nutrient use efficiency in plants. In *Plant Macronutrient Use Efficiency: Molecular and Genomic Perspectives in Crop Plants* (pp. 285–306). Elsevier Inc. https://doi.org/10.1016/B978-0-12-811308-0.00016-8

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Rg Peplies, J., & Glö Ckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, *35*(21), 7188–7196. https://doi.org/10.1093/nar/gkm864

R Core Team. (2019). No Title.

Radhakrishnan, R., Hashem, A., & Abd Allah, E. F. (2017). Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, *8*(SEP), 667. https://doi.org/10.3389/fphys.2017.00667

Rakotoson, T., Dusserre, J., Letourmy, P., Ramonta, I. R., Cao, T. V., Ramanantsoanirina, A., … Raboin, L. M. (2017). Genetic variability of nitrogen use efficiency in rainfed upland rice. *Field Crops Research*, *213*(May), 194–203. https://doi.org/10.1016/j.fcr.2017.07.023

Reasoner, D. J., & Geldreich, E. E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Applied and Environmental Microbiology*, *49*(1), 1–7. https://doi.org/10.1128/aem.49.1.1-7.1985

Reeve, W., Ardley, J., Tian, R., Eshragi, L., Yoon, J. W., Ngamwisetkun, P., … Kyrpides, N. C. (2015, February 1). A Genomic Encyclopedia of the root nodule bacteria: Assessing genetic diversity through a systematic biogeographic survey. *Standards in Genomic Sciences*. BioMed Central Ltd. https://doi.org/10.1186/1944-3277-10-14

Reid, J. B., Hunt, A. G., Johnstone, P. R., Searle, B. P., & Jesson, L. K. (2017). On the responses of carrots (*Daucus carota* L.) to nitrogen supply. *New Zealand Journal of Crop and Horticultural Science*, *0*(0), 1–21. https://doi.org/10.1080/01140671.2017.1402790

Rintala, A., Pietilä, S., Munukka, E., Eerola, E., Pursiheimo, J. P., Laiho, A., … Huovinen, P. (2017). Gut microbiota analysis results are highly dependent on the 16s rRNA gene target region, whereas the impact of DNA extraction is minor. *Journal of Biomolecular Techniques*, *28*(1), 19–30. https://doi.org/10.7171/jbt.17-2801-003

Ros, M., Hernandez, M. T., & García, C. (2003). Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biology and Biochemistry*, *35*(3), 463–469. https://doi.org/10.1016/S0038-0717(02)00298-5

Rozpądek, P., Domka, A., Ważny, R., Nosek, M., Jędrzejczyk, R., Tokarz, K., & Turnau, K. (2018). How does the endophytic fungus Mucor sp. improve Arabidopsis arenosa vegetation in the degraded environment of a mine dump? *Environmental and Experimental Botany*, *147*(August), 31–42. https://doi.org/10.1016/j.envexpbot.2017.11.009

Rudisill, M. A., Turco, R. F., & Hoagland, L. A. (2016). Fertility practices and rhizosphere effects alter ammonia oxidizer community structure and potential nitrification activity in pepper production soils. *Applied Soil Ecology*, *99*, 70–77. https://doi.org/10.1016/j.apsoil.2015.10.011

Sagan, L. (1967). On the origin of mitosing cells. *Journal of Theoretical Biology*, *14*(3), 225-IN6. https://doi.org/10.1016/0022-5193(67)90079-3

Sambo, F., Finotello, F., Lavezzo, E., Baruzzo, G., Masi, G., Peta, E., … Di Camillo, B. (2018). Optimizing PCR primers targeting the bacterial 16S ribosomal RNA gene. *BMC Bioinformatics*, *19*(1), 343. https://doi.org/10.1186/s12859-018-2360-6

Sasse, J., Martinoia, E., & Northen, T. (2018). Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in Plant Science*, *23*(1), 25–41. https://doi.org/10.1016/j.tplants.2017.09.003

Schenk, H. J. (2006). Root competition: beyond resource depletion. *Journal of Ecology*, *94*(4), 725–739. https://doi.org/10.1111/j.1365-2745.2006.01124.x

Schulz, B., & Boyle, C. (2007). What are Endophytes? In *Microbial Root Endophytes* (pp. 1–13). Springer Berlin Heidelberg. https://doi.org/10.1007/3-540-33526-9_1

Shannon, C. E., & Weaver, W. (1964). *The mathematical theory of communication*. University of Illinois Press.

Shaver, J. M., Oldenburg, D. J., & Bendich, A. J. (2006). Changes in chloroplast DNA during development in tobacco, Medicago truncatula, pea, and maize. *Planta*, *224*(1), 72–82. https://doi.org/10.1007/s00425-005-0195-7

Simon, P. W. (2019). Economic and Academic Importance (pp. 1–8). https://doi.org/10.1007/978-3-030-03389-7_1

Sinclair, L., Osman, O. A., Bertilsson, S., & Eiler, A. (2015). Microbial community composition and diversity via 16S rRNA gene amplicons: Evaluating the illumina platform. *PLoS ONE*, *10*(2), 1–18. https://doi.org/10.1371/journal.pone.0116955

Stein, L. Y., & Klotz, M. G. (2016). The nitrogen cycle. *Current Biology*, *26*(3), R94–R98. https://doi.org/10.1016/j.cub.2015.12.021

Strokal, M., & Kroeze, C. (2014). Nitrous oxide (N2O) emissions from human waste in 1970-2050. *Current Opinion in Environmental Sustainability*, *9*(3), 108–121. https://doi.org/10.1016/j.cosust.2014.09.008

Subbarao, G. V., Sahrawat, K. L., Nakahara, K., Ishikawa, T., Kishii, M., Rao, I. M., … Lata, J. C. (2012). *Biological nitrification inhibition-a novel strategy to regulate nitrification in agricultural systems*. *Advances in Agronomy* (1st ed., Vol. 114). Elsevier Inc. https://doi.org/10.1016/B978-0-12-394275-3.00001-8

Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X., & Song, W. (2008). Endophytic bacterial diversity in rice (Oryza sativa L.) roots estimated by 16S rDNA sequence analysis. *Microbial Ecology*, *55*(3), 415–424. https://doi.org/10.1007/s00248-007-9287-1

Surette, M. A., Sturz, A. V, Lada, R. R., & Nowak, J. (2003). Bacterial endophytes in processing carrots (Daucus carota L. var. sativus): their localizations, population density, biodiversity and their effects on plant growth. *Plant and Soil*, *253*, 381–390.

Terrazas, R. A., Pietrangelo, L., Corral, A. M., Torres-Cortés, G., Robertson-Albertyn, S., Balbirnie-Cumming, K., … Bulgarelli, D. (2019). Nitrogen Availability Modulates the Host Control of the Barley Rhizosphere Microbiota. *BioRxiv*, 605204. https://doi.org/10.1101/605204

Theobald, J. C., Mitchell, R. A. C., Parry, M. A. J., & Lawlor, D. W. (1998). Estimating the excess investment in ribulose-1, 5-bisphosphate carboxylase/oxygenase in leaves of spring wheat grown under elevated CO2. *Plant Physiology*, *118*(3), 945–955. https://doi.org/10.1104/pp.118.3.945

Thijs, S., Op De Beeck, M., Beckers, B., Truyens, S., Stevens, V., Van Hamme, J. D., … Vangronsveld, J. (2017). Comparative Evaluation of Four Bacteria-Specific Primer Pairs for 16S rRNA Gene Surveys. *Frontiers in Microbiology*, *8*(MAR), 494. https://doi.org/10.3389/fmicb.2017.00494

Thorup-Kristensen, K. (2006). Root growth and nitrogen uptake of carrot, early cabbage, onion and lettuce following a range of green manures. *Soil Use and Management*, *22*(1), 29–38. https://doi.org/10.1111/j.1475-2743.2005.00012.x

Tian, B., Zhang, C., Ye, Y., Wen, J., Wu, Y., Wang, H., … Zhang, K. (2017). Beneficial traits of bacterial endophytes belonging to the core communities of the tomato root microbiome. *Agriculture, Ecosystems and Environment*, *247*(2), 149–156. https://doi.org/10.1016/j.agee.2017.06.041

Tiemens-Hulscher, M., Lammerts van Bueren, E. T., & Struik, P. C. (2014). Identifying nitrogen-efficient potato cultivars for organic farming. *Euphytica*, *199*(1–2), 137–154. https://doi.org/10.1007/s10681-014-1143-z

Toju, H., Peay, K. G., Yamamichi, M., Narisawa, K., & Hiruma, K. (2018). Core microbiomes for sustainable agroecosystems. *Nature Plants*, *4*(May). https://doi.org/10.1038/s41477-018-0139-4

Turner, T. R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D., … Poole, P. S. (2013). Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *ISME Journal*, *7*(12), 2248–2258. https://doi.org/10.1038/ismej.2013.119

Upreti, R., & Thomas, P. (2015). Root-associated bacterial endophytes from Ralstonia solanacearum resistant and susceptible tomato cultivars and their pathogen antagonistic effects. *Frontiers in Microbiology*, *6*(MAR). https://doi.org/10.3389/fmicb.2015.00255

Van Bueren, E. T. L., Struik, P. C., & Jacobsen, E. (2002). Ecological concepts in organic farming and their consequences for an organic crop ideotype. *Netherlands Journal of Agricultural Science*, *50*(1), 1–26. https://doi.org/10.1016/s1573-5214(02)80001-x

van der Voort, M., Kempenaar, M., van Driel, M., Raaijmakers, J. M., & Mendes, R. (2016, April 1). Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. *Ecology Letters*. Blackwell Publishing Ltd. https://doi.org/10.1111/ele.12567

https://doi.org/10.3389/fmicb.2017.00494

Veitch, R. S., Lada, R. R., Adams, A., & MacDonald, M. T. (2014). Carrot Yield and Quality as Influenced by Nitrogen Application in Cut-and-Peel Carrots. *Communications in Soil Science and Plant Analysis*, *45*(7), 887–895. https://doi.org/10.1080/00103624.2014.880713

Vestheim, H., & Jarman, S. N. (2008). Blocking primers to enhance PCR amplification of rare sequences in mixed samples - A case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology*, *5*, 1–11. https://doi.org/10.1186/1742-9994-5-12

Vibbert, H. B., Ku, S., Li, X., Liu, X., Ximenes, E., Kreke, T., … Gehring, A. G. (2015). Accelerating sample preparation through enzyme-assisted microfiltration of Salmonella in chicken extract. *Biotechnology Progress*, *31*(6), 1551–1562. https://doi.org/10.1002/btpr.2167

von Wirén, N., Gazzarrini, S., Gojon, A., & Frommer, W. B. (2000). The molecular physiology of ammonium uptake and retrieval. *Current Opinion in Plant Biology*, *3*(3), 254–261. https://doi.org/10.1016/s1369-5266(00)80074-6

Walitang, D. I., Kim, C.-G., Jeon, S., Kang, Y., & Sa, T. (2018). Conservation and transmission of seed bacterial endophytes across generations following crossbreeding and repeated inbreeding of rice at different geographic locations. *MicrobiologyOpen*, (February), e00662. https://doi.org/10.1002/mbo3.662

Wang, D., Wang, Z., He, F., Kinchla, A. J., & Nugen, S. (2016). Enzymatic Digestion for Improved Bacteria Separation from Leafy Green Vegetables. *Journal of Food Protection*, *79*(8), 1378–1386. https://doi.org/10.4315/0362-028x.jfp-15-581

Wang, S. S., Liu, J. M., Sun, J., Sun, Y. F., Liu, J. N., Jia, N., … Dai, X. F. (2019). Diversity of culture-independent bacteria and antimicrobial activity of culturable endophytic bacteria isolated from different Dendrobium stems. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-46863-9

Weaver, R. W., Angle, J. S., & Bottomley, P. S. (1994). *Methods of soil analysis, Part 2*. *American Society of Agronomy*. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:methods+of+soil+analysis+part2#1%5Cnhttp://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Methods+of+soil+analysis,+Part+2#1

Westerveld, S. M., McKeown, A. W., & McDonald, M. R. (2006). Seasonal nitrogen partitioning and nitrogen uptake of carrots as affected by nitrogen application in a mineral and an organic soil. *HortScience*, *41*(5), 1332–1338. https://doi.org/10.21273/hortsci.41.5.1332

Wicaksono, W. A., Eirian Jones, E., Monk, J., & Ridgway, H. J. (2017). Using bacterial endophytes from a New Zealand native medicinal plant for control of grapevine trunk diseases. *Biological Control*, *114*(August), 65–72. https://doi.org/10.1016/j.biocontrol.2017.08.003

Wick, K., Heumesser, C., & Schmid, E. (2012). Groundwater nitrate contamination: Factors and indicators. *Journal of Environmental Management*, *111*, 178–186. https://doi.org/10.1016/j.jenvman.2012.06.030

Ximenes, E., Hoagland, L., Ku, S., Li, X., & Ladisch, M. (2017). Human pathogens in plant biofilms: Formation, physiology, and detection. *Biotechnology and Bioengineering*, *114*(7), 1403–1418. https://doi.org/10.1002/bit.26247

Yao, H., & Wu, F. (2010). Soil microbial community structure in cucumber rhizosphere of different resistance cultivars to fusarium wilt. *FEMS Microbiology Ecology*, *72*(3), 456–463. https://doi.org/10.1111/j.1574-6941.2010.00859.x

Yergeau, E., Bell, T. H., Champagne, J., Maynard, C., Tardif, S., Tremblay, J., & Greer, C. W. (2015). Transplanting soil microbiomes leads to lasting effects on willow growth, but not on the rhizosphere microbiome. *Frontiers in Microbiology*, *6*(DEC). https://doi.org/10.3389/fmicb.2015.01436

Yergeau, E., Sanschagrin, S., Maynard, C., St-Arnaud, M., & Greer, C. W. (2014). Microbial expression profiles in the rhizosphere of willows depend on soil contamination. *ISME Journal*, *8*(2), 344–358. https://doi.org/10.1038/ismej.2013.163

Yin, H., Wheeler, E., & Phillips, R. P. (2014). Root-induced changes in nutrient cycling in forests depend on exudation rates. *Soil Biology and Biochemistry*, *78*, 213–221. https://doi.org/10.1016/j.soilbio.2014.07.022

Yin, L., Dijkstra, F. A., Wang, P., Zhu, B., & Cheng, W. (2018). Rhizosphere priming effects on soil carbon and nitrogen dynamics among tree species with and without intraspecific competition. *New Phytologist*, *218*(3), 1036–1048. https://doi.org/10.1111/nph.15074

