RESEARCH ARTICLE

Carrot genotypes differentially alter soil bacterial communities and decomposition of plant residue in soil

Narda J. Triviño · Alejandro Rodriguez-Sanchez · Timothy Filley · James J. Camberato · Micaela Colley · Phillip Simon · Lori Hoagland

Abstract

Purpose Farmers need alternative approaches to manage nitrogen (N) that meet crop needs while reducing loss to the environment. Identifying crop genotypes that promote decomposition of organic materials and understanding the potential mechanisms responsible could help address this challenge. Consequently, we aimed to 1) determine whether carrot genotypes differ in their potential to facilitate organic matter decomposition in soil, and 2) identify bacterial taxa that are stimulated by carrot roots, and thus could play a role in these processes.

Methods We grew five genotypes expected to differ in N use efficiency in a nutrient-poor soil amended with [15] N-enriched corn residue, tracked changes in carbon (C) and N pools, quantified microbial activity and bacterial community composition, and predicted the potential expression of microbial genes involved in soil C and N cycles.

Results Experimental genotype 8503 had the greatest capacity to promote decomposition of corn residues. This genotype had the highest % of N from the corn residue in its taproots and on average, promoted higher *b-glucosidase* activity in soils. Distinct bacterial communities from the families *Micromonosporaceae*, *Chromatiaceae*, and *Rhodospirillaceae* were also enriched in the soils of genotypes like 8503 that were most effective in obtaining N from the corn residue, and this was correlated with greater potential expression of genes responsible for β-glucosidase and nitrification activity.

Conclusions Carrot genotypes do differ in their potential to alter soil bacterial communities and stimulate microbially-mediated decomposition of organic materials indicating that it may be possible to begin selecting for this important trait.

Keywords Priming · Cellulose degradation · Nitrogen metabolism · Stable isotopes

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Introduction

Nitrogen (N) is an essential macronutrient required by all plants in large quantities to fulfill critical processes such as photosynthesis. Farmers apply substantial amounts of N fertilizers to meet crop plant needs, however, only 50% is generally captured by plants (Fageria and Baligar 2005). The remaining fertilizer is subject to loss via leaching and emissions of nitrous oxide (N_2O), which negatively impacts environmental health (Erisman et al. 2013). One way to address this challenge is by improving crop nitrogen-use efficiency (NUE), which is broadly defined as the yield that a plant produces per unit of N fertilizer applied (Fageria and Baligar 2005). NUE can be further separated into components, such as a plants ability to recover N in soil, and its capacity to utilize N efficiently to support physiological functions. The former process is likely to be particularly critical for reducing N loss to the environment. Plant root traits most commonly known for their potential to influence N acquisition in soil include growth rate, architecture, amount and duration of root hairs, and ability to host symbiotic microbes such as arbuscular mycorrhizal fungi (AMF) (Bardgett et al. 2014; Blagodatskaya et al. 2014; Poirier et al. 2018). However, the ideal set of traits may vary given the type of fertilizer applied. For example, in agricultural systems that rely exclusively on inorganic forms of N fertililzers such as ammonium (NH_4^+) and nitrate (NO_3^-), the ideal root ideotype has been referred to as "steep, deep and cheap" (Lynch 2013). In this case, roots that grow quickly and deeply into the soil profile are useful in acquiring inorganic N compounds subject to leaching. In contrast, in systems where plants are more reliant on decomposing organic matter pools to obtain N, producing roots closer to the soil surface, and releasing compounds from roots that can stimulate microbially-mediated decomposition and N cycling processes in soils could be more important.

Farmers have long relied on organic nutrient sources such as animal manures, animal byproducts and residues from cover crops to help meet N fertility needs (Carr et al. 2020). While the use of these organic fertility sources fell out of favor during the twentieth century due to the widespread availability of inexpensive inorganic N fertilizers derived using the Haber–Bosch process, rising costs and environmental concerns have led to a resurgence in their use

(Carr et al. 2020; Hirel et al. 2011). Plants can take up dissolved low molecular weight organic N compounds like urea, amino acids and oligopeptides to help fulfill their N needs (Moreau et al. 2019), however, this is energetically expensive so plants generally prefer NH_4^+ and NO_3^- since these compounds require less processing internally (Laberge et al. 2006). Decomposition and cycling of organic materials into these inorganic N compounds is carried out by a diverse set of soil macro and microfauna and these processes are dependent on environmental conditions such as temperature and moisture (Frouz 2018). This makes synchronizing plant available N with critical periods of plant uptake when relying on organic fertility sources challenging (Baresel et al. 2008; Hirel et al. 2011; Hoagland et al. 2008; Rudisill et al. 2016). Evidence is accumulating that plants can also influence decomposition of organic materials in soil to help meet their nutrient needs (Heijboer et al. 2016; Moreau et al. 2019; Yin et al. 2018), and this could aid in N acquisition from organic nutrient sources. Plants release up to 40% of their photosynthetically derived C from their roots through both active and passive processes (Bais et al. 2006). These rhizodeposits can influence decomposition of organic matter within soils in a phenomenon known as priming. This phenomenon has been extensively studied for its effects on C fluxes from soil and recent studies indicate that it could also be important for N cycling (Blagodatsky et al. 2010; Finzi et al. 2015; Meier et al. 2017; Murphy et al. 2015; Mwafulirwa et al. 2017; Paterson et al. 2016). As plants grow and N demand increases, plants that are able to exude more C from their roots to stimulate N cycling under situations where is limited, would have greater productivity.