Yiridoe, E. K., Bonti-Ankomah, S., & Martin, R. C. (2005). Comparison of consumer perceptions and preference toward organic versus conventionally produced foods: A review and update of the literature. *Renewable Agriculture and Food Systems*, *20*(4), 193–205. https://doi.org/10.1079/raf2005113

Zavalloni, C., Jenkins, J. R., Fornasier, F., Arnold, E. C., Girardin, C., Miglietta, F., … Rumpel, C. (2016). Biochar alters the soil microbiome and soil function: results of next-generation amplicon sequencing across Europe. *GCB Bioenergy*, *9*(3), 591–612. https://doi.org/10.1111/gcbb.12371

Zhang, J., Ding, X., Guan, R., Zhu, C., Xu, C., Zhu, B., … Lu, Z. (2018). Evaluation of different 16S rRNA gene V regions for exploring bacterial diversity in a eutrophic freshwater lake. *Science of the Total Environment*, *618*, 1254–1267. https://doi.org/10.1016/j.scitotenv.2017.09.228

Zhu, B., Gutknecht, J. L. M., Herman, D. J., Keck, D. C., Firestone, M. K., & Cheng, W. (2014). Rhizosphere priming effects on soil carbon and nitrogen mineralization. *Soil Biology and Biochemistry*, *76*, 183–192. https://doi.org/10.1016/j.soilbio.2014.04.033

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, *32*(5), 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x

CHAPTER 3. IDENTIFYING THE BEST APPROACH TO CHARACTERIZE BACTERIAL ENDOPHYTES IN CARROTS

3.1 Introduction

Interactions between plants and microorganisms play an important role in the health and productivity of plants. Microbial communities living inside and around plants can enhance plant performance by helping them acquire nutrients like phosphorous (P) from insoluble forms in soil (Rozpądek et al., 2018), fix atmospheric nitrogen (N) (Meena et al., 2017), and protect them from pathogens by acting as biological control entities (Liu et al., 2018; Wicaksono et al., 2017). These plant associated microbes can also help plants alleviate abiotic stresses like salinity, drought and UV radiation (Cheng et al., 2007; Eida et al., 2018). Bacterial endophytes, which are microbes that spend at least part of their life cycle living within plants, are one component of a plants microbiome with the greatest potential to directly benefit their plant hosts (Hardoim et al., 2008). This is because of the intimate relationships these microbes are able to form with their plant hosts.

Soil properties are generally one of the biggest drivers of soil and plant microbial complexity, though there are clear differences in microbial biomass, activity and diversity between bulk soil and the plant rhizosphere and endosphere, demonstrating that plants have some degree of control over these microbial communities (Giagnoni et al., 2016). For example, specific plant factors that can alter the composition of endophytes include root morphology, plant developmental stage, and composition of root exudates, which act as signaling compounds in microbe-plant interactions, and support microbes by providing them with carbon (C) and various nutrients (Shi et al., 2017; Sasse et al., 2018). Soil management practices can also induce changes in rhizosphere and endosphere bacterial composition. For example, the type and amount of fertilizer inputs can alter nutrient availability, which can affect the composition of microbes available in soil to utilize those substrates, as well as affect plant-microbe relationships because it can change how they compete for substrates in soil (Zavalloni et al., 2016). Finally, plants can shape the composition of their bacterial endophytes by vertically transmitting them through their seeds (Walitang et al., 2018). These vertically transmitted microbes are thought to be a critical component of a plants core microbiome that is conserved over generations (Fitzpatrick et al., 2018). Consequently, it has been suggested that plants and all of their associated microorganisms should be treated as a single unit of selection in evolution called a “holobiont”, where the plant host and its microbiota are a

single dynamic entity in which each organism contributes to its survival and fitness (Card et al., 2016; Zilber-Rosenberg & Rosenberg, 2008). Learning more about the composition of bacterial endophytes, and specific factors driving their complexity is critical to learning how these plant-microbial relationships evolved, and might be manipulated to enhance plant performance in agriculture.

Carrots (*Daucus carota* L.) are one of the most highly consumed fresh vegetables around the world, and they are an important source of carotenoids and vitamin A for the human diet. They also make an excellent model crop to study endophyte dynamics. This is because these plants produce a long-lived taproot, which must be able to acquire sufficient resources to survive a winter dormancy period, and withstand assault by a diverse group of soil pathogens and insect pests. Moreover, these taproots are often consumed raw, so they could be an important factor contributing to the composition of human gut microbiomes which could enhance or harm human health (Hoagland et al., 2018; Kõiv et al., 2019). Despite their importance, only a few studies have investigated endophytes in carrot taproots to date (Abdelrazek et al., under review; Abdelrazek, 2018; Kõiv et al., 2019; Louarn et al., 2013; Surette et al., 2003). Most of these studies were conducted using traditional culture based studies. For example, our lab used selective media to determine that carrot taproots are colonized by bacteria belonging to *Pseudomonas*, *Xanthomonas, Stenotrophomonas*, *Rhizobium*, *Bacillus*, *Paenibacillus* and *Methylobacterium* genera, and fungi belonging to *Cladosporium, Plectosphaerella, Colletotrichum* and *Epicoccum* genera, (Abdelrazek, 2018; Abdelrazek et al., under review). These endophytes were able to suppress *Alternaria dauci*, a critical plant pathogen, and some had other properties, such as potential to solubilize P, which could aid in carrot growth and productivity. However, these endophyte communities are likely to be much more diverse. Because of limitations associating with culturing endophytes, these studies are expected to reveal only 1% of the true diversity of these communities, which leaves the remaining 99% to be discovered (Nithya & Babu, 2017; Surette et al., 2003).

New culture independent technologies such as next-generation sequencing conducted using platforms such as Illumina MiSeq (J. Gregory Caporaso et al., 2011), have demonstrated that endophytic communities in plants such as tomato (*Solanum lycopersicum.* L) (Tian et al., 2017), barley (*Hordeum vulgare.* L) (Bianco et al., 2018), sugarcane *(Saccharum officinarum.* L) (Arruda et al., 2018), and three medicinal plant species (Köberl et al., 2013) are incredibly diverse. Others have begun using these technologies to demonstrate how soil and plant factors can influence the

composition of plant endophytes (Toju et al., 2018). For example, our lab recently used Illumina sequencing to demonstrate that carrot taproot fungal communities are incredibly diverse and change given crop management system and carrot genotype (Abdelrazek, 2018; Abdelrazek et al., under review). Kõiv et al. (2019) recently used this approach to investigate the composition of bacterial endophytes in carrots as well as other root crops. However, while this study demonstrated that bacterial endophytes in carrot taproots are more diverse than previous studies using culture based approaches (Abdelrazek, 2018, Abdelrazek et al., 2018; Surette et al., 2003). We suspect that this study could be biased. This is due to the fact that many of the primer sets designed to amplify bacteria can also amplify tissues belonging to several plant organelles (Ghyselinck et al., 2013). Plant organelles such as mitochondria, chloroplasts and other plastids (e.g. chromoplasts) were acquired by plants from on an endosymbiotic α-proteobacterium–like ancestor, as eukaryotic cells developed more than a billion years ago (Dyall et al., 2004). This homology between these plant organelles and bacteria leads to problems associated with co-amplification of mitochondrial and chloroplast/plastids (Ghyselinck et al., 2013).

Choosing the correct primer set to amplify the 16 rRNA hypervariable regions (V1-V9) in bacteria is critical to accurately determining the composition of bacterial communities. Primers targeting V3-V4 region, are the most commonly used in bacterial microbiome studies. This is because the V3 region has the greatest variability, and the V4 region has good domain specificity, resulting in high coverage and high confidence (Bowman et al., 2012; Sinclair et al., 2015; Zhang et al., 2018). Most sequences in bacterial databases such as Greengenes and Silva were generated using these primer sets, so you can obtain the most comprehensive assessment of bacterial communities using these primer sets. However, when we used V3-V4 primer sets to amplify bacterial endophytes in carrot taproots, the vast majority of sequences belonged to plant materials. Consequently, scientists have begun designing alternative primer sets to investigate bacterial endophytes. For examples, primers targeting the V5-V7 region were designed to exclude plant host DNA because they have low affinity for chloroplast DNA (Chelius & Triplett, 2001), and this has allowed researchers to obtain more accurate assessments of bacterial endophyte diversity in plants such as poplar trees (Beckers et al., 2016). Others have designed so-called ‘blocking primers’, which were initially designed to target prey DNA in diet krill studies (Vestheim & Jarman, 2008). These primers included a C3 spacer modification on the 3’ end, which prevents amplification of host DNA. Arenz et al. (2015) adapted this approach to design a set of V5-V6

primers that would block plant chloroplast and mitochondrial DNA for a study investigating endophyte communities *Sorghum* leaves. However, while these alternative primer sets have potential to aid in studies of bacterial endophytes, they could lead to an underestimation of the true bacterial diversity present within plants.

To overcome challenges associated with primer set design, it may be possible to optimize culture independent surveys of bacterial endophytes in carrots by subjecting carrot tissues to extraction procedures that separate plant and microbial tissues. For example, a group of engineers and microbiologists recently collaborated to develop a new hollow-fiber microfiltration system to separate bacteria from food products such as meat and eggs to aide in detection of foodborne pathogens (Ku et al., 2016; X. Liu et al., 2013; Vibbert et al., 2015). During this technique, food products are passed through a hollow fiber microfiltration system (HF), which concentrates cells to increase the probability of bacterial cell detection. This system was adapted for use in plants and an extra enzymatic digestion step was added to further concentrate microbial cells (Wang et al., 2016; Ximenes et al., 2017). During this digestion step, enzymes (e.g. cellulases, pectinases) that degrade large sugars and proteins in plants and bacterial biofilms are added to degrade these materials, but are not expected to affect the microbial cells (Wang et al., 2016; Ximenes et al., 2016).

Having a clear picture of the bacterial endophyte community present in carrot taproots is critical to learning how to leverage these communities to enhance carrot productivity and possibly human health. Consequently, the primary goal of this study was to determine whether subjecting carrots to hollow fiber microfiltration and an enzymatic digestion step could be used to enhance recovery of bacterial endophytes. Second, we aimed to determine how the choice of primer set affect estimates of bacterial endophyte composition in carrot taproots, and identify the best primer set for these studies.

3.2 Materials and Methods

3.2.1 Sample collection and surface sterilization

The carrots (experimental breeding line Exp3999), were collected from an on-going breeding trial (Simon et al., 2017). The carrot were sown in May 2017 at Purdue University's Student Farm in West Lafayette, IN. Soils at this site are classified as part of the Mahalasville

Treaty Complex and characterized as silty, clay loam and generally poorly drained. Seeds (provided by Dr. Phil Simon, USDA-ARS Vegetable Crops Research Unit, Madison, WI) were planted on raised beds in in rows with a density of approximately 60 plants m^{-1}. After 110 days**,** five healthy carrot taproots were randomly collected with no physical damage or pathogen symptoms. The carrot taproots were transferred to the lab and stored at 4°C until processing on the following day. The carrot taproots were rinsed and surface sterilized using 5.2% bleach for 3 minutes, 3% peroxide solution for an additional 3 minutes, and a final rinse with sterile water and 1ml of tween (Surette et al., 2003). Surface disinfestation was confirmed by culturing the final rinse water in selective media for three bacterial groups: LGI (diazotrophic bacteria), R2A (oligotrophic bacteria), Triptic soy agar (heterotrophic bacteria) (Curtis et al., 2011; Reasoner & Geldreich, 1985). After incubating at 28ºC for one week, there was no sign of colony forming units confirming that the surface sterilization procedure was effective.

3.2.2 Pre-treatment of carrot taproot tissue

Cylinders of 15 mm size were collected from the core carrot taproots using a sterile core borer. The carrot taproot cylinders were then mixed and divided equally for the different endophyte extraction methods described below. Stock solutions of the carrot taproot cylinders were created using 5 g of carrot tissue and 25 ml of sodium phosphate buffer (pH 7), which were homogenized using an Omni Tissue Master Homogenizer (OMNI International, GA, U. S. A). After homogenization, a control treatment (C), received no additional processing, prior to DNA extraction described below. Stock solutions for the other treatments, hollow-fiber microfiltration along (F), and hollow-fiber microfiltration followed by enzymatic treatment (E) were filtered through 90 mm filter paper before further processing described below.

3.2.3 Hollow-fiber microfiltration (F) and enzymatic treatment (E)

To try and increase recovery of endophytes, carrot stock solutions were subject to a hollow-fiber microfiltration technique, which was originally developed to recover and detect low levels of food-borne pathogens in various plant and animal products (Ximenes et al., 2017). 50 ml of the carrot stock solutions were passed through the automated unit, which further filtered the blended carrot tissue through a 2.7 µm glass microfiber to remove colloidal plant particles, before further

passing the tissue through a 0.2 μmm polyether hollow fiber membrane. The fluid passed through the hollow fiber filters at a flow rate of 10 ml/min, reducing the initial sample's total volume. After filtration, half of the carrot filtrate processed using the microfiltration technique was subject to an enzymatic technique in an attempt to further increase recovery of endophytic microbes in the carrot tissue. The carrot filtrates were subject to enzymes that specifically target plant and not microbial tissues, using a process that was originally developed by Ku et al. (2016). This included subjecting carrot filtrates to a 0.5% concentration of protease, cellulase and pectinases enzymes for 2 h at 37ºC and 200rpm. All treatments (C), (F), and (E) were processed in triplicate.

3.2.4 DNA extraction and library preparation

DNA was extracted from 500 μl carrot tissues processed as above using the DNeasy plant mini kit (Qiagen, Venlo, Netherlands) in triplicate from each treatment and replicate. Next, DNA from each treatment and replicate was subject to PCR using one of three primer sets targeting the 16S hypervariable region (Table 3.1). This included a standard (S) treatment using V3-V4 (Caporaso et al., 2011; Muyzer et al., 1993) which are the most commonly used primers for community analyses of bacteria. A second treatment designated (B) which used a set of primers targeting the V4-V6 region, which were designed to block amplification of plant compounds (Arenz et al., 2015; Caporaso et al., 2011). Finally, the third treatment designated (M) used a primer set target the V5-V7 region, which were also designed to prevent amplification of plant compounds (Hanshew et al., 2013). All DNA extracts were first diluted to concentrations of 1 ng/ul before PCR described below. All PCR reactions were conducted in triplicate for each treatment.