While the health of soils and availability of organic materials is likely to be critical in mediating decomposition of organic materials in soil, plant genetics could also play an important role in this process (Dijkstra et al. 2013; Sasse et al. 2018; Terrazas et al. 2019). For example, wheat (*Triticum aestivum* L.) genotypes with high C investment in roots and root growth rates had greater capacity to stimulate decomposition and obtain N from compost to promote plant growth (Junaidi et al. 2018). Other researchers have demonstrated that the total amount of C released to soil through root exudates can vary among plant genotypes (Kuzyakov 2002; Schenk 2006; Yin et al. 2018), providing evidence that it may be possible to

identify genotypes that can preferentially stimulate decomposition of organic residues in soil, thereby increasing NUE. Carrot (*Daucus carota* L.) is an ideal model crop to investigate how plant genetics can interact with microbial communities to facilitate N acquisition from organic sources in soil. Carrot is an important component of the rapidly growing organic vegetable industry (Kim et al. 2019), and this crop has one of the greatest market shares of organic vegetables with 14.3% of the total U.S. carrot crop grown on organic farms (Lucier and Lin 2011). Carrot is well known for its ability to scavenge N in soil and this crop is commonly included in crop rotation and intercropping strategies to help prevent N loss (Thorup-Kristensen 2006; Westerveld et al. 2006). Standard recommendations for N in carrots range from 50 to 220 kg/ha (Boskovic-Rakocevic et al. 2012; Veitch et al. 2014), though N uptake from inorganic fertilizers is notoriously low with only 8 to 33% of the N actually making it into carrot crops (Reid et al. 2017). Moreover, carrots do not respond to increased rates of inorganic N fertilizers to enhance yield suggesting that they can obtain sufficient N from other sources such as decomposing organic matter (Veitch et al. 2014).

In our organic carrot breeding program, we have observed potential differences in NUE among entries grown in our field trials (Simon et al. 2017). We have also determined that carrot genotypes host distinct microbial assemblages in their taproots which can play a role in helping carrot plants resist pathogens (Abdelrazek et al. 2020a, b), and we suspect that these microbes could also influence decomposition of plant residues and therefore NUE. To test these hypotheses, we grew five carrot genotypes suspected to differ in NUE from our field trials in a nutrient poor soil amended with a ^{15}N labeled corn residue and tracked changes in C and N cycling, soil bacterial composition and potential functional activity, and N uptake into carrot plants.

Materials and Methods

Greenhouse experiment

The experiment was conducted in the greenhouse using an artificial soil constructed from a 50:50 mix of agricultural field soil and sand. Initial characteristics of this soil were determined using standard procedures at the Midwest Soil Testing Laboratory in Omaha, NE (midwestlabs.com). Briefly, total soil organic matter was determined based on loss on ignition; pH was determined using a 1:1 soil water mixture; available phosphorous (P) was extracted as weak Bray and analyzed colorimetrically; exchangeable potassium (K), calcium (Ca) and magnesium (Mg) were extracted with neutral ammonium acetate (1 N) and quantified using inductively coupled argon plasma-mass spectrometry (ICP-MS); and base saturation and cation exchange capacity (CEC) were estimated from the results of exchangeable cations (Moore 2001). The soil mixture contained 1% total organic matter (OM), a pH of 7.2, and 110, 359, 327, and 1598 mg/kg available P and exchangeable K, Mg and Ca, respectively. Cation exchange capacity was 11.6 mmol (+) /kg soil and the % base saturation on CEC sites was 8, 24 and 61 for K, Mg, and Ca respectively. Initial NO_3-N and NH_4-N values were 6.5 and 1.2 mg N/kg, respectively, which were determined using techniques described in Section "Quantification of plant available nitrogen in soils". Total elemental N, organic C and δ^{15}N values of the soil and organic amendments (Table 1.) were determined using techniques described in Section "Soil and plant carbon and nitrogen elemental composition".

Table 1. %C, %N, δ15N isotopic composition and N contribution from the soil and amendments used in calculations and in the greenhouse study

Material	Material added per pot (g)	C (g/kg)	N (g/kg)	C:N ratio	δ^{15}N (‰)	Total N contribution (g)
Initial soil	8000	7	1.0	7.0	8.3	8.00
Feather meal	1.2	456	138	3.3	3.9	0.17
^{15}N enriched corn residue	1.0	425	6.9	61.6	603.0	0.01
Unlabeled corn residue	-	430	6.9	62.3	3.5	-

The artificial soil mixture was distributed into 15.2 × 40.6 cm pots, each with a total volume of 7.7 L and a soil bulk density of 1.03 g cm^{-3}. Each pot was amended with 1.0 g of ^{15}N enriched corn material that was previously generated in a field trial by growing corn plants with ^{15}N enriched N fertilizer. The corn biomass was harvested from the field, dried and ground, and the total element N, organic C and δ^{15}N values δ^{15}N values determined using techniques described in Section "Soil and plant carbon and nitrogen elemental composition". Next, 1.2 g of organic feather meal fertilizer (Down to Earth, Eugene, Oregon) was added to each both and both substrates were mixed evenly in the soil prior to planting 7 seeds in each pot from one of five carrot genotypes (Table 2). The control experiment was only amended with 1.2 g of organic feather meal fertilizer. Soil, enriched corn material and feather meal fertilizer C: N ratios were 7:1, 62:1 and 3:1, respectively. These carrot genotypes were chosen because they have similar taproot morphological characteristics but are expected to vary in NUE based on our field trials. This included two experimental breeding lines and three commercial varieties. The pots were placed in the Purdue Greenhouse Horticulture Facility in West Lafayette, IN during the first week of June. The average day and nighttime temperatures were set at 22 °C and 18° C ± 1° C, respectively, and relative humidity was maintained at between 50 and 70%. Auto-drippers were placed in each pot, and all pots were irrigated daily as needed with the same amount of water throughout the experiment.

After germination, carrot seedlings were thinned to one per pot. The pots were arranged in a complete randomized block design with the five carrot genotypes and one unplanted control treatment, each with six replicates for a total of 36 pots. 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 1.27 cm probe to minimize any potential damage to carrot roots. The three soil cores from each pot were mixed and homogenized. Every time the soil samples were recovered from the pot, the holes were covered to minimize the effects of soil disturbance. A subsample of the pooled soil cores collected from each pot was stored in a plastic bag at 4° C until microbial analysis and another was air-dried for two days and sieved (< 2 mm) in preparation for elemental analyses described below. Carrots were harvested in September, 90 days after seeding, when they reached maturity.

The total wet weight of above and below ground biomass was determined at harvest and the plant materials were oven dried at 70 °C until they no longer changed in moisture content (4 days) to quantify the dry weight of each fraction. The dry plant samples were ground using a UDY cyclone sample mill (UDY Corp., Boulder, Col) to obtain fragments of approximately 1 mm for analyses described below.