Table 3.1: Sequence and region of the primer sets targeting the 16S hypervariable region

Primer Name		Primer sequence (5'- 3')	16S region	Reference
Standard (S)	341-F	ACTCCTACGGGAGGCAGCAG	V3 - V4	(Muyzer et al., 1993)
	806-R	GGACTACHVGGGTWTCTAAT		(Caporaso et al., 2011)
Blocking (B)	515-F	GTGCCAGCMGCCGCGGTAA	V4-V6	(Caporaso et al., 2011)
	1064-R	CGACRRCCATGCANCACCT		(Arenz et al., 2015)
	V6RMitoBlkC3	AGCACCTGTATGAAAGTCAGTAC/3SpC3/		(Arenz et al., 2015)
	V6RChlorBlkC3	GCACCACCTGTGTCCGCG/3SpC3/		(Arenz et al., 2015)
Mismatch (M)	799-F	AACMGGATTAGATACCCKG	V5-V6-V7	(Chelius & Triplett, 2001)
	1193-R	ACGTCATCCCCACCTTCC		(Bodenhausen et al., 2013)

3.2.5 Control V3-V4 region (S)

To amplify the V3-V4 region, 1 µl of DNA was used as a template in 25 µl PCR reactions that consisted of 10.5 µl H_2O, 12.5 µl Q5® High-fidelity Master Mix (NEB), and 0.5 µl of each primer (10 µM). PCR thermocycler conditions including an initial 98°C denaturing step for 2 minutes, followed by 25 cycles of: 98°C for 10 sec, 55 °C for 30 sec, 72°C for 30 sec, and a final extension step of 72°C for 2 min. After PCR, 4 µl of the amplification products (~ 490 pb) were visualized on a 1 % agarose gel to confirm that the PCR reaction was successful. Following this 1st round of PCR, 20 µl of amplification product was purified using a PCR purification kit (Qiagen, Venlo, Netherlands) before being used as template in a 2nd PCR reaction, where the products were amplified with primers that included a barcode for processing using the Illumina sequencing

pipeline. This second PCR used the same condition as the first, though was only run for only 5 cycles. Again, amplification were run on a 1 % agarose gel to confirm that the PCR reaction was successful.

3.2.6 V4-V6 region plus blocking primers (B)

PCR was conducted using the protocol described by Arenz et al., (2015) with some modifications. 1 µl of DNA was used as a template in 37 µl PCR reactions that included 12.5 µl H_2O, 12.5 µl Q5® High-fidelity Master Mix (NEB), 0.5 µl primer V4 (10 µM), 0.5 µl primer V6 (10 µM), 5 µl primer V6RChlorBLK(10 µM), and 5 µl primer V6RMitoBLK primer (10 µM). PCR thermocycler conditions including an initial 98°C denaturing step for 2 minutes, followed by 35 cycles of: 98°C for 10 sec, 59 °C for 30 sec, 72°C for 30 sec, and a final extension step of 72°C for 2 min. After PCR, 4 µl of amplification products (~ 500 pb) were visualized on a 1 % agarose gel and cleaned and subject to a 2nd PCR using the same procedures described above.

3.2.7 Mismatch V5-V7 region (M)

PCR was conducted using the protocol developed by Hanshew et al. (2013), but modified using the 799F/1193R primer pair. 1 µl of DNA was used as a template in 25 µl PCR reactions consisting of 9 µl H2O, 12.5 µl Q5® High-fidelity Master Mix (NEB), 1.25 µl primer V5 (10 µM), and 1.25 µl primer V7 (10 µM). PCR thermocycler conditions including an initial 94°C for denaturing step for 2 minutes, followed by 30 cycles of: 94°C for 30 sec, 55 °C for 30 sec, 72°C for 60 sec, and a final extension step of 72°C for 10 min. After PCR, 12 µl of amplification products were run on a 2 % agarose gel, to visualize two distinct bands that included microbial products of ~500 bp, and plant plastids/mitochondria of ~ 800 bp. The band representing the microbial products were excised using a non-UV transilluminator, Dark Reader® (Clare Chemical Research) and purified using a gel extraction kit (Qiagen, Venlo, Netherlands). The product was then subject to a 2nd PCR using the same protocol described above.

3.2.8 Next generation sequencing

After pooling the PCR products from each treatment replicate, 50 µl of final purified and tagged PCR products were sent to the Purdue Genomics Facility for sequencing on the Illumina MiSeq platform. The sample were diluted and combined in equimolar concentrations prior to sequencing.

3.2.9 Bioinformatic analyses

Sequences generated by Illumina sequencing were demultiplexed based on the different barcodes and trimmed according to a 25 phred quality value using the TrimGalore tool (Krueger, 2015), and forward and reverse reads were merged and quality checked using the FastQC tool (Andrews, 2010). The sequences were then imported into the QIIME v2. platform (Caporaso et al., 2010) for OTUs picking, assigning, and aligning using the SILVA bacterial database (Pruesse et al., 2007). The QIIME pipeline was modified to run the sequences with and without mitochondrial and chloroplast/plastid filters, to calculate the type of sequences generated in each treatment.

3.2.10 Statistical analyses

Statistical analysis were performed using QIIME v2. (Caporaso et al., 2010) to calculate α diversity (Shannon and chao1 indexes) using the alpha_rarefaction.py script that uses a nonparametric index to measure total number of OTUs and evenness (relative abundance of OTUs) found in QIIME documentation (http://qiime.org/scripts/alpha_diversity.html). Differences in the number of bacterial OTUs were compared among the nine treatment combinations. Differences in the indices and bacterial OTUs, plastids and mitochondria were determined using a two way analysis of variance (ANOVA) using the R version 3.6.2 (R Core Team, 2019). In addition, a pairwise Tukey test was performed to determine statistical differences among the bacterial OTUs and alpha diversity indices obtained from the ANOVA test.

3.3 Results

3.3.1 Recovery of chloroplasts/plastids and mitochondrial sequences

There were significant differences in the percentage of plastids and mitochondrial sequences recovered from the carrot taproots using the different primer set evaluated in this trial (Table 3.2; Figure 3.1). Regardless of the pre-processing treatment to isolate endophytic microbes from carrot taproot tissues, amplification using the V4-V6 blocking primer pair (B), resulted in the vast majority of sequences amplified belonging to chloroplast/plastid sequences and very little mitochondria. In contrast, amplification using the V5-V7 mismatch primer set (M) primers resulted in a greater total percentage of bacterial sequences. The greatest percentage of bacterial sequences were amplified when carrot taproots were processed using hollow fiber filtration followed by enzymatic digestion (E), and amplified using the V5-V7 mismatch (M) primer pair.

Table 3.2: Two-way ANOVA models evaluating differences in percentage of plastids and mitochondrial sequences recovered from the carrot taproots using the different extraction and primer set combinations

type of sequence		df	sum sq	mean sq	F value	pr(>F)
plastids	tissue treatment	2	4.05E+09	2.03E+09	2.045	0.158
	primer	2	2.86E+10	1.43E+10	14.454	<0.001***
	tissue treatment x primer type	4	4.26E+09	1.07E+09	1.076	0.397
	residuals	18	1.78E+10	9.90E+08		
mitochondria	treatment	2	8.04E+08	4.02E+08	0.413	0.667
	primer	2	1.61E+11	8.05E+10	82.770	<0.001***
	tissue treatment x primer type	4	1.47E+09	3.68E+08	0.378	0.820
	residuals	18	1.75E+10	9.73E+08		

Significant p-values correspond to ***p<0.001 (alpha value = 0.05).

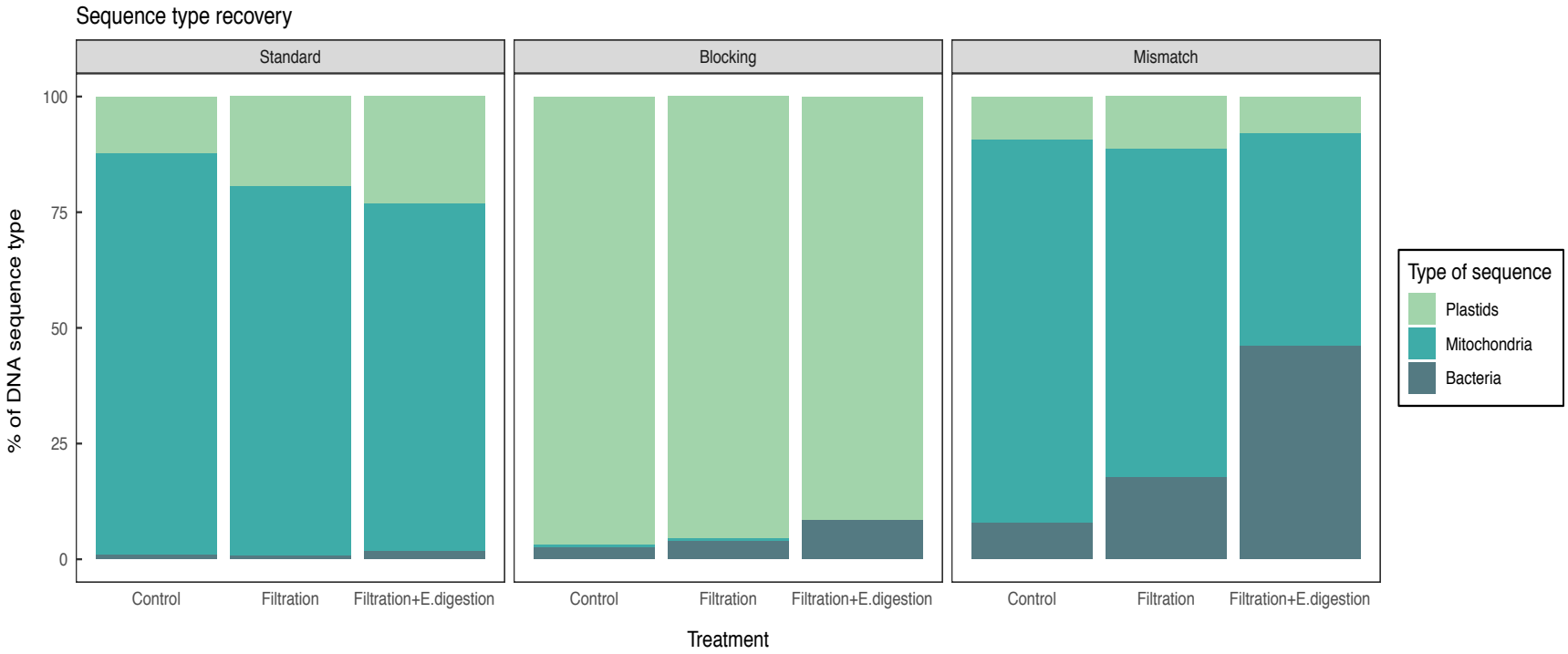

Figure 3.1: Relative abundance of plastids, mitochondria and bacterial OTUs recovered from each treatment combination before a bioinformatic sequence filter was applied to the data to remove mitochondria and plastids.

3.3.2 Recovery of bacterial OTUs

Results of the two-way ANOVA and subsequent post-hoc pairwise analyses indicated that there were significant differences in the number of bacterial OTUs amplified among the carrot taproot treatment combination (Table 3.3; Figure 3.2). The greatest number of bacterial endophytic OTUs were present in carrot taproots that were processed using hollow fiber microfiltration followed by enzymatic digestion, and amplified using the V5-V7 mismatch primer set (EM). Carrot taproots that were processed using hollow fiber microfiltration followed by enzymatic digestion and then amplified using the V4-V6 blocking primers, also had a significantly greater number of bacterial OTUs than any of the carrot taproots processed using a standard approach to recover endophytes regardless of the primer set used for amplification.

Table 3.3: Two-way ANOVA model evaluating differences in bacterial endophyte OTUs present in carrot taproots after subjecting taproot cores to three treatments using one of three primer

	df	sum sq	mean sq	F value	pr(>F)
tissue treatment	2	549072208	274536104	11.451	<0.001***
primer type	2	1139095091	569547545	23.756	<0.001***
tissue treatment x primer type	4	253403949	63350987	2.642	0.067.
Residuals	18	431544205	23974678		

Significant p-values correspond to ***p<0.001. Marginal significant differences correspond to (.)<0.10 (alpha value = 0.05).

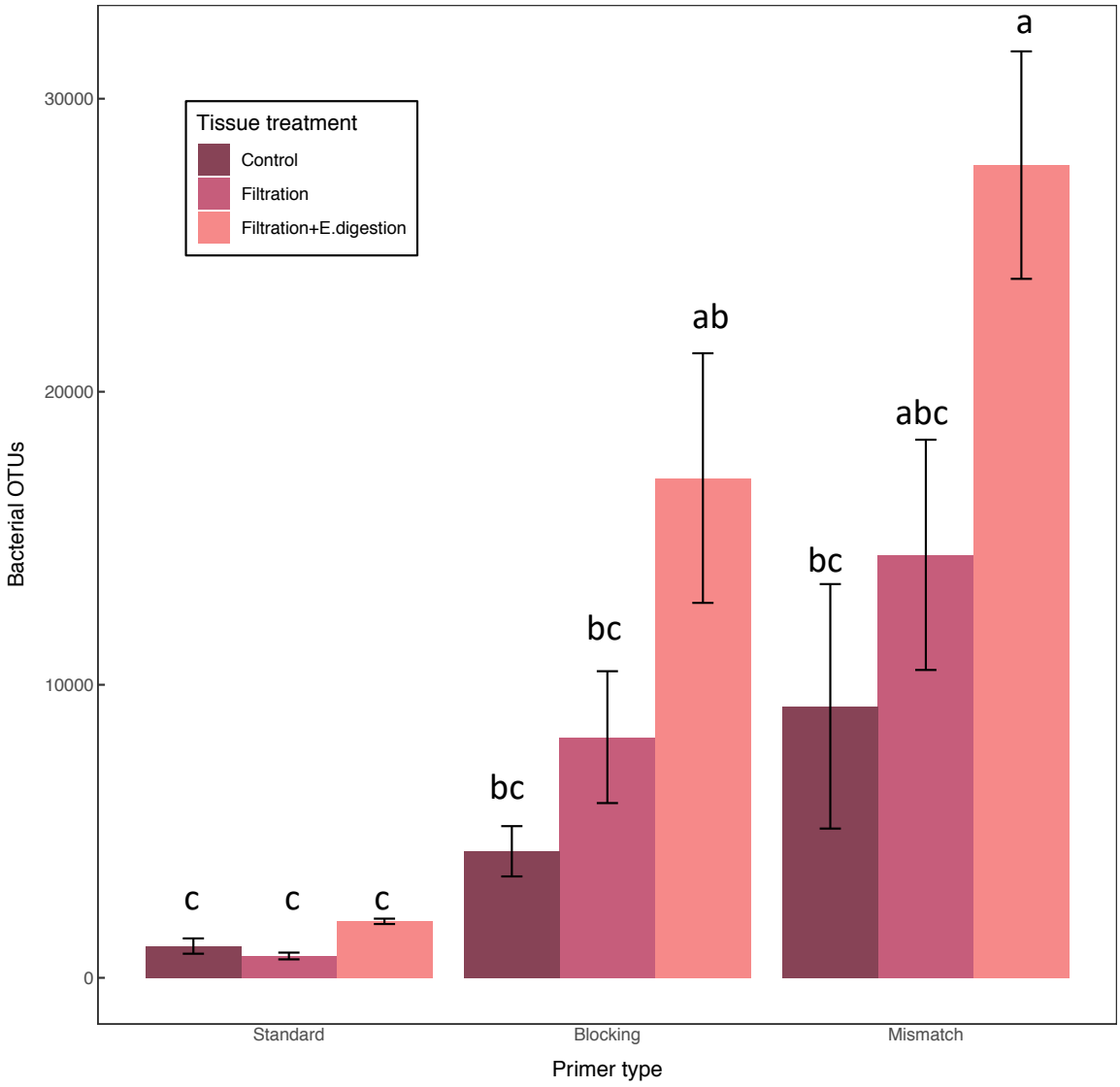

Figure 3.2: Number of bacterial endophyte OTUs present in carrot taproots after subjecting taproot cores to three treatments to isolate endophytes and amplification using one of three primer sets .*Columns that share the same letters indicate no significant differences (alpha value = 0.05).

3.3.3 Alpha diversity (richness)

Results of the alpha diversity analysis of bacterial endophytes estimated using the Shannon diversity index (Shannon & Weaver, 1964), indicated there were no significant differences ($P<0.05$) between the treatment combinations (Table 3.4; Figure 3.3). However, when alpha diversity was evaluated using the chao1 index, there were significant different between the treatment combinations (Table 3.4; Figure 3.4). Carrot taproots processed using hollow fiber microfiltration

followed by enzymatic digestion and amplified using either the V4-V6 blocking primer set (EB) or the V5-V7 mismatch primer set (EM) had significantly greater alpha diversity than most of the other treatments. Carrot taproots processed using hollow fiber microfiltration alone and amplified using the V4-V6 blocking primer set (FB) also had greater alpha diversity than the CS, CM, FS and ES treatments.

Table 3.4: Two-way ANOVA models evaluating differences in shannon and chao1 alpha diversity index in carrot taproots after subjecting taproot cores to three treatments using one of three primers

Alpha diversity index		df	sum sq	mean sq	F value	pr(>F)
shannon	treatment	2	0.135	0.067	0.288	0.752
	primer type	2	2.8572	1.428	6.114	0.009**
	tissue treatment x primer type	4	1.6811	0.420	1.798	0.173
	residuals	18	4.2056	0.233		
chao1	treatment	2	259812	129906	30.083	<0.001***
	primer type	2	286221	143111	33.141	<0.001***
	tissue treatment x primer type	4	122670	30668	7.101	<0.001***
	residuals	18	77728	4318		

Significant p-values correspond to *** p <0.001 and ** p<0.01 (alpha value=0.05)

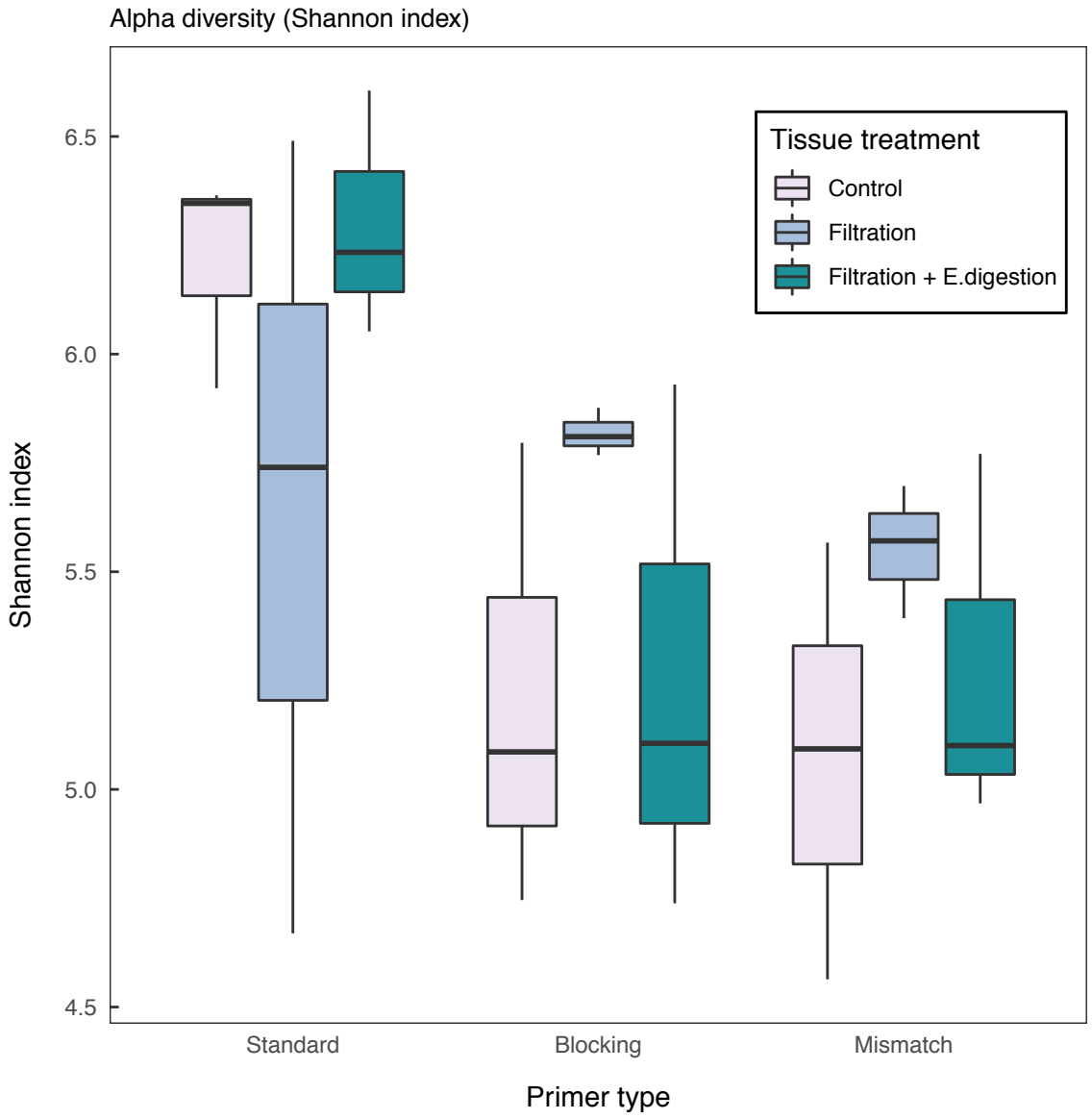

Figure 3.3: Alpha diversity of bacterial endophytes in carrot taproots using the Shannon index after subjecting taproot cores to three treatments to isolate endophytes and amplification using one of three primer sets. There were no significant statistically significant differences among the treatment combinations

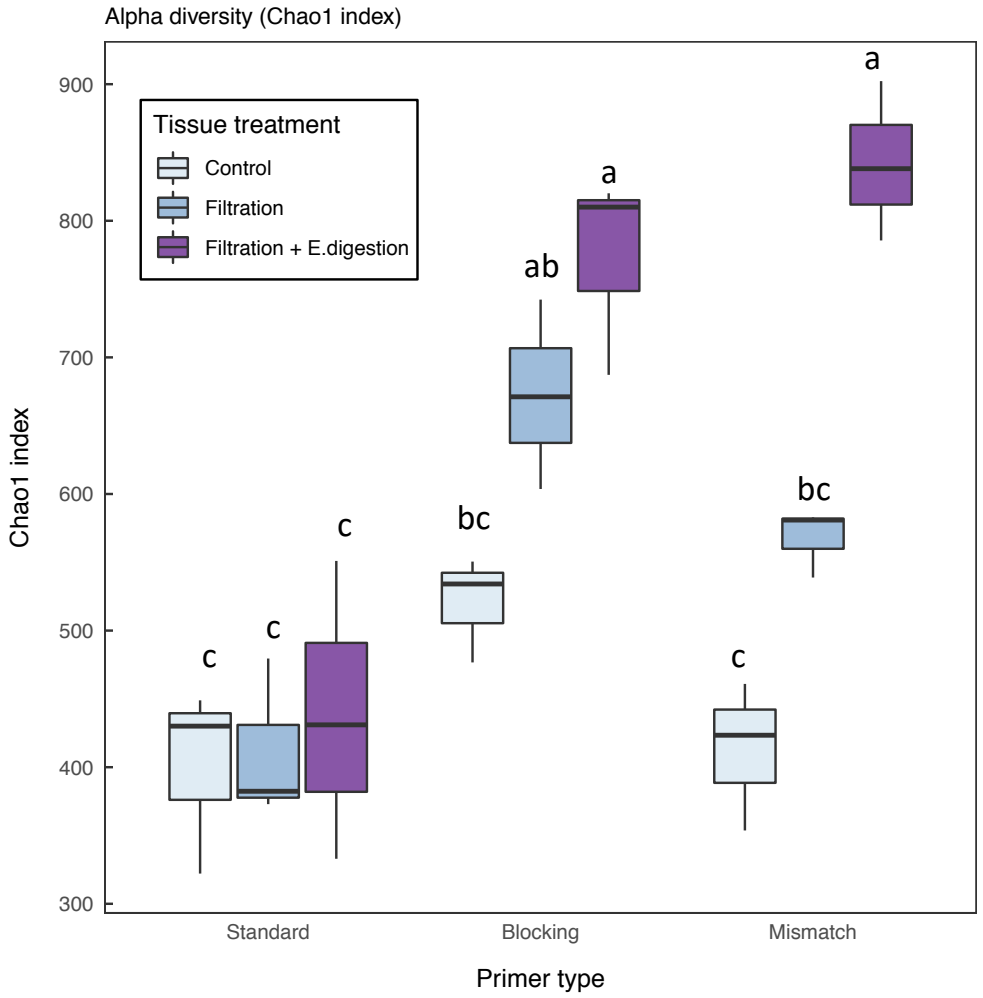

Figure 3.4: Alpha diversity of bacterial endophytes in carrot taproots using the chao1 index after subjecting taproot cores to three treatments to isolate endophytes and amplification using one of three primer sets. *Categories that share the same letter indicate no statistically significant differences (alpha value=0.05).

3.3.4 Bacterial relative abundance

The type and relative abundance of bacterial phyla varied greatly between the nine treatment combinations (Figure 3.5). OTUs representing *Actinobacteria*, *Bacteriodes*, *Firmicutes, Proteobacteria,* and Unassigned were present regardless of the primer sets used. However, the relative proportion of bacteria in these phyla differed among the primer sets used. For example, most of the sequences amplified using the standard V3-V4 (S) primer set were unassigned, while only a very small fraction were in this group when using the V5-V7 mismatch (M) primer set. The relative proportions of *Proteobacteria* and *Firmicutes* were also greater in using the V4-V6 blocking (B) or V5-V7 mismatch (M) primer sets than when using the standard V3-V4 (S) primer set. There were also unique phyla amplified among the different primer sets. For example, Chloroflexi were only present in carrot tissues amplified using the standard V3-V4 primer set (S) and the V5-V7 mismatch (M) primer set. In addition, candidate division TM7 and *Acidobacteria* were only present in carrot taproot tissues amplified using the standard V3-V4 (S) primer set. In contrast, *Archaeplastida, Armatimonadetes*, *BD1_5*, and *Chlamydiae* were only present in carrot taproot tissues amplified using the V4-V6 blocking primers.

Differences in the assignment of OTUs in response to the preprocessing treatment to isolate endophytes depended on the primer set used. For example, when using the standard V3-V4 (S) primer set, there were very few differences in the OTU assignments. When using the V4-V6 blocking (B) primers, a greater number of bacterial phyla were amplified when using the standard treatment to obtain endophytes (CB) than when using the microfiltration treatment with or without enzymatic digestion. In addition, when following the microfiltration treatment with enzymatic digestion, the total number of phyla was lowest, and the relative proportion of *Firmicutes* decreased. Finally, the relative proportion of endophytes assigned to different phyla when using the V5-V7 mismatch (M) primer sets also varied given the preprocessing treatment to isolate endophytes, with more *Actinobacteria* when using the standard (CM) treatment, and the most *Firmicutes* when using the microfiltration technique alone (FM).

When endophytic OTUs in carrot taproot tissues subject to the nine treatment combinations were analyzed at the level of genera, differences became even more dramatic (Figure 3.6).

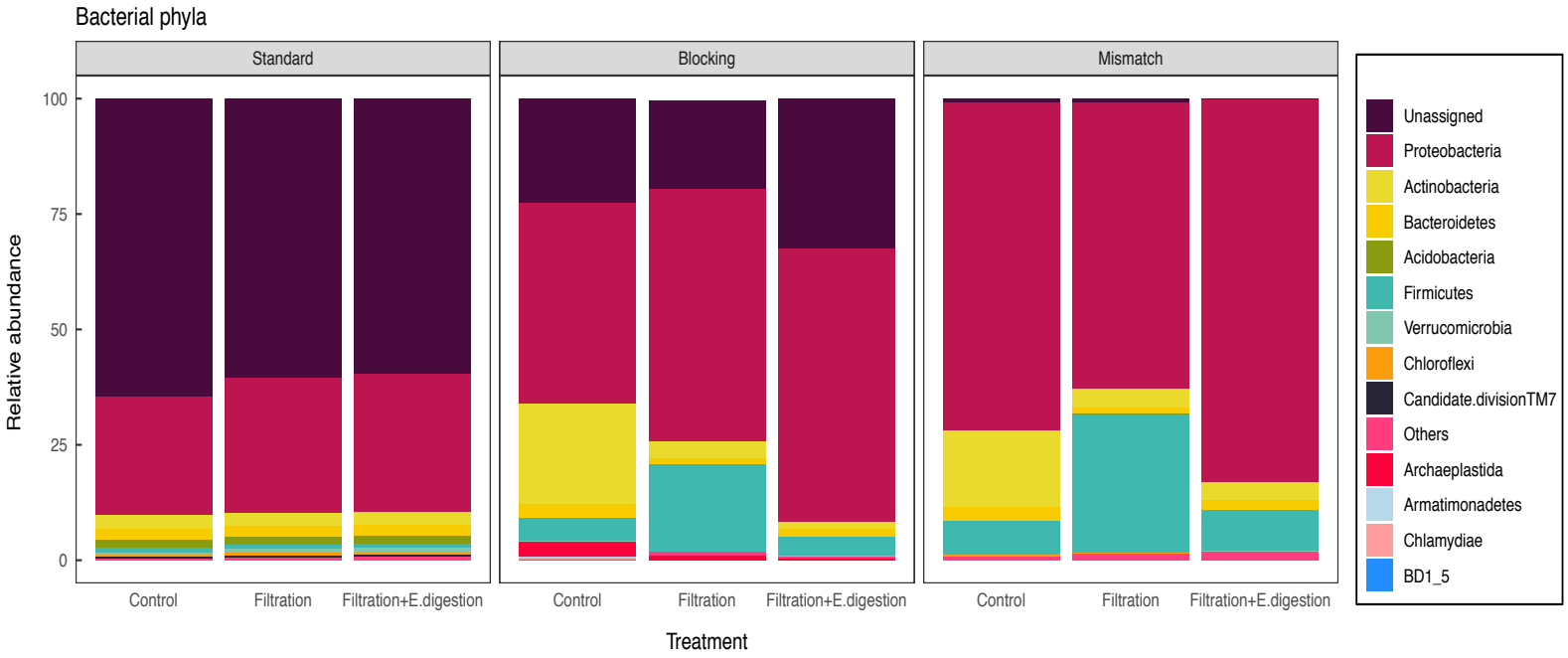

Figure 3.5: Relative abundance of bacterial endophytes by phyla in carrot taproots subject to three protocols to isolate endophytes and amplify them using three 16S primer sets.