Quantification of plant available nitrogen in soils

A modified protocol by Bottomley et al. (1994) was used to quantify NH_4^+-N and NO_3-N in each soil sample. Briefly, 12.5 mL of KCl (1 M) was added to 5 g soil samples and the slurries were incubated at room temperature for 30 min while shaking at 250 rpm. Samples were then filtered through a #42 Whatman filter paper for 10–15 min and the filtrate was stored at -20° C until analysis using an AQ2 Discrete SEAL Analyzer (Seal Analytical, Southampton, Hampshire, United Kingdom) to quantify nitrate (NO_3-N) and ammonium (NH_4-N) according to EPA methods AGR-231-A-Rev. 0 and AGR-210-A Rev. 0, respectively. All analyses were conducted in duplicate.

To estimate the corn residue decomposition, we calculated the mg of inorganic N per kg of soil made

Table 2 Name, characteristics and origins of the carrot genotypes planted in the greenhouse study

Entry	Color	Shape	Length	Top Size	Nematode resistance	Origin
Exp 3999	Orange	Cylindrical	Long	Small	Yes	Brazil/Europe
Exp 8503	Orange	Thin Cylindrical	Long	Medium	Yes	Brazil/Europe
Sun255	Orange	Conical	Long	Medium	No	Europe
Karotan	Orange	Conical	Medium-Long	Large	No	Europe
Uppercut	Orange	Conical	Long	Large	No	Europe

available from organic sources, or N made available (NRO), during the experiment as follows:

$$NRO = ((IN(harvest) - IN(\text{initial})) \times kg\ soil) + total\ plant\ N\ uptake \quad (1)$$

where *IN(harvest)* is the soil inorganic N (NH_4-N + NO_3-N) at harvest, *IN(initial)* is the initial soil inorganic N (NH_4-N + NO_3-N) in each pot, *kg soil* is the total soil in each pot, and *total plant N uptake* (total % N x total plant dry weight).

Soil ß -glucosidase enzyme assay

Soil ß-glucosidase activity was quantified at three time points (6 and 10 weeks after seeding and at harvest) in triplicate for each soil sample. The assay was based on the determination of p-nitrophenol released after incubating soil with p-nitrophenyl (Eivazi and Tabatabai 1988). Briefly, 1 g of moist soil per sample was incubated for 1 h with a solution containing 0.25 mL of toluene, 4 mL of modified universal buffer (MUB) and 1 mL of p-nitrophenyl ß-d-glucoside (PNG). After the incubation, a solution containing 1 mL of $CaCl_2$ and 4 mL of tris buffer at pH 12 were added to stop the enzymatic reaction. The soil suspensions were then filtered immediately through #42 Whatman filter paper. Three technical replicates of each sample were then diluted by half and the color intensity of the filtrate at 400 nm was quantified. During each measurement an additional blank suspension with no soil was included as a control.

Soil and plant carbon and nitrogen elemental composition

Approximately 30 mg of the initial soil mixture was analyzed for total C and N with 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 quantify C and N. The total elemental (C and N) isotope composition of carrot root and shoot biomass, soil, organic feather meal fertilizer as well as values of delta ^{15} N ($\delta^{15}N$ ‰), were 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.

Nitrogen isotope ratio calculations

The δ ^{15} N ‰ value is calculated as the proportional amount of ^{15} N (‰) in the sample relative to an international isotope standard of air (0.00366 atom ‰ ^{15} N) according to the following equation:

$$\delta^{15}N\ sample\ (\%_o) = \left(\frac{R\ sample}{R\ standard} - 1 \right) \times 1000 \quad (2)$$

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 et al. 2008).

Using the δ^{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 (*%NFR*) using the following calculation:

$$\%N\ FR = 100 \times \frac{\delta^{15}N\ enriched\ carrot - \delta^{15}N\ control\ carrot}{\delta^{15}N\ enriched\ corn - \delta^{15}N\ control\ corn} \quad (3)$$

Equation (3) calculates %N obtained from ^{15} N labeled corn residue based on the δ ^{15} N values from carrot roots and shoots and δ ^{15} N values of enriched corn residue and the unlabeled corn residue and carrot roots and shoots controls.

Statistical analyses of soil and plant assays

Differences in soil ß-glucosidase activity, NH_4-N, NO_3-N, %N and %C 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 and outliers were removed using the R function boxplot.stats with a 1.8 coefficient. A two-way ANOVA analysis was then conducted to determine if the effect of time, genotype and the genotype x time interaction were significantly different. Inorganic N made available and %N from residue during the experiment was evaluated using a linear model and a one-way ANOVA to determine if genotype was significant. Data from root and shoot dry weights that did not meet the normality assumptions were fitted to a linear model and a non-parametric Kruskal–Wallis test was conducted to determine

if there were differences among the carrot genotypes. Then the root:shoot ratio (R:S), %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 an ANOVA analysis was performed to determine if there were differences in *%NFR* uptake among the genotypes.

Bacterial soil microbiome analysis

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 108 extractions. The quantity and quality of each extraction was estimated using a Nanodrop 2000 microvolume spectrophotometer (Thermo Fischer Scientific, Waltman, MA, U.S.A), before pooling the three technical replicates from each pot. The concentration of DNA in each sample was then quantified using a Qubit Fluorometer (Thermofisher Scientific) to obtain a more accurate concentration and samples were diluted to 1 ng/µl in preparation for use as a template in PCR.

PCR reactions were conducted using primers targeting the V3-V4 16 s rRNA bacterial region: V3_341F: CCTACGGGAGGCAGCAG and V4_806R: GGA CTACHVGGGTWTCTAAT. In addition, each primer contained a so-called "CS" linker sequence which is necessary to run a 2nd PCR with Illumina adapters and barcodes. 1 µl of DNA was used as a template in each 25 µl PCR reaction, which also included 10.5 µl H$_2$O, 12.5 µl Q5® High-fidelity Master Mix (NEB), 0.5 µl primer V3 (10 µM), and 0.5 µl primer V4 (10 µM). All PCR reactions were conducted 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 s, 50 °C for 30 s, 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 and the three replicate PCR reactions were pooled. 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 Illumina MiSeq platform.

Bioinformatic analysis of the soil microbiome data

Sequences obtained from the Illumina platform were demultiplexed based on individual barcodes assigned to each sample and trimmed according to a 25 phred quality value using the TrimGalore tool (Krueger 2015). Reads from forward and reverse primers were then merged using the join_paired_ends.py script found in QIIME v2. (Bolyen et al. 2019) and quality checked using the FastQC tool (Andrews 2010). QIIME v2. was used for picking, identifying chimeric sequences, assigning bacterial taxonomy, and aligning final operational taxonomic units (OTUs) with the SILVA 119 version bacterial data base (Pruesse et al. 2007), and then OTUs were filtered for chloroplasts and mitochondria sequences. Statistical analyses were conducted to calculate bacterial α-diversity (Shannon indexes) using the alpha_rarefaction.py script that uses a nonparametric index to measure the total number of OTUs and evenness (relative abundance of OTUs) using QIIME v2. A post-hoc Tukey pairwise comparison was performed to test for differences in α-diversity for each pair of means, and differences in individual families between genotypes. 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 if there were statistical differences between the treatments using the R package "vegan" (Oksanen 2007), in R version 3.6.2 (R Development Core Team 2019).

The PICRUSt2 program was used to predict the abundance of genes from protein families involved in cellulose degradation and N metabolism using normalized OTU counts (Langille et al. 2013) (Table 3). Differences in the abundance of genes predicted between treatments were evaluated using a permutational analysis of variance (PERMANOVA) in the software PAST, based on Bray–Curtis distance and with 9999 bootstrap replications. Potential enrichment of specific genes among treatments were determined using both a correlation-based indicator species analysis and a differential abundance analysis. The correlation-based indicator species analysis was conducted using the "indicspecies" package implemented in R (De Cáceres and Legendre 2009), and was computed under a point-biserial correlation coefficient with 10,000 replications and a confidence of p < 0.05 (Hartman et al. 2018). The differential gene expression analysis was calculated using the edgeR

Table 3 Genes involved in cellulose degradation and N metabolism included in the PICRUSt analysis of soil bacterial communities

KEGG Number	Gene	Encoded enzyme	Description	Reference
Cellulose degradation				
K02760	*celA*	cellobiose PTS system EIIB component	Phosphorylation and translocation of β-glucosides across the cytoplasmic membrane	(Fauré et al. 2001)
K01225	*CBH1*	cellulose 1,4-β -cellobiosidase	Cleave the ends of cellulose chains resulting and release of oligosaccharides	(Yeoman et al. 2010)
K01222	*celF*	phospho (P)-β-glucosidase	Hydrolyzes O-β-linked phosphorylated derivatives to generate glucose-6-phosphate (G6P)	(Thompson et al. 1999)
K01188	*β-glucosidase*	β-glucosidase	Hydrolyzing β-glucosidic linkages present in either disaccharides, oligosaccharides, or glucosides	(Fan and Conn 1985)
K01199	*EGLC*	glucan endo-1,3- β -D-glucosidase	Liberation of α-glucose from the nonreducing ends of β-1,3-glucan and β-1,3-glucoside	(Hasper et al. 2002)
K01210	*glucan 1,3- β -glucosidase*	glucan 1,3- β -glucosidase	Removes glucose units from the nonreducing ends of polymeric and oligomeric *β* -glucans	(Vázquez-Garcidueñas et al. 1998)
K01223	*bglA*	6-phospho- β -glucosidase	Hydrolysis of β-1,4-linked cellobiose 6-phosphate (cellobiose-6′P) to yield glucose and glucose 6-phosphate	(Yu et al. 2013)
Nitrogen metabolism				
K04561	*norB*	nitric oxide reductase	Reduction of NO to N_2O	(Braker and Tiedje 2003)
K10944	*pmoA-amoA*	ammonia monooxygenase	Oxidizing NH_3 to NO_2^- through the intermediate NH_2OH	(Sinigalliano et al. 1995)
K10535	*hao*	hydroxylamine oxidoreductase	Conversion of NH_2OH to NO_2^-	(Sayavedra-Soto et al. 1994)
K00376	*nosZ*	nitrous oxide reductase	Reduction of N_2O to N_2	(Zumft et al. 1992)
K00370	*narG-narZ- nxrA*	membrane bound nitrate reductase / nitrite oxidoreductase	Reduction of NO_3^- to NO_2^-	(Smith et al. 2007)
K03385	*nrfA*	cytochrome c nitrite reductase	Reduction of NO_2^- to NH_3	(Welsh et al. 2014)
K00368	*nirK*	copper containing nitrite reductase	Reduction of NO_2^- to NO	(Oakley et al. 2007)
K02584	*nifA*	nitrogen reductase	Reduction of N_2 to NH_3	(Gussin et al. 1986)

package implemented in R (Robinson et al. 2009), computed using likelihood ration tests, and results were considered significantly different if they had a false discovery rate of p < 0.05 (Hartman et al. 2018).

Results

Soil ß-glucosidase enzyme assay

Soil ß-glucosidase activity differed based on the interaction between carrot genotype and time (p < 0.01) (Table S1). At 6 weeks after planting, there were no differences among the genotypes, however, at 10 weeks after seeding there were (Fig. 1a; Table S2). Specifically, carrot genotype 8503 had greater soil ß-glucosidase activity than Karotan and the unplanted control, and 3999 had greater levels than the unplanted control. At harvest there were no differences among genotypes (Fig. 1a; Table S2).

Plant available N (NH_4-N and NO_3-N) in soil

Soil NH_4-N differed based on the interaction between carrot genotype and time (p < 0.001) (Table S1). Soil NH_4-N levels were similar among carrot genotypes at 6 weeks after seeding, but differed at 10 weeks after seeding and harvest (Fig. 1b; Table S2). Specifically, at 10 weeks after seeding, there was greater concentrations of soil NH_4-N in Karotan than Uppercut (Fig. 1b; Table S2). At harvest, 3999 had greater soil NH_4-N values than 8503, Uppercut and the unplanted control, and Karotan had greater values than Uppercut or the unplanted control (Fig. 1b; Table S2).