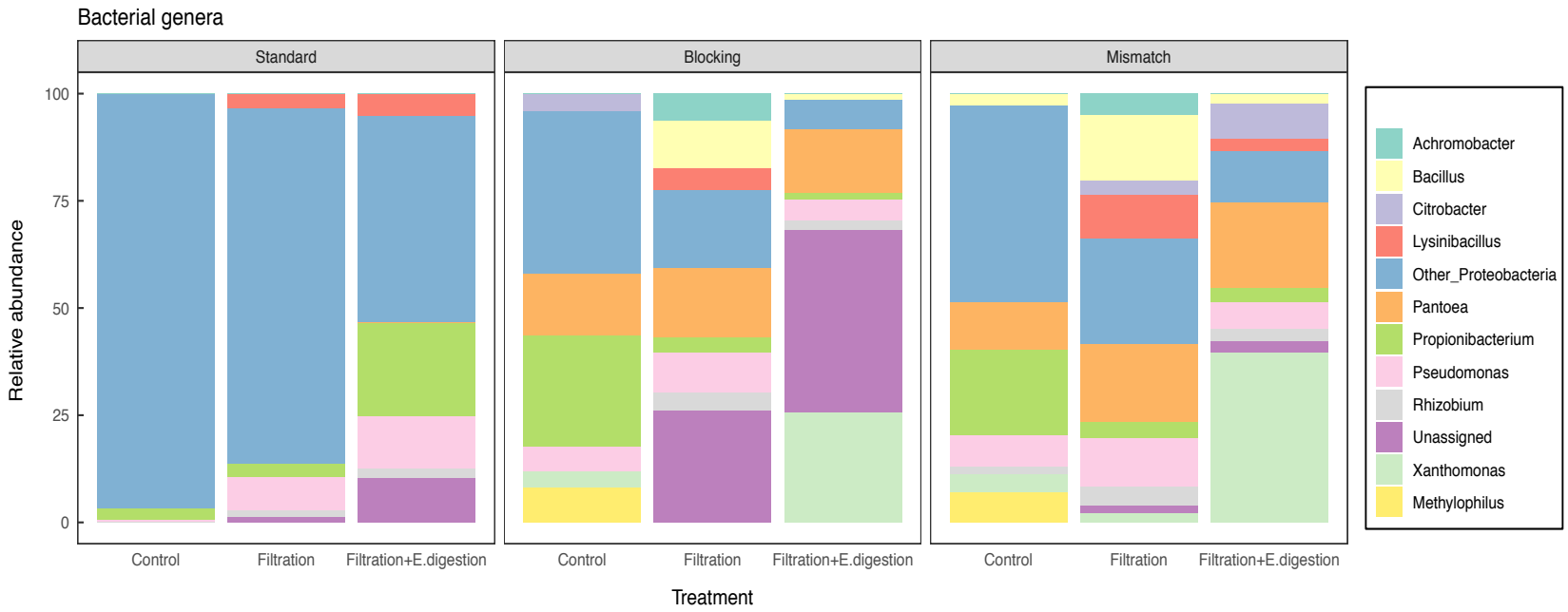

Figure 3.6: Relative abundance of bacterial endophytes by genera in carrot taproots cores after subjecting to three treatments using one of three primer

3.4 Discussion

Carrots are an important vegetable crop, but they suffer from many biotic and abiotic challenges that reduce the productivity of this crop. The presence of endophytic bacteria can improve the health of plants (Malfanova et al., 2013), so learning more about bacterial endophytes in carrots could help identify strategies to overcome production challenges. New next generation sequencing technologies are allowing researchers to begin to uncover the immense diversity of microbiomes found in nature, though using these tools to study bacterial endophytes in plants is challenging due to the presence of plant tissue DNA that is co-amplified along with bacterial DNA. The DNA copy number of mitochondrial and chloroplast cells vary with plant tissue conditions, cell type and plant age (Shaver et al., 2006). Our lab previously determined that co-amplification of plant plastids and mitochondrial DNA is abundant in carrot taproot tissues, making sequencing of bacterial communities particularly difficult. Although a high percentage of plant mitochondrial and chloroplast/plastids sequences can be filtered using bioinformatic tools, this introduces biases in the interpretation of the bacterial sequences, and increases costs due to the need for greater sequencing depth (Van Hamme et al., 2017). Consequently, the primary goal of this study was to determine if microfiltration and enzymatic digestion could be used to increase recovery of bacterial DNA from carrot taproot tissues.

Results indicate that subjecting carrot taproots to hollow fiber microfiltration alone or with a subsequent enzymatic digestion step did not result in significant reductions in the relative abundance of plastids or mitochondria (Figure 3.1; Table 3.2). However, there was a trend towards greater concentrations of bacterial cells following these treatments, especially when the two treatments were combined. In addition, using these tools did influence the assignment of bacterial communities using subsequent bioinformatic assays. For example, bacterial endophytes were assigned to a greater number of total OTUs when carrot taproot tissues were processed using hollow fiber microfiltration with enzymatic digestion, than when using the standard approach to isolate endophytes from plant tissues (Table 3.3; Figure 3.2). In addition, alpha diversity estimated using the ChaoI index was higher (Table 3.4; Figure 3.3). This indicates that coupling hollow fiber microfiltration with enzymatic digestion (Ximenes et al., 2017), can enhance recovery of bacterial endophytes in plants, and could be further optimized to maximize recovery of bacterial endophytes in carrot taproots.

The second goal of this study was to determine how 16S primer sets can influence the interpretation of bacterial communities in carrot taproots, as well as identify the carrot taproot treatment and primer set combination, that maximizes the potential for researchers to characterize these communities. Targeted amplicon sequencing of the 16S ribosomal RNA gene (16S rRNA) has become a cornerstone in microbial ecology research. The 16S rRNA gene is present in all prokaryotes. It is desirable for taxonomic assignment because it contains both fast-evolving regions, which can be used to classify organisms at different taxonomic levels, as well as slowly-evolving regions, which are relatively conserved throughout many species (Sambo et al., 2018). The 16S rRNA gene is approximately 1600 base pairs long, and includes nine hypervariable regions (V1-V9) that vary in conservation. One primer set targeting the V4 region was widely used in many soil ecology studies as part of the Earth Microbiome Project, because it allowed researchers to target both bacteria and archaea (Gilbert et al., 2014). As tools for next generation sequencing evolved and the ability to sequence longer fragments became available, many researchers switched to primer sets targeting both the V3–V4 region (Thijs et al., 2017; Yergeau et al., 2015; Yergeau et al., 2014) because they yielded greater resolution of taxa, especially when studies were conducted in the rhizospheres of plants. Consequently, the V3-V4 region is now well represented in taxonomic databases, and unless the DNA belongs to very rare taxa, it is generally possible to assign these taxa at the phylum level using this primer set.

In this study, there were dramatic differences in the amplification of plastid and mitochondrial sequences based on the three primer sets used (Figure 3.1; Table 2). The blocking primers significantly reduced amplification of mitochondria compared to the standard V3-V4 primer set, but instead, amplification of plastids increased substantially. These results differ from those of Arenz et al. (2015), who used these blocking primers to characterize bacterial endophytes in *Sorghum* leaves, and observed almost zero recovery of chloroplasts and mitochondrial DNA. This difference could be due to the composition of chloroplasts within carrot taproots in comparison with other plant species and tissues. For example, chromoplasts, which aid in the synthesis and storage of carotenes, are the most abundant non-photosynthetic plastids found in carrots (Egea et al., 2010). Despite their common origin and apparent similarities in DNA content with chloroplasts, there is evidence that methylation and inversions during the development of various plastid types does alter DNA structure (Hansmann, 1987), and therefore could affect the efficacy of blocking primers in reducing the amplification of different types of plastids.

Mismatch primers (799F-1193R) have been previously been shown to completely eliminate chloroplast/plastid DNA sequences in the roots, stems and leaf tissues of several plant species including *Populus spp., Oriza sativa* and *Arabidopsis thaliana* (Beckers et al., 2016; Bulgarelli et al., 2012; Sun et al., 2008). This was not the case in our carrot taproots. However, these primers did dramatically reduce the amplification of mitochondria when compared with the standard V3-V4 primer set, and resulted in greater amplification of bacteria, especially when they were coupled with the hollow fiber microfiltration and enzymatic digestion protocol.

The choice of primer set also interacted with the carrot taproot processing treatment to influence how the composition of bacteria in carrot taproots were assigned using the SILVA taxonomic database. The primer set targeting the V3-V4 region, resulted in amplification of the lowest total number of OTUs (Figure 3.2; Table 3.3), which was likely due to the fact that these primers amplified so many plant co-products and the sequencing coverage used was too low. The preprocessing filtration and enzymatic digestion procedure did not significantly increase the total number of OTUs amplified with this primer set. However, at the phylum level, unique OTUs corresponding to *Acidobacteria*, candidate *TM7* and *Verrumicrobia* were only amplified when using this primer set (Figure 3.5). Bacteria in these phyla are all thought to be ecologically important bacteria in soil, though they have not yet been well studied because they tend to be present in low concentrations, and are difficult to culture. Using the V3-V4 primer set also resulted in the amplification of many sequences that were unassigned. The reason for this is unclear. It is possible that these primer sets were able to amplify a diverse set of bacteria present in carrot taproots that have not yet been identified in other studies. Alternatively, it could indicate that the high amount of non-target DNA amplified using these primers altered the quality of the bacterial sequences resulting in poor assignment specificity within the SILVA reference database. Consequently, we conclude that these primers are valuable for amplifying unique taxa in carrot taproots, though further research to optimize preprocessing will be required to eliminate co-products and increase amplification of bacteria.

Coupling treatment of the carrot taproots using hollow fiber microfiltration followed by enzymatic digestion with the use of either the blocking or mismatch primers resulted in the highest total number of OTUs (Figure 3.2; Table 3.3) and greatest overall diversity (Figure 3.4; Table 3.4). However, the assignment of bacterial OTUs was different when using these primer sets than the V3-V4 (Figure 3.5). For example, unique bacteria belonging to *Archaeplastida, Armatimonadetes*,

BD1_5, and *Chlamydiae* were assigned when using the blocking primers, and the total number of phyla was reduced to only seven phyla when using the mismatch primers. Modifications used to reduce chloroplast amplification in primer sets have previously been shown to introduce biases against bacterial groups that are abundant in soil (Lundberg et al., 2013). Since most endophytic bacteria residing in carrot taproots are likely to have come from the soil, it is not surprising that these primer sets might introduce the same biases in carrot roots. Primer sets targeting the V3-V4 and V5-V7 rRNA regions have also been shown to lead to different interpretation of bacterial communities in gut microbiome studies. For example, *Lenthispherae* and *Bacteroidetes* appeared more abundant using primers targeting the V4-V5 region, while *Actinobacteria* and *Proteobacteria* were appeared higher with using primers targeting the V3-V4 region (Rintala et al., 2017). When moving to the level of genera, differences in the assignment of bacterial communities became even more divergent when using the three primer sets evaluated in this trial (Figure 3.6). These results highlight the challenge associated with identifying the best primer set for use in microbial studies, and it may be necessary to use multiple primer sets to uncover the true diversity of taxa present within plant roots. Future studies should consider using qPCR to amplify individual bacterial groups, in order to determine which primer sets provide the most accurate assignment of bacterial taxa.

While subjecting carrot taproots to hollow fiber microfiltration followed by enzymatic digestion increased the total amount (Figure 3.2; Table 3.3) and diversity (Figure 3.4; Table 3.4) of OTUs amplified in carrot taproots, it is possible that the enzymatic digestion step increased the relative abundance of certain taxa during this processing step. This procedure was originally developed to isolate *Proteobacteria* like *Salmonella* and *Escherchia* (Wang et al., 2016; Ximenes et al., 2017), but it has not yet been determined how this processing step could affect other bacteria, such as those belonging to *Firmicutes* or *Actinobacteria.* In this study, this processing step appeared to increase the relative abundance of Proteobacteria (Figure 3.5), specifically increasing the abundance of individual genera such as *Xanthomonas*, but not others such as *Pantoea.* Again, follow up studies using qPCR are needed to confirm these changes, however based on the results of this study, it is plausible to assume this might be occurring. While this is not desirable for studies investigating the total composition of bacterial endophytes in plant roots in general, it could be exploited in studies trying to isolate specific genera from carrot tissue, for example, genera with potential plant growth promoting activities.

Finally, given the differences in bacterial communities amplified using the different carrot tissue and primer set treatments evaluated in this study, we cannot make definitive conclusions about the true composition of bacterial endophytes in the carrot taproots used in this trial. However, because *Proteobacteria*, *Actinobacteria*, *Bacteroidetes, and Firmicutes* phyla were abundant regardless of the combination of treatments used, we can conclude that these bacteria are important in carrot taproots. Other studies investing bacterial endophytes in *Populus spp.* (Beckers et al., 2016; Thijs et al., 2017) and *A. thaliana* (Bodenhausen et al., 2013) have also found that these phyla dominated root bacterial communities, indicating that they are important root endophytes regardless of the plant species. Since many individual genera within these broad phyla have been demonstrated to have antagonistic activity against pathogens, and/or provide other plant growth promoting activities for plants (Reeve et al., 2015), it would be worth following up to investigate their role in carrot plants. For example, the *Firmicutes* phyla contains genera such as *Bacillus,* which often have plant growth promoting properties (Radhakrishnan et al., 2017)

3.5 Conclusions

New approaches are needed to study bacterial endophytes in carrot taproots. Subjecting carrot taproot tissues to a technique previously developed to isolate foodborne pathogens from plant tissues (Ximenes et al., 2017) has potential to better separate bacterial cells in carrot taproots, allowing for greater resolution of the endophyte community using NGS. However, the procedure may need to be further optimized to prevent enrichment of individual microbial taxa during this processing step. Accurately characterizing the composition of bacterial endophytes in carrot taproots may require multiple primer sets to ensure that rare taxa can be identified. However, if researchers are interested in relative differences in the composition of these communities in response to some particular treatment, then a previously developed mismatch primer set (Hanshew et al., 2013) has the greatest potential to distinguish between a large number of bacterial OTUs.