Soil NO_3-N values differed based on genotype (p < 0.01) and time (p < 0.01), but there was no interaction between these two factors (Fig. 1c; Table S1). With the exception of genotype Karotan, soil NO_3-N values decreased across time. Soil NO_3-N values were greater across time in the unplanted control relative to all pots planted with carrots, but there were no

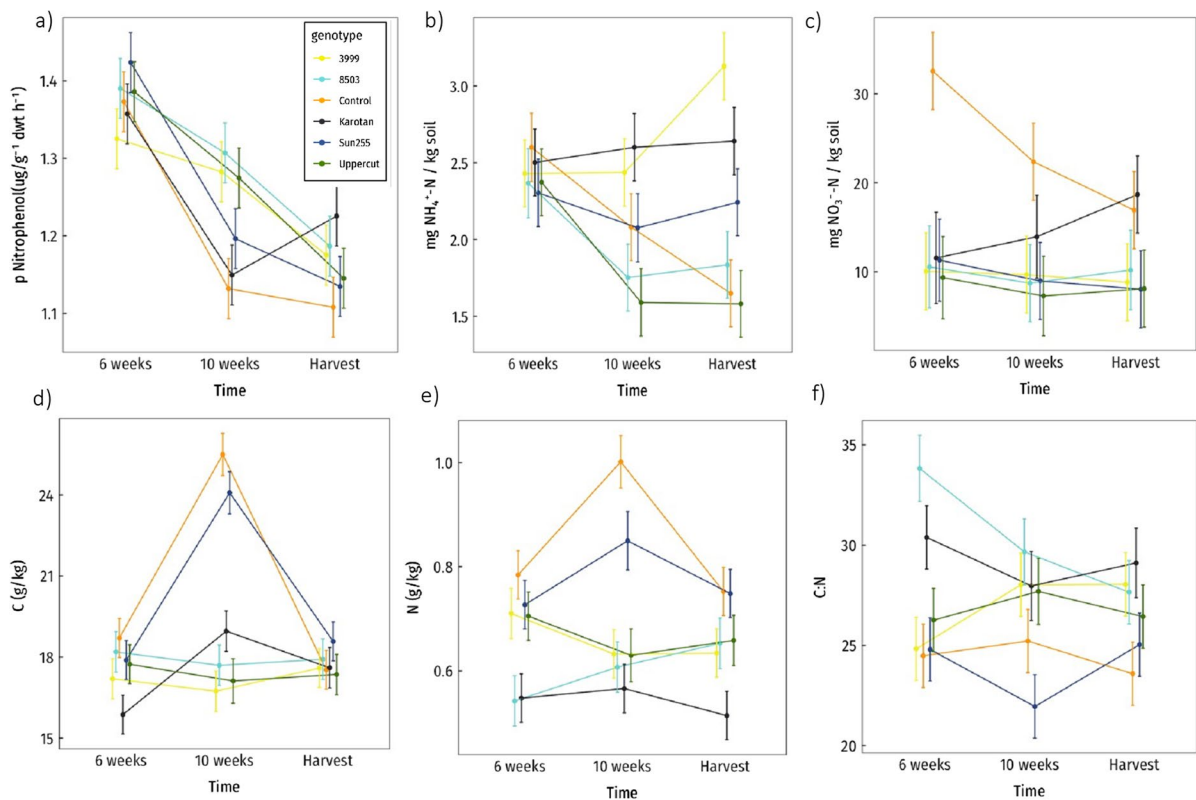**Fig. 1** Soil ß-glucosidase activity (**a**), NH_4-N, (**b**), NO_3-N, (**c**), C (**d**), N (**e**) and the C:N ratio (**f**) in pots grown with five carrot genotypes and an unplanted control at 6 and 10 weeks after seeding and at harvest. The legend for all frames is indicated in (a). Points are means and bars indicate S.E

differences among genotypes within the individual time points (Fig. 1c; Table S2).

Soil total C and N, C:N ratio and elemental composition

Soil C concentration differed based on the interaction between carrot genotype and time ($p < 0.001$) (Table S1). There were no differences in soil %C levels among the genotypes at 6 weeks after seeding or at harvest, however, there were differences at 10 weeks after seeding (Fig. 1d; Table S2). Specifically, Sun255 and the unplanted control had higher soil %C levels than 3999, 8503 and Karotan.

Soil %N also differed based on an interaction between carrot genotype and time ($p < 0.01$) (Table S1). At 6 weeks after seeding, the unplanted control had higher %N levels than 8503 and Karotan (Fig. 1e; Table S2). At 10 weeks after seeding, Sun255 and the unplanted control had greater %N values than 8503, 3999, Uppercut and Karotan (Fig. 1e; Table S2). Finally, at harvest Sun255 and the unplanted control had greater soil %N values than Karotan (Fig. 1e; Table S2).

Soil C:N ratio was significantly different based on genotype only ($p < 0.001$) (Table S1). At 6 weeks, genotype 8503 had significantly greater soil C:N than 3999, Sun255 and Uppercut, while at 10 weeks genotype 8503 had significantly greater soil C:N than Sun255. There were no significant differences in the soil C:N ratio at harvest (Fig. 1f; Table S2).

Root and shoot dry weight, root:shoot ratio and carbon and nitrogen composition of plant tissues

There were no significant differences in total root, shoot or combined root and shoot dry weight (Table 4; Table S4). There were also no differences among genotypes in %C in roots or shoots, %N in roots, or C:N ratio in roots, however, there were significant differences in %N and the C:N ratio in shoots (Table 4). Specifically, there was greater %N in the shoots of 3999 than Karotan, Sun255 and Uppercut, and 8503 had greater %N than Uppercut, and Karotan, Sun255 and Uppercut had greater C:N ratios than 3999 and 8503 in carrot shoots.

There were no significant differences in the total amount of N in carrot roots, shoots or total plant biomass (Table 4; Table S4). Between 171–323

total mg N/kg was made available from the corn residue and taken up by carrot plants during the course of the experiment, and carrots obtained between 33–44% of the total N from this material (Table 4; Table S4). There were differences in the % of N in roots from the corn residue based on genotype, with greater % NFR in 8503 than Uppercut, but there were no differences in carrot shoots (Table 4).

Soil bacterial microbiome composition and diversity at harvest

Twenty-two unique bacterial families were identified in the soils collected at harvest and there were significant differences in families between carrot genotypes (Fig. 2; Table S5). For example, *Oxalobacteraceae, Opitutaceae, Micrococcaceae* families were detected in Karotan, Sun255 and Uppercut but not the other genotypes, whereas the *Chromatiacea* family was detected only in 3999, 8503 and Sun255. The *Intrasporangiaceae* family was found in Karotan and Sun255 and the *Familyl* family was detected only in 3999, Sun255 and Uppercut. 3999 and the unplanted control were the only treatments in which the *Rhodospirillaceae* family was found. Finally, *Nitrosomonadaceae* was greater in 3999 and 8503 than Karotan, Sun255 and Uppercut.

When comparing alpha diversity among the bacterial communities there were significant differences ($p < 0.01$) between 3999, 8503, Sun255 and the unplanted control treatment (Fig. 3a). Results of the principal components analysis (PCoA) and ADONIS test indicated that there were also significant differences in β diversity among the soil bacteria communities in the carrot genotypes ($p < 0.001$). 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 (Fig. 3b). Specifically, 3999 and 8503 clustered together in group A, while Karotan, Uppercut and Sun255 clustered in group B, and the unplanted control treatment clustered in group C (Fig. 3b).

3.6 PICRUSt's prediction analysis at harvest

Results of the PICRUSt's gene prediction indicated there were significant differences in the

Table 4 Total weight, %C, %N, C:N content, total N content, N from ^{15}N labeled corn residue, and N made available from all sources during the experiment at harvest in above and belowground biomass of five carrot genotypes

Genotype	Weight (g/plant)	% C	% N	C:N	Total N (mg/plant)	N from residue (mg/plant)	% N from residue (% NFR)	δ^{15}N labeled exp	δ^{15}N unlabeled exp
Roots									
3999	6.42±1.9	40.5±0.3	1.45±0.1	29.3±3.5	92±27.5	0.50±0.1	0.49±0.1 ab	13.2±0.5	10.3±0.4
8503	7.35±1.9	39.8±0.3	1.18±0.1	35±3.5	78±27.5	0.58±0.1	0.73±0.1 a	15.1±0.5	10.6±0.4
Karotan	7.28±1.9	39.7±0.3	1.25±0.1	35.7±3.5	104±27.5	0.41±0.1	0.40±0.1 ab	13.3±0.5	10.9±0.4
Sun255	8.55±1.9	40.5±0.3	1.18±0.1	35.5±3.5	108±27.5	0.72±0.1	0.57±0.1 ab	14±0.5	10.5±0.4
Uppercut	5.4±1.9	39.7±0.3	0.97±0.1	43.6±3.5	59±27.5	0.17±0.1	0.24±0.1 b	13.4±0.5	12±0.4
p-value	n.s	n.s	n.s	n.s	n.s		*	n.s	n.s
Shoots									
3999	3.77±1.1	38±0.4	2.33±0.1 a	16.8±1.7 b	90±24.7	0.50±0.1	0.59±0.1	14.7±0.5	11.1±0.5
8503	3.52 1.1	37.3±0.4	2.04±0.1 a	18.6±1.7 b	74±24.7	0.35±0.1	0.60±0.1	14.5±0.5	10.8±0.5
Karotan	5.95 1.1	38.2±0.4	1.51±0.1 bc	26.4±1.7 a	95±24.7	0.42±0.1	0.44±0.1	13.7±0.5	11.0±0.5
Sun255	6.17 1.1	38.6±0.4	1.45±0.1 bc	27±1.7 a	93±24.7	0.42±0.1	0.45±0.1	13.2±0.5	10.5±0.5
Uppercut	5.82 1.1	37.5±0.4	1.37±0.1 c	28.3±1.7 a	88±24.7	0.57±0.1	0.70±0.1	14.3±0.5	10.1±0.5
p-value	n.s	n.s	***	***	n.s	n.s	n.s	n.s	n.s

Significant p-values correspond to *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and n.s.: not significant. Different letters indicate significant differences between genotypes in the Tukey test pairwise comparison ($\alpha = 0.05$). Values are mean ± S.E
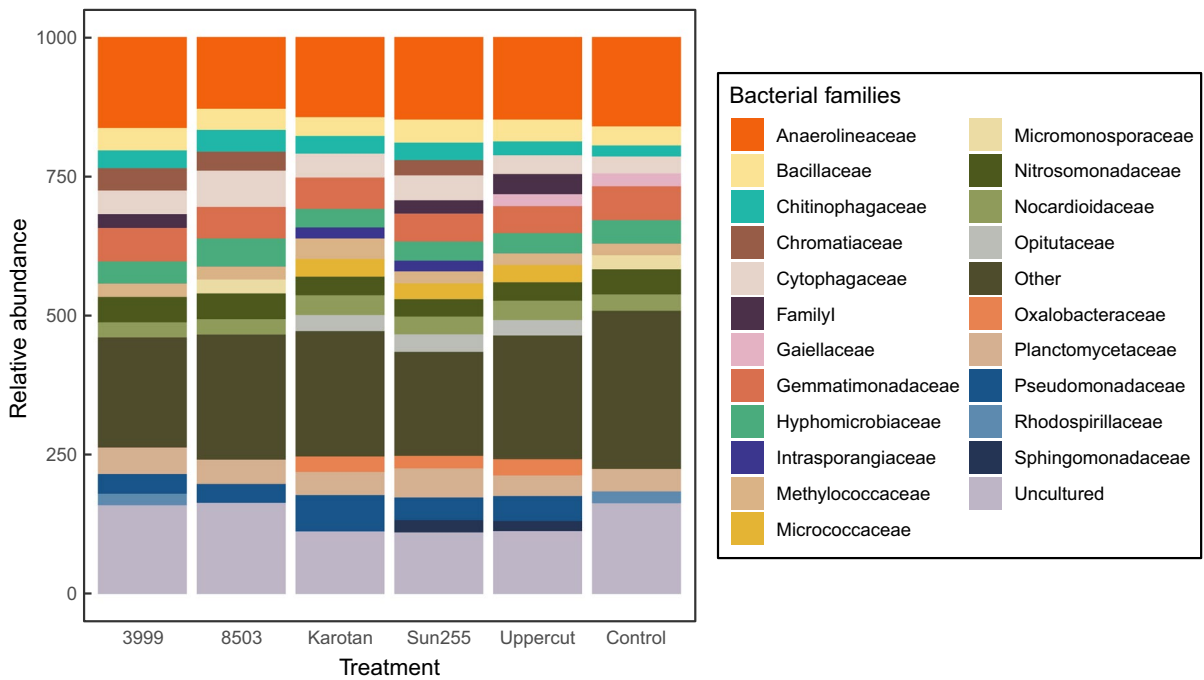
Fig. 2 Taxonomic families of bacteria in the soil of pots grown with five carrot genotypes and an unplanted control at harvest
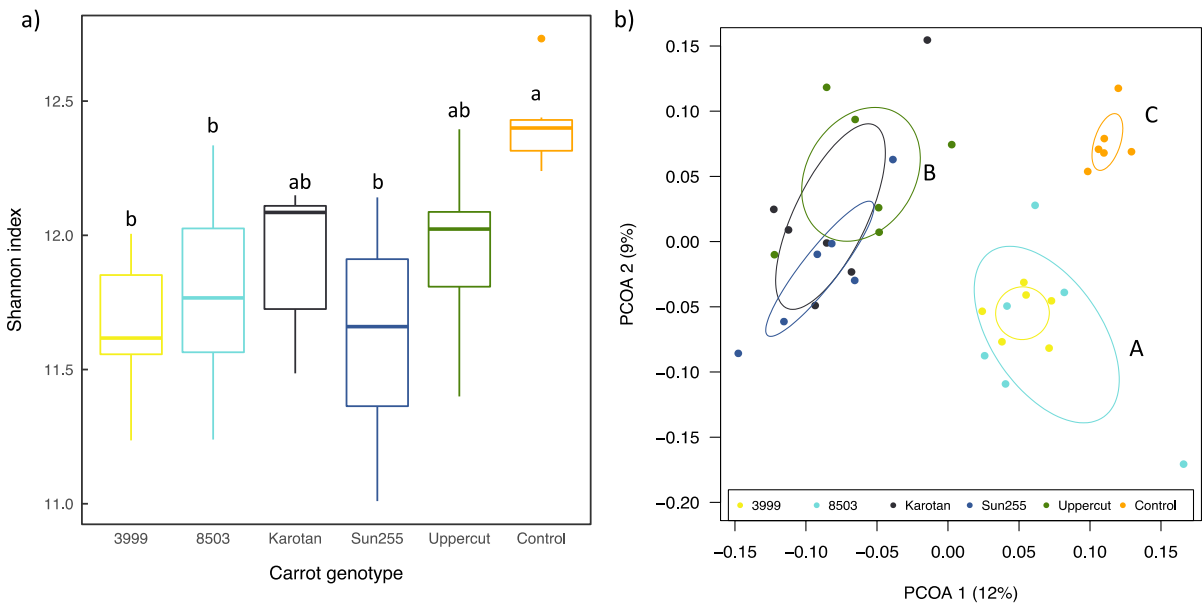