3.6 References

Abdelrazek, S. (n.d.). Crop management system and carrot genotype affect endophyte composition and Alternaria dauci suppression. *PLoS ONE*.

Abdelrazek, S. (2018). *Carrot endophytes: Diversity, ecology and function*. Purdue University.

Ahmad, T., Cawood, M., Iqbal, Q., & Batool, A. (2017). Phytochemicals in Daucus carota and their importance in nutrition – Review article, 1–22. https://doi.org/10.7287/peerj.preprints.3187v1

Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data.

Arenz, B. E., Schlatter, D. C., Bradeen, J. M., & Kinkel, L. L. (2015). Blocking primers reduce co-amplification of plant DNA when studying bacterial endophyte communities. *Journal of Microbiological Methods*, *117*, 1–3. https://doi.org/10.1016/j.mimet.2015.07.003

Arruda, P., Imperial, J., de Araújo, L. M., de Souza, R. S. C., Armanhi, J. S. L., & Damasceno, N. de B. (2018). A Community-Based Culture Collection for Targeting Novel Plant Growth-Promoting Bacteria from the Sugarcane Microbiome. *Frontiers in Plant Science*, *8*(January), 1–17. https://doi.org/10.3389/fpls.2017.02191

Bardgett, R. D., Mommer, L., & De Vries, F. T. (2014). Going underground: Root traits as drivers of ecosystem processes. *Trends in Ecology and Evolution*, *29*(12), 692–699. https://doi.org/10.1016/j.tree.2014.10.006

Baresel, J. P., Zimmermann, G., & Reents, H. J. (2008). Effects of genotype and environment on N uptake and N partition in organically grown winter wheat (Triticum aestivum L.) in Germany. *Euphytica*, *163*(3), 347–354. https://doi.org/10.1007/s10681-008-9718-1

Bassi, D., Menossi, M., & Mattiello, L. (2018). Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. *Scientific Reports*, *8*(1), 1–13. https://doi.org/10.1038/s41598-018-20653-1

Bateman, A. S., Kelly, S. D., & Woolfe, M. (2007). Nitrogen Isotope Composition of Organically and Conventionally Grown Crops. https://doi.org/10.1021/jf0627726

Beckers, B., Op De Beeck, M., Thijs, S., Truyens, S., Weyens, N., Boerjan, W., & Vangronsveld, J. (2016). Performance of 16s rDNA primer pairs in the study of rhizosphere and endosphere bacterial microbiomes in metabarcoding studies. *Frontiers in Microbiology*, *7*(MAY). https://doi.org/10.3389/fmicb.2016.00650

Bell, C. W., Asao, S., Calderon, F., Wolk, B., & Wallenstein, M. D. (2015). Plant nitrogen uptake drives rhizosphere bacterial community assembly during plant growth. *Soil Biology and Biochemistry*, *85*, 170–182. https://doi.org/10.1016/j.soilbio.2015.03.006

Bianco, A., Fancello, F., Balmas, V., Zara, G., Dettori, M., & Budroni, M. (2018). The microbiome of Sardinian barley and malt. *Journal of the Institute of Brewing*, *124*(4), 344–351. https://doi.org/10.1002/jib.522

Blagodatskaya, E., Littschwager, J., Lauerer, M., & Kuzyakov, Y. (2014). Plant traits regulating N capture define microbial competition in the rhizosphere. *European Journal of Soil Biology*, *61*, 41–48. https://doi.org/10.1016/j.ejsobi.2014.01.002

Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., & Kuzyakov, Y. (2010). Model of apparent and real priming effects: Linking microbial activity with soil organic matter decomposition. *Soil Biology and Biochemistry*, *42*(8), 1275–1283. https://doi.org/10.1016/j.soilbio.2010.04.005

Bodenhausen, N., Horton, M. W., & Bergelson, J. (2013). Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. *PLoS ONE*, *8*(2), e56329. https://doi.org/10.1371/journal.pone.0056329

Bowman, J. S., Rasmussen, S., Blom, N., Deming, J. W., Rysgaard, S., & Sicheritz-Ponten, T. (2012). Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. *ISME Journal*, *6*(1), 11–20. https://doi.org/10.1038/ismej.2011.76

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., … Schulze-Lefert, P. (2012). Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature*, *488*(7409), 91–95. https://doi.org/10.1038/nature11336

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., … Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, *108*(Supplement_1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

Caporaso, J. Gregory, Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., … Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(SUPPL. 1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

Caporaso, J Gregory, Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). correspondence QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. *Nature Publishing Group*, *7*(5), 335–336. https://doi.org/10.1038/nmeth0510-335

Card, S., Johnson, L., Teasdale, S., & Caradus, J. (2016). Deciphering endophyte behaviour: The link between endophyte biology and efficacious biological control agents. *FEMS Microbiology Ecology*, *92*(8), 1–19. https://doi.org/10.1093/femsec/fiw114

Chang, E.-H., Chung, R.-S., & Tsai, Y.-H. (2007). Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. *Soil Science and Plant Nutrition*, *53*(2), 132–140. https://doi.org/10.1111/j.1747-0765.2007.00122.x

Chelius, M. K., & Triplett, E. W. (2001). The diversity of archaea and bacteria in association with the roots of Zea mays L. *Microbial Ecology*, *41*(3), 252–263. https://doi.org/10.1007/s002480000087

Cheng, W. (2009). Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. *Soil Biology and Biochemistry*, *41*(9), 1795–1801. https://doi.org/10.1016/j.soilbio.2008.04.018

Cheng, Z., Park, E., & Glick, B. R. (2007). 1-Aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Canadian Journal of Microbiology*, *53*(7), 912–918. https://doi.org/10.1139/W07-050

Compant, S., Clément, C., & Sessitsch, A. (2010, May 1). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*. Pergamon. https://doi.org/10.1016/j.soilbio.2009.11.024

Coskun, D., Britto, D. T., Shi, W., & Kronzucker, H. J. (2017). How Plant Root Exudates Shape the Nitrogen Cycle. *Trends in Plant Science*, *22*(8), 661–673. https://doi.org/10.1016/j.tplants.2017.05.004

Cretoiu, M. S., Korthals, G. W., Visser, J. H. M., & Van Elsas, J. D. (2013). Chitin amendment increases soil suppressiveness toward plant pathogens and modulates the actinobacterial and oxalobacteraceal communities in an experimental agricultural field. *Applied and Environmental Microbiology*, *79*(17), 5291–5301. https://doi.org/10.1128/AEM.01361-13

Curtis, M. A., Zenobia, C., & Darveau, R. P. (2011, October 4). The relationship of the oral microbiotia to periodontal health and disease. *Cell Host and Microbe*. NIH Public Access. https://doi.org/10.1016/j.chom.2011.09.008

da Silva Dias, J. C. (2014). Nutritional and Health Benefits of Carrots and Their Seed Extracts. *Food and Nutrition Sciences*, *05*(22), 2147–2156. https://doi.org/10.4236/fns.2014.522227

de Vries, F. T., & Wallenstein, M. D. (2017, July 1). Below-ground connections underlying above-ground food production: a framework for optimising ecological connections in the rhizosphere. (R. Bardgett, Ed.), *Journal of Ecology*. Blackwell Publishing Ltd. https://doi.org/10.1111/1365-2745.12783

Dechorgnat, J., Nguyen, C. T., Armengaud, P., Jossier, M., Diatloff, E., Filleur, S., & Daniel-Vedele, F. (2011). From the soil to the seeds: The long journey of nitrate in plants. *Journal of Experimental Botany*, *62*(4), 1349–1359. https://doi.org/10.1093/jxb/erq409

Dijkstra, F. A., Carrillo, Y., Pendall, E., & Morgan, J. A. (2013). Rhizosphere priming: A nutrient perspective. *Frontiers in Microbiology*, *4*(JUL), 1–8. https://doi.org/10.3389/fmicb.2013.00216

Dijkstra, F. A., Morgan, J. A., Blumenthal, D., & Follett, R. F. (2010). Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. *Soil Biology and Biochemistry*, *42*(7), 1073–1082. https://doi.org/10.1016/j.soilbio.2010.02.026

Dyall, S. D., Brown, M. T., & Johnson, P. J. (2004). Ancient Invasions: From Endosymbionts to Organelles. *Science*, *304*(5668), 253–257. https://doi.org/10.1126/science.1094884

Egea, I., Barsan, C., Bian, W., Purgatto, E., Latché, A., Chervin, C., … Pech, J. C. (2010). Chromoplast differentiation: Current status and perspectives. *Plant and Cell Physiology*, *51*(10), 1601–1611. https://doi.org/10.1093/pcp/pcq136

Eida, A. A., Ziegler, M., Lafi, F. F., Michell, C. T., Voolstra, C. R., Hirt, H., & Saad, M. M. (2018). Desert plant bacteria reveal host influence and beneficial plant growth properties. *Plos One*, *13*(12), e0208223. https://doi.org/10.1371/journal.pone.0208223

Eivazi, F., & Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, *20*(5), 601–606. https://doi.org/10.1016/0038-0717(88)90141-1

Fageria, N. K., & Baligar, V. C. (2005, January 1). Enhancing Nitrogen Use Efficiency in Crop Plants. *Advances in Agronomy*. Academic Press. https://doi.org/10.1016/S0065-2113(05)88004-6

FAO. (2018). Food and Agriculture Organization of the United Nations Statistics database. Retrieved February 10, 2020, from http://www.fao.org/faostat/en/#data/QC/visualize

Finzi, A. C., Abramoff, R. Z., Spiller, K. S., Brzostek, E. R., Darby, B. A., Kramer, M. A., & Phillips, R. P. (2015). Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Global Change Biology*, *21*(5), 2082–2094. https://doi.org/10.1111/gcb.12816

Fitzpatrick, C. R., Copeland, J., Wang, P. W., Guttman, D. S., Kotanen, P. M., & Johnson, M. T. J. (2018). Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences*, 201717617. https://doi.org/10.1073/pnas.1717617115

Frank, A., Saldierna Guzmán, J., & Shay, J. (2017). Transmission of Bacterial Endophytes. *Microorganisms*, *5*(4), 70. https://doi.org/10.3390/microorganisms5040070

Funk, J. L., Glenwinkel, L. A., & Sack, L. (2013). Differential Allocation to Photosynthetic and Non-Photosynthetic Nitrogen Fractions among Native and Invasive Species. *PLoS ONE*, *8*(5). https://doi.org/10.1371/journal.pone.0064502

Gallart, M., Adair, K. L., Love, J., Meason, D. F., Clinton, P. W., Xue, J., & Turnbull, M. H. (2018). Host Genotype and Nitrogen Form Shape the Root Microbiome of Pinus radiata. *Microbial Ecology*, *75*(2), 419–433. https://doi.org/10.1007/s00248-017-1055-2

GARNETT, T., CONN, V., & KAISER, B. N. (2009). Root based approaches to improving nitrogen use efficiency in plants. *Plant, Cell & Environment*, *32*(9), 1272–1283. https://doi.org/10.1111/j.1365-3040.2009.02011.x

Ghyselinck, J., Pfeiffer, S., Heylen, K., Sessitsch, A., & De Vos, P. (2013). The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies. *PloS One*, *8*(8), 1–14. https://doi.org/10.1371/journal.pone.0071360

Giagnoni, L., Pastorelli, R., Mocali, S., Arenella, M., Nannipieri, P., & Renella, G. (2016). Availability of different nitrogen forms changes the microbial communities and enzyme activities in the rhizosphere of maize lines with different nitrogen use efficiency. *Applied Soil Ecology*, *98*, 30–38. https://doi.org/10.1016/j.apsoil.2015.09.004

Gilbert, J. A., Jansson, J. K., & Knight, R. (2014, August 22). The Earth Microbiome project: Successes and aspirations. *BMC Biology*. BioMed Central Ltd. https://doi.org/10.1186/s12915-014-0069-1

Grime, J. P. (1977). Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. *The American Naturalist*, *111*(982), 1169–1194. https://doi.org/10.1086/283244

Hadas, A., Kautsky, L., Goek, M., & Kara, E. E. (2004). Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. *Soil Biology and Biochemistry*, *36*(2), 255–266. https://doi.org/10.1016/j.soilbio.2003.09.012

Haichar, F. el Z., Santaella, C., Heulin, T., & Achouak, W. (2014). Root exudates mediated interactions belowground. *Soil Biology and Biochemistry*, *77*, 69–80. https://doi.org/10.1016/j.soilbio.2014.06.017

Hanshew, A. S., Mason, C. J., Raffa, K. F., & Currie, C. R. (2013). Minimization of chloroplast contamination in 16S rRNA gene pyrosequencing of insect herbivore bacterial communities. *Journal of Microbiological Methods*, *95*(2), 149–155. https://doi.org/10.1016/j.mimet.2013.08.007

Hansmann, P. (1987). Daffodil Chromoplast DNA : Comparison with Chloroplast DNA , Physical Map and Gene Localization, *122*(1987), 118–122.