Fig. 3 Differences in α diversity (**a**) and β diversity (**b**) of soil bacterial communities in pots planted with five carrot genotypes and an unplanted control. Points in (a) indicate outliers for each group. Different letters in (a) indicate significant differences between genotypes in the Tukey test pairwise comparison (α value = 0.05). Principal components analysis (PcoA) in (b) explained 21% of the variability based on Bray–Curtis distances

potential for expression of cellulose degradation and N metabolism genes in soil bacterial communities present at harvest after cultivation of the five carrot genotypes and unplanted control (Fig. 4; Table 5; Table S6). Specifically, the genes *celA* (p < 0.001), *celF* (p < 0.01), *β-glucosidase* (p < 0.01), and *bglA* (p < 0.001) involved in cellulose degradation were significantly different, as were genes *norB* (p < 0.01), *pmoA-amoA* (p < 0.01), *hao* (p < 0.01), *narG-narZ-nxrA* (p < 0.05), *nirK* (p < 0.001) and *nifA* (p < 0.01), which are involved in N metabolism.

Results of the indicator species analysis indicated that several cellulose degradation and N metabolism genes could be considered to be unique, or had potential for greater expression, in soil bacterial communities associated with the individual carrot genotypes (Table 5). Among the cellulose degradation genes, *β-glucosidase*, *celF*, *bglA*, and *celA* genes were associated with Sun255; *β-glucosidase* and *celF* were associated with 3999 and 8503; and, *celF*, *bglA,* and *celA* with Uppercut. Among the N metabolism genes, *nirK*, *norB*, *narG-narZ-nxrA*, *nifA*, *p-amoA-amoA*, and *hao* genes were associated with 3999 and 8503; *nirK*, *norB*, *narG-narZ-nxrA* and *nifA* were associated with Sun255; and, *p-amoA-amoA* was the only gene associated with the untreated control.

Results of the differential gene expression analysis were similar to those of the indicator species analysis, also indicating that there were potential differences in gene expression among soil bacterial communities following cultivation with the five carrot genotypes (Fig. 4; Table 5). Among the cellulose degradation genes, *celF* expression was expected to have greater expression in 3999, 8503, Sun255 and Uppercut relative to the other genotypes; and, *blglA* and *celA* expression was likely to have greater expression in Sun255 and Uppercut. Among the N metabolism genes, *p-amoA-amoA* and *nifA* expression were likely to have greater expression in 3999, 8503 and the untreated control; and, *nifA* was greater in Sun255.
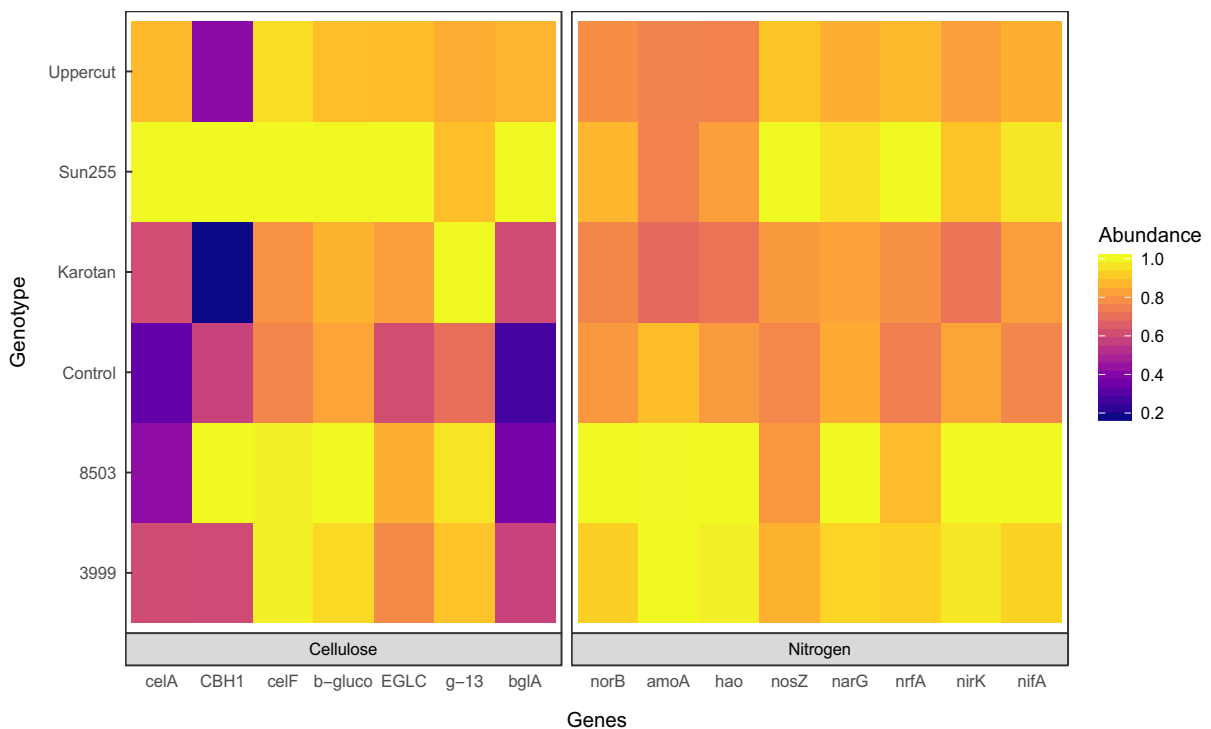