Hardoim, P. R., van Overbeek, L. S., & Elsas, J. D. van. (2008). Properties of bacterial endophytes and their proposed role in plant growth. *Trends in Microbiology*, *16*(10), 463–471. https://doi.org/10.1016/j.tim.2008.07.008

Hargreaves, S. K., Williams, R. J., & Hofmockel, K. S. (2015). Environmental filtering of microbial communities in agricultural soil shifts with crop growth. *PLoS ONE*, *10*(7), e0134345. https://doi.org/10.1371/journal.pone.0134345

Hartz, T. K., & Johnstone, P. R. (2006). Nitrogen availability from high-nitrogen-containing organic fertilizers. *HortTechnology*, *16*(1), 39–42. https://doi.org/10.21273/horttech.16.1.0039

Heijboer, A., ten Berge, H. F. M., de Ruiter, P. C., Jørgensen, H. B., Kowalchuk, G. A., & Bloem, J. (2016). Plant biomass, soil microbial community structure and nitrogen cycling under different organic amendment regimes; a 15N tracer-based approach. *Applied Soil Ecology*, *107*, 251–260. https://doi.org/10.1016/j.apsoil.2016.06.009

Hirel, B., Tétu, T., Lea, P. J., & Dubois, F. (2011). Improving nitrogen use efficiency in crops for sustainable agriculture. *Sustainability*, *3*(9), 1452–1485. https://doi.org/10.3390/su3091452

Huo, C., Luo, Y., & Cheng, W. (2017). Rhizosphere priming effect: A meta-analysis. *Soil Biology and Biochemistry*, *111*, 78–84. https://doi.org/10.1016/j.soilbio.2017.04.003

Junaidi, J., Kallenbach, C. M., Byrne, P. F., & Fonte, S. J. (2018). Root traits and root biomass allocation impact how wheat genotypes respond to organic amendments and earthworms. *PLoS ONE*, *13*(7), 1–24. https://doi.org/10.1371/journal.pone.0200646

Kant, S. (2017). Understanding nitrate uptake, signaling and remobilisation for improving plant nitrogen use efficiency. *Seminars in Cell and Developmental Biology*, *74*, 89–96. https://doi.org/10.1016/j.semcdb.2017.08.034

Kim, G. S., Seok, J. H., Mark, T. B., & Reed, M. R. (2019). The price relationship between organic and non-organic vegetables in the U.S.: evidence from Nielsen scanner data. *Applied Economics*, *51*(10), 1025–1039. https://doi.org/10.1080/00036846.2018.1524570

Köberl, M., Schmidt, R., Ramadan, E. M., Bauer, R., & Berg, G. (2013). The microbiome of medicinal plants: Diversity and importance for plant growth, quality, and health. *Frontiers in Microbiology*, *4*(DEC), 1–9. https://doi.org/10.3389/fmicb.2013.00400

Kõiv, V., Arbo, K., Maiväli, Ü., Kisand, V., Roosaare, M., Remm, M., & Tenson, T. (2019). Endophytic bacterial communities in peels and pulp of five root vegetables. *PLoS ONE*, *14*(1), 1–17. https://doi.org/10.1371/journal.pone.0210542

Krueger, F. (2015). Wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files. *The Babraham Institute, Bioinformatics Group*.

Ku, S., Ximenes, E., Kreke, T., Foster, K., Deering, A. J., & Ladisch, M. R. (2016). Microfiltration of enzyme treated egg whites for accelerated detection of viable Salmonella. *Biotechnology Progress*, *32*(6), 1464–1471. https://doi.org/10.1002/btpr.2343

Kuzyakov, Y. (2002). Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition & Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, *165*(4), 382–396. https://doi.org/10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2-#

Kwak, M.-J., Kong, H. G., Choi, K., Kwon, S.-K., Song, J. Y., Lee, J., … Kim, J. F. (2018). Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nature Biotechnology*, *36*(11). https://doi.org/10.1038/nbt.4232

Laberge, G., Ambus, P., Hauggaard-Nielsen, H., & Jensen, E. S. (2006). Stabilization and plant uptake of N from 15N-labelled pea residue 16.5 years after incorporation in soil. *Soil Biology and Biochemistry*, *38*(7), 1998–2000. https://doi.org/10.1016/j.soilbio.2005.11.023

Lammerts van Bueren, E. T., & Struik, P. C. (2017). Diverse concepts of breeding for nitrogen use efficiency. A review. *Agronomy for Sustainable Development*, *37*(5). https://doi.org/10.1007/s13593-017-0457-3

Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: Mechanisms are the key to understanding production systems. In *Journal of Experimental Botany* (Vol. 53, pp. 773–787). Oxford University Press. https://doi.org/10.1093/jxb/53.370.773

Liu, L., Liang, M., Li, L., Sun, L., Xu, Y., Gao, J., … Huang, S. (2018). Synergistic effects of the combined application of Bacillus subtilis H158 and strobilurins for rice sheath blight control. *Biological Control*, *117*(August 2017), 182–187. https://doi.org/10.1016/j.biocontrol.2017.11.011

Liu, T., Ren, T., White, P. J., Cong, R., & Lu, J. (2018). Storage nitrogen co-ordinates leaf expansion and photosynthetic capacity in winter oilseed rape. *Journal of Experimental Botany*, *69*(12), 2995–3007. https://doi.org/10.1093/jxb/ery134

Liu, X., Vibbert, H. B., Foster, K., Ladisch, M. R., Amalaradjou, M. A. R., Jones, J., … Li, X. (2013). Rapid Sample Processing for Detection of Food-Borne Pathogens via Cross-Flow Microfiltration. *Applied and Environmental Microbiology*, *79*(22), 7048–7054. https://doi.org/10.1128/aem.02587-13

Ljiljana Boskovic-Rakocevic. (2012). Effect of nitrogen fertilization on carrot quality. *African Journal of Agricultural Reseearch*, *7*(18), 2884–2890. https://doi.org/10.5897/ajar11.1652

Louarn, S., Nawrocki, A., Thorup-Kristensen, K., Lund, O. S., Jensen, O. N., Collinge, D. B., & Jensen, B. (2013). Proteomic changes and endophytic micromycota during storage of organically and conventionally grown carrots. *Postharvest Biology and Technology*, *76*, 26–33. https://doi.org/10.1016/j.postharvbio.2012.08.011

Lucier, G., & Lin, B. H. (2011). Factors affecting carrot consumption in the United States. *Eating Right: The Consumption of Fruits and Vegetables*, 199–220.

Lundberg, D. S., Yourstone, S., Mieczkowski, P., Jones, C. D., & Dangl, J. L. (2013). Practical innovations for high-throughput amplicon sequencing. *Nature Methods*, *10*(10), 999–1002. https://doi.org/10.1038/nmeth.2634

Madigan, M., Cox, S. S., & Stegeman, R. A. (1984). Nitrogen fixation and nitrogenase activities in members of the family Rhodospirillaceae. *Journal of Bacteriology*, *157*(1), 73–78. https://doi.org/10.1128/jb.157.1.73-78.1984

Malfanova, N., Lugtenberg, B. J. J., & Berg, G. (2013). Bacterial Endophytes: Who and Where, and What are they doing there? *Molecular Microbial Ecology of the Rhizosphere*, *1*, 391–403. https://doi.org/10.1002/9781118297674.ch36

Meena, V. S., Meena, S. K., Verma, J. P., Kumar, A., Aeron, A., Mishra, P. K., … Dotaniya, M. L. (2017). Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: A review. *Ecological Engineering*, *107*, 8–32. https://doi.org/10.1016/j.ecoleng.2017.06.058

Meier, I. C., Finzi, A. C., & Phillips, R. P. (2017). Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. *Soil Biology and Biochemistry*, *106*, 119–128. https://doi.org/10.1016/j.soilbio.2016.12.004

Menneer, J. C., Sprosen, M. S., & Ledgard, S. F. (2016). Effect of timing and formulation of dicyandiamide (DCD) application on nitrate leaching and pasture production in a Bay of Plenty pastoral soil, *8233*(October). https://doi.org/10.1080/00288230809510468

Mikkelsen, R., & Hartz, T. K. (2008). Nitrogen Sources for Organic Crop Production. Better Crops. Vol. 92, No. 4. *Better Crops*, *92*(4), 16–19.

Moreau, D., Bardgett, R. D., Finlay, R. D., Jones, D. L., & Philippot, L. (2019). A plant perspective on nitrogen cycling in the rhizosphere. *Functional Ecology*, *33*(4), 540–552. https://doi.org/10.1111/1365-2435.13303

Murphy, C. J., Baggs, E. M., Morley, N., Wall, D. P., & Paterson, E. (2015). Rhizosphere priming can promote mobilisation of N-rich compounds from soil organic matter. *Soil Biology and Biochemistry*, *81*, 236–243. https://doi.org/10.1016/j.soilbio.2014.11.027

Musyoka, M. W., Adamtey, N., Muriuki, A. W., Bautze, D., Karanja, E. N., Mucheru-Muna, M., … Cadisch, G. (2019). Nitrogen leaching losses and balances in conventional and organic farming systems in Kenya. *Nutrient Cycling in Agroecosystems*, *114*(3), 237–260. https://doi.org/10.1007/s10705-019-10002-7

Muyzer, G., De Waal,' And, E. C., & Uitierlinden2, A. G. (1993). *Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16S rRNA Downloaded from*. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*. Retrieved from http://aem.asm.org/

Mwafulirwa, L., Baggs, E. M., Russell, J., George, T., Morley, N., Sim, A., … Paterson, E. (2016). Barley genotype influences stabilization of rhizodeposition-derived C and soil organic matter mineralization. *Soil Biology and Biochemistry*, *95*(April), 60–69. https://doi.org/10.1016/j.soilbio.2015.12.011

Mwafulirwa, L. D., Baggs, E. M., Russell, J., Morley, N., Sim, A., & Paterson, E. (2017). Combined effects of rhizodeposit C and crop residues on SOM priming, residue mineralization and N supply in soil. *Soil Biology and Biochemistry*, *113*, 35–44. https://doi.org/10.1016/j.soilbio.2017.05.026

Navarrete, A. A., Soares, T., Rossetto, R., van Veen, J. A., Tsai, S. M., & Kuramae, E. E. (2015). Verrucomicrobial community structure and abundance as indicators for changes in chemical factors linked to soil fertility. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *108*(3), 741–752. https://doi.org/10.1007/s10482-015-0530-3

Navazio, J. (2014). How to Breed Carrots for Organic Agriculture. *Organic Seed Alliance*.

Nithya, A., & Babu, S. (2017). Prevalence of plant beneficial and human pathogenic bacteria isolated from salad vegetables in India. *BMC Microbiology*, *17*(1), 1–16. https://doi.org/10.1186/s12866-017-0974-x

Oksanen, J. (2007). vegan : Community Ecology Package. R package version 1.8-5.

Pajares, S., & Bohannan, B. J. M. (2016). Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. *Frontiers in Microbiology*, *7*(JUL), 1–20. https://doi.org/10.3389/fmicb.2016.01045

Pausch, J., Loeppmann, S., Kï¿½hnel, A., Forbush, K., Kuzyakov, Y., & Cheng, W. (2016). Rhizosphere priming of barley with and without root hairs. *Soil Biology and Biochemistry*, *100*, 74–82. https://doi.org/10.1016/j.soilbio.2016.05.009

Pausch, J., Zhu, B., Kuzyakov, Y., & Cheng, W. (2013). Plant inter-species effects on rhizosphere priming of soil organic matter decomposition. *Soil Biology and Biochemistry*, *57*, 91–99. https://doi.org/10.1016/j.soilbio.2012.08.029

Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., … Ley, R. E. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(16), 6548–6553. https://doi.org/10.1073/pnas.1302837110

Poirier, V., Roumet, C., & Munson, A. D. (2018). The root of the matter: Linking root traits and soil organic matter stabilization processes. *Soil Biology and Biochemistry*, *120*(August 2017), 246–259. https://doi.org/10.1016/j.soilbio.2018.02.016

Prieto, K. R., Echaide-Aquino, F., Huerta-Robles, A., Valério, H. P., Macedo-Raygoza, G., Prado, F. M., … Beltran-García, M. J. (2017). Endophytic bacteria and rare earth elements; promising candidates for nutrient use efficiency in plants. In *Plant Macronutrient Use Efficiency: Molecular and Genomic Perspectives in Crop Plants* (pp. 285–306). Elsevier Inc. https://doi.org/10.1016/B978-0-12-811308-0.00016-8

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Rg Peplies, J., & Glö Ckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, *35*(21), 7188–7196. https://doi.org/10.1093/nar/gkm864

R Core Team. (2019). No Title.

Radhakrishnan, R., Hashem, A., & Abd Allah, E. F. (2017). Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, *8*(SEP), 667. https://doi.org/10.3389/fphys.2017.00667

Rakotoson, T., Dusserre, J., Letourmy, P., Ramonta, I. R., Cao, T. V., Ramanantsoanirina, A., … Raboin, L. M. (2017). Genetic variability of nitrogen use efficiency in rainfed upland rice. *Field Crops Research*, *213*(May), 194–203. https://doi.org/10.1016/j.fcr.2017.07.023

Reasoner, D. J., & Geldreich, E. E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Applied and Environmental Microbiology*, *49*(1), 1–7. https://doi.org/10.1128/aem.49.1.1-7.1985

Reeve, W., Ardley, J., Tian, R., Eshragi, L., Yoon, J. W., Ngamwisetkun, P., … Kyrpides, N. C. (2015, February 1). A Genomic Encyclopedia of the root nodule bacteria: Assessing genetic diversity through a systematic biogeographic survey. *Standards in Genomic Sciences*. BioMed Central Ltd. https://doi.org/10.1186/1944-3277-10-14

Reid, J. B., Hunt, A. G., Johnstone, P. R., Searle, B. P., & Jesson, L. K. (2017). On the responses of carrots (*Daucus carota* L.) to nitrogen supply. *New Zealand Journal of Crop and Horticultural Science*, *0*(0), 1–21. https://doi.org/10.1080/01140671.2017.1402790

Rintala, A., Pietilä, S., Munukka, E., Eerola, E., Pursiheimo, J. P., Laiho, A., … Huovinen, P. (2017). Gut microbiota analysis results are highly dependent on the 16s rRNA gene target region, whereas the impact of DNA extraction is minor. *Journal of Biomolecular Techniques*, *28*(1), 19–30. https://doi.org/10.7171/jbt.17-2801-003

Ros, M., Hernandez, M. T., & García, C. (2003). Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biology and Biochemistry*, *35*(3), 463–469. https://doi.org/10.1016/S0038-0717(02)00298-5

Rozpądek, P., Domka, A., Ważny, R., Nosek, M., Jędrzejczyk, R., Tokarz, K., & Turnau, K. (2018). How does the endophytic fungus Mucor sp. improve Arabidopsis arenosa vegetation in the degraded environment of a mine dump? *Environmental and Experimental Botany*, *147*(August), 31–42. https://doi.org/10.1016/j.envexpbot.2017.11.009

Rudisill, M. A., Turco, R. F., & Hoagland, L. A. (2016). Fertility practices and rhizosphere effects alter ammonia oxidizer community structure and potential nitrification activity in pepper production soils. *Applied Soil Ecology*, *99*, 70–77. https://doi.org/10.1016/j.apsoil.2015.10.011

Sagan, L. (1967). On the origin of mitosing cells. *Journal of Theoretical Biology*, *14*(3), 225-IN6. https://doi.org/10.1016/0022-5193(67)90079-3

Sambo, F., Finotello, F., Lavezzo, E., Baruzzo, G., Masi, G., Peta, E., … Di Camillo, B. (2018). Optimizing PCR primers targeting the bacterial 16S ribosomal RNA gene. *BMC Bioinformatics*, *19*(1), 343. https://doi.org/10.1186/s12859-018-2360-6

Sasse, J., Martinoia, E., & Northen, T. (2018). Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? *Trends in Plant Science*, *23*(1), 25–41. https://doi.org/10.1016/j.tplants.2017.09.003

Schenk, H. J. (2006). Root competition: beyond resource depletion. *Journal of Ecology*, *94*(4), 725–739. https://doi.org/10.1111/j.1365-2745.2006.01124.x

Schulz, B., & Boyle, C. (2007). What are Endophytes? In *Microbial Root Endophytes* (pp. 1–13). Springer Berlin Heidelberg. https://doi.org/10.1007/3-540-33526-9_1

Shannon, C. E., & Weaver, W. (1964). *The mathematical theory of communication*. University of Illinois Press.

Shaver, J. M., Oldenburg, D. J., & Bendich, A. J. (2006). Changes in chloroplast DNA during development in tobacco, Medicago truncatula, pea, and maize. *Planta*, *224*(1), 72–82. https://doi.org/10.1007/s00425-005-0195-7

Simon, P. W. (2019). Economic and Academic Importance (pp. 1–8). https://doi.org/10.1007/978-3-030-03389-7_1

Sinclair, L., Osman, O. A., Bertilsson, S., & Eiler, A. (2015). Microbial community composition and diversity via 16S rRNA gene amplicons: Evaluating the illumina platform. *PLoS ONE*, *10*(2), 1–18. https://doi.org/10.1371/journal.pone.0116955

Stein, L. Y., & Klotz, M. G. (2016). The nitrogen cycle. *Current Biology*, *26*(3), R94–R98. https://doi.org/10.1016/j.cub.2015.12.021

Strokal, M., & Kroeze, C. (2014). Nitrous oxide (N2O) emissions from human waste in 1970-2050. *Current Opinion in Environmental Sustainability*, *9*(3), 108–121. https://doi.org/10.1016/j.cosust.2014.09.008

Subbarao, G. V., Sahrawat, K. L., Nakahara, K., Ishikawa, T., Kishii, M., Rao, I. M., … Lata, J. C. (2012). *Biological nitrification inhibition-a novel strategy to regulate nitrification in agricultural systems*. *Advances in Agronomy* (1st ed., Vol. 114). Elsevier Inc. https://doi.org/10.1016/B978-0-12-394275-3.00001-8

Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X., & Song, W. (2008). Endophytic bacterial diversity in rice (Oryza sativa L.) roots estimated by 16S rDNA sequence analysis. *Microbial Ecology*, *55*(3), 415–424. https://doi.org/10.1007/s00248-007-9287-1

Surette, M. A., Sturz, A. V, Lada, R. R., & Nowak, J. (2003). Bacterial endophytes in processing carrots (Daucus carota L. var. sativus): their localizations, population density, biodiversity and their effects on plant growth. *Plant and Soil*, *253*, 381–390.

Terrazas, R. A., Pietrangelo, L., Corral, A. M., Torres-Cortés, G., Robertson-Albertyn, S., Balbirnie-Cumming, K., … Bulgarelli, D. (2019). Nitrogen Availability Modulates the Host Control of the Barley Rhizosphere Microbiota. *BioRxiv*, 605204. https://doi.org/10.1101/605204

Theobald, J. C., Mitchell, R. A. C., Parry, M. A. J., & Lawlor, D. W. (1998). Estimating the excess investment in ribulose-1, 5-bisphosphate carboxylase/oxygenase in leaves of spring wheat grown under elevated CO2. *Plant Physiology*, *118*(3), 945–955. https://doi.org/10.1104/pp.118.3.945

Thijs, S., Op De Beeck, M., Beckers, B., Truyens, S., Stevens, V., Van Hamme, J. D., … Vangronsveld, J. (2017). Comparative Evaluation of Four Bacteria-Specific Primer Pairs for 16S rRNA Gene Surveys. *Frontiers in Microbiology*, *8*(MAR), 494. https://doi.org/10.3389/fmicb.2017.00494

Thorup-Kristensen, K. (2006). Root growth and nitrogen uptake of carrot, early cabbage, onion and lettuce following a range of green manures. *Soil Use and Management*, *22*(1), 29–38. https://doi.org/10.1111/j.1475-2743.2005.00012.x

Tian, B., Zhang, C., Ye, Y., Wen, J., Wu, Y., Wang, H., … Zhang, K. (2017). Beneficial traits of bacterial endophytes belonging to the core communities of the tomato root microbiome. *Agriculture, Ecosystems and Environment*, *247*(2), 149–156. https://doi.org/10.1016/j.agee.2017.06.041

Tiemens-Hulscher, M., Lammerts van Bueren, E. T., & Struik, P. C. (2014). Identifying nitrogen-efficient potato cultivars for organic farming. *Euphytica*, *199*(1–2), 137–154. https://doi.org/10.1007/s10681-014-1143-z

Toju, H., Peay, K. G., Yamamichi, M., Narisawa, K., & Hiruma, K. (2018). Core microbiomes for sustainable agroecosystems. *Nature Plants*, *4*(May). https://doi.org/10.1038/s41477-018-0139-4

Turner, T. R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D., … Poole, P. S. (2013). Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. *ISME Journal*, *7*(12), 2248–2258. https://doi.org/10.1038/ismej.2013.119

Upreti, R., & Thomas, P. (2015). Root-associated bacterial endophytes from Ralstonia solanacearum resistant and susceptible tomato cultivars and their pathogen antagonistic effects. *Frontiers in Microbiology*, *6*(MAR). https://doi.org/10.3389/fmicb.2015.00255

Van Bueren, E. T. L., Struik, P. C., & Jacobsen, E. (2002). Ecological concepts in organic farming and their consequences for an organic crop ideotype. *Netherlands Journal of Agricultural Science*, *50*(1), 1–26. https://doi.org/10.1016/s1573-5214(02)80001-x

van der Voort, M., Kempenaar, M., van Driel, M., Raaijmakers, J. M., & Mendes, R. (2016, April 1). Impact of soil heat on reassembly of bacterial communities in the rhizosphere microbiome and plant disease suppression. *Ecology Letters*. Blackwell Publishing Ltd. https://doi.org/10.1111/ele.12567

Veitch, R. S., Lada, R. R., Adams, A., & MacDonald, M. T. (2014). Carrot Yield and Quality as Influenced by Nitrogen Application in Cut-and-Peel Carrots. *Communications in Soil Science and Plant Analysis*, *45*(7), 887–895. https://doi.org/10.1080/00103624.2014.880713

Vestheim, H., & Jarman, S. N. (2008). Blocking primers to enhance PCR amplification of rare sequences in mixed samples - A case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology*, *5*, 1–11. https://doi.org/10.1186/1742-9994-5-12

Vibbert, H. B., Ku, S., Li, X., Liu, X., Ximenes, E., Kreke, T., … Gehring, A. G. (2015). Accelerating sample preparation through enzyme-assisted microfiltration of Salmonella in chicken extract. *Biotechnology Progress*, *31*(6), 1551–1562. https://doi.org/10.1002/btpr.2167

von Wirén, N., Gazzarrini, S., Gojon, A., & Frommer, W. B. (2000). The molecular physiology of ammonium uptake and retrieval. *Current Opinion in Plant Biology*, *3*(3), 254–261. https://doi.org/10.1016/s1369-5266(00)80074-6

Walitang, D. I., Kim, C.-G., Jeon, S., Kang, Y., & Sa, T. (2018). Conservation and transmission of seed bacterial endophytes across generations following crossbreeding and repeated inbreeding of rice at different geographic locations. *MicrobiologyOpen*, (February), e00662. https://doi.org/10.1002/mbo3.662

Wang, D., Wang, Z., He, F., Kinchla, A. J., & Nugen, S. (2016). Enzymatic Digestion for Improved Bacteria Separation from Leafy Green Vegetables. *Journal of Food Protection*, *79*(8), 1378–1386. https://doi.org/10.4315/0362-028x.jfp-15-581

Wang, S. S., Liu, J. M., Sun, J., Sun, Y. F., Liu, J. N., Jia, N., … Dai, X. F. (2019). Diversity of culture-independent bacteria and antimicrobial activity of culturable endophytic bacteria isolated from different Dendrobium stems. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-46863-9

Weaver, R. W., Angle, J. S., & Bottomley, P. S. (1994). *Methods of soil analysis, Part 2*. *American Society of Agronomy*. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:methods+of+soil+analysis+part2#1%5Cnhttp://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Methods+of+soil+analysis,+Part+2#1

Westerveld, S. M., McKeown, A. W., & McDonald, M. R. (2006). Seasonal nitrogen partitioning and nitrogen uptake of carrots as affected by nitrogen application in a mineral and an organic soil. *HortScience*, *41*(5), 1332–1338. https://doi.org/10.21273/hortsci.41.5.1332

Wicaksono, W. A., Eirian Jones, E., Monk, J., & Ridgway, H. J. (2017). Using bacterial endophytes from a New Zealand native medicinal plant for control of grapevine trunk diseases. *Biological Control*, *114*(August), 65–72. https://doi.org/10.1016/j.biocontrol.2017.08.003

Wick, K., Heumesser, C., & Schmid, E. (2012). Groundwater nitrate contamination: Factors and indicators. *Journal of Environmental Management*, *111*, 178–186. https://doi.org/10.1016/j.jenvman.2012.06.030

Ximenes, E., Hoagland, L., Ku, S., Li, X., & Ladisch, M. (2017). Human pathogens in plant biofilms: Formation, physiology, and detection. *Biotechnology and Bioengineering*, *114*(7), 1403–1418. https://doi.org/10.1002/bit.26247

Yao, H., & Wu, F. (2010). Soil microbial community structure in cucumber rhizosphere of different resistance cultivars to fusarium wilt. *FEMS Microbiology Ecology*, *72*(3), 456–463. https://doi.org/10.1111/j.1574-6941.2010.00859.x

Yergeau, E., Bell, T. H., Champagne, J., Maynard, C., Tardif, S., Tremblay, J., & Greer, C. W. (2015). Transplanting soil microbiomes leads to lasting effects on willow growth, but not on the rhizosphere microbiome. *Frontiers in Microbiology*, *6*(DEC). https://doi.org/10.3389/fmicb.2015.01436

Yergeau, E., Sanschagrin, S., Maynard, C., St-Arnaud, M., & Greer, C. W. (2014). Microbial expression profiles in the rhizosphere of willows depend on soil contamination. *ISME Journal*, *8*(2), 344–358. https://doi.org/10.1038/ismej.2013.163

Yin, H., Wheeler, E., & Phillips, R. P. (2014). Root-induced changes in nutrient cycling in forests depend on exudation rates. *Soil Biology and Biochemistry*, *78*, 213–221. https://doi.org/10.1016/j.soilbio.2014.07.022

Yin, L., Dijkstra, F. A., Wang, P., Zhu, B., & Cheng, W. (2018). Rhizosphere priming effects on soil carbon and nitrogen dynamics among tree species with and without intraspecific competition. *New Phytologist*, *218*(3), 1036–1048. https://doi.org/10.1111/nph.15074

Yiridoe, E. K., Bonti-Ankomah, S., & Martin, R. C. (2005). Comparison of consumer perceptions and preference toward organic versus conventionally produced foods: A review and update of the literature. *Renewable Agriculture and Food Systems*, *20*(4), 193–205. https://doi.org/10.1079/raf2005113

Zavalloni, C., Jenkins, J. R., Fornasier, F., Arnold, E. C., Girardin, C., Miglietta, F., … Rumpel, C. (2016). Biochar alters the soil microbiome and soil function: results of next-generation amplicon sequencing across Europe. *GCB Bioenergy*, *9*(3), 591–612. https://doi.org/10.1111/gcbb.12371

Zhang, J., Ding, X., Guan, R., Zhu, C., Xu, C., Zhu, B., … Lu, Z. (2018). Evaluation of different 16S rRNA gene V regions for exploring bacterial diversity in a eutrophic freshwater lake. *Science of the Total Environment*, *618*, 1254–1267. https://doi.org/10.1016/j.scitotenv.2017.09.228

Zhu, B., Gutknecht, J. L. M., Herman, D. J., Keck, D. C., Firestone, M. K., & Cheng, W. (2014). Rhizosphere priming effects on soil carbon and nitrogen mineralization. *Soil Biology and Biochemistry*, *76*, 183–192. https://doi.org/10.1016/j.soilbio.2014.04.033

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, *32*(5), 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x

CHAPTER 4. CONCLUSIONS

The role that plant microbiomes play in the health and productivity of agricultural crops is becoming increasingly apparent. The studies outlined in this thesis demonstrate that carrot plants interact with a diverse assortment of microbes in the soil surrounding their root systems, and some of these can colonize internal taproot tissues. Carrot plants have long been noted as N scavenging crops, and the results of these trials demonstrate that this could be due, at least in part, to their potential to facilitate positive priming of organic materials in soil. However, not all carrot genotypes are capable of accessing these nutrients, and some may contribute to negative priming. These results highlight the importance of varietal development and selection in optimizing nutrient dynamics in different types of farming systems. For example, the experimental genotypes 3999 and 8503 can potentially perform better under low input productions systems that rely on organic fertility sources. In contrast, because Karotan is slow to develop, growing this variety could promote N loss in early developmental stages in systems supplied with mineral fertilizers. Consequently, by selecting varieties best suited to a particular farming system, farmers can obtain greater production at a lower input cost, and reduce N loss to the environment.

Plants can have feedback affects that alter the health of soil in positive and negative ways, and the results of this trial demonstrate that carrots are no exception. In addition, results showed that carrot genotypes differ in how they alter soil microbial communities. The implications for these changes are unclear at this time, but should be followed up as they could have important implications for the health and productivity of future crops. For example, this could influence how subsequently planted crops interact with microbes to fight disease, acquire nutrients or promote plant growth. In addition, this further highlights how plant breeding programs can alter plant and soil microbiomes in agricultural systems.

New approaches to studying plant microbiomes are needed to begin to understand the implications of these communities on plant and environmental health. Next generation sequencing technologies have revolutionized the study of soil and plant microbiomes, but subtle changes in methodology can have dramatic effects on the interpretation of data obtained from these studies. Subjecting carrot taproot tissues to microfiltration and enzymatic digestion have the potential to increase resolution when characterizing endophyte communities in carrots. However, further work is needed to determine which combination of approaches best characterizes endophyte

communities in crops like carrots. Further research also should be conducted to determine possible functions of specific endophytic bacterial taxa, how management practices alter the composition of these communities, and the ecological role they could play in soil C and N cycles.