Fig. 4 PICRUSt's prediction for abundance of bacterial genes involved in cellulose degradation and N metabolism in soil from pots grown with five carrot genotypes and an unplanted control. *b-gluco: β-glucosidase, g-13: glucan 1,3- β -glucosidase, amoA: pmoA-amoA*

Table 5 Correlation-based indicator species analysis and differential gene expression for PICRUSt's gene abundance prediction of cellulose degradation and N metabolism of bacteria in soil from pots grown with five carrot genotypes and an unplanted control

Cellulose				Nitrogen			
KEGG	Enzyme	Species indicator p-value	Differential expression p-value	KEGG	Enzyme	Species indicator p-value	Differential expression p-value
	Genotypes: 3999, 8503, Sun 255				Genotypes: 3999, 8503, Sun 255		
K01188	β -glucosidase	*	n.s	K00368	nirK	***	n.s
				K04561	norB	**	n.s
				K00370	narG-narZ-nxrA	*	n.s
				K02584	nifA	**	**
	Genotypes: 3999, 8503, Sun255, Uppercut				Genotypes: Control, 3999, 8503		
K01222	celF	**	*	K10944	pmoA-amoA	***	*
	Genotypes: Sun 255, Uppercut				Genotypes: 3999, 8503		
K01223	bglA	*	***	K10535	hao	***	n.s
K02760	celA	*	***				

10,000 replications. Significant p-values correspond to *** $p < 0.001$, ** $p < 0.01$, *$p < 0.05$, and n.s.: not significant. ($\alpha = 0.05$). Genotypes included in the table represent those that could be considered unique for the potential expression of the genes relative to all carrot genotypes

Discussion

Determining how plants interact with soil microbial communities to facilitate C and N cycling has potential to increase the health and yield of crops while reducing N losses to the environment (de Vries and Wallenstein 2017; Dijkstra et al. 2013; Meier et al. 2017). One way that plants can influence C and N cycling processes is through priming. To quantify the potential for carrot plants to promote decomposition of organic materials in soil, we amended soils with an ^{15} N -isotopically labeled corn residue. The vast majority of N in the environment is present in the form ^{14} N, so introducing materials enriched in ^{15} N form allows researchers to track the movement of specific N compounds in the environment (de Oliveira Silva et al. 2017). The corn residue used in this study had an initial δ^{15}N value of 603‰. This residue also had a high C:N ratio (62:1), so it should have been slow to decompose relative to the other N source added in this study, and thus serve as a good material to quantify priming activity by the carrot plants. The δ^{15}N. values of the carrots from the control experiment ranged from 10.1 to 12, similar to other published values for carrots (Bateman et al. 2005). The δ ^{15} N values from the enriched experiments exhibited higher values ranging 13.2 to 15.1, confirming that the carrot plants did obtain N from the corn residue

(Table 4). Specifically, carrots obtained around 4% of their total N from the corn residue and took up from 33–44% of the N that was added in the residue (Table 4; Table S4).

Determining whether genotypes differ in their potential to enhance decomposition of organic materials to obtain N is critical to being able to leverage this capacity to improve NUE in crop breeding programs. In this study, the experimental genotype 8503 appeared to have the greatest potential to obtain N from organic materials given the higher concentrations of N obtained from the corn residue in its roots at harvest (Table 4). Other evidence that experimental genotype 8503 was better in stimulating organic matter priming and obtaining N is provided by differences in soil β-glucosidase activity and C and N pools over the course of this study (Fig. 1; Tables S1 & S2). Soil β-glucosidase is an extracellular enzyme produced by many soil microbes to break down cellulose, the primary component of most plant materials, making it a valuable indicator for tracking rhizosphere induced priming activity (Chang et al. 2007). For example, Zhu et al. (2014) observed 19–56% increases in β-glucosidase activity in the presence of living sunflower and soybean roots that were positively correlated with gross N mineralization rates in soil. While there were no differences in ß-glucosidase activity at 6 weeks after seeding in this study when

the carrots were still very small, 8503 had lower total soil N and a higher soil C:N ratio, indicating that it was better at obtaining N from more easily degraded organic matter pools. As the experiment progressed, differences in soil ß-glucosidase activity and C and N pools became much more dramatic providing stronger evidence of varietal differences in N mineralization processes (Fig. 1; Tables S1 & S2). For example, at 10 weeks after planting, carrot genotype 8503 had the highest ß-glucosidase activity and soil C:N ratio, supporting our assertion that this genotype was most effective at stimulating organic matter decomposition and obtaining N from organic residues. Genotypes 3999 and Uppercut also had higher ß-glucosidase activity and Uppercut had lower NH_4-N at this time point, indicating that these genotypes were also becoming more effective than genotypes like Karotan, which had the highest levels of NH_4-N, and thus lowest uptake. Finally, since Sun255 had higher soil C and N than the other treatments, we suspect that this genotype had the lowest capacity to promote soil priming processes.

One of the reasons that experimental genotype 8503 may have had greater potential to promote decomposition and obtain N from organic materials in this study was that it had a moderately higher root:shoot ratio (although it was non-significant, $p < 0.10$) than the other genotypes (Table S3). This phenomenon has been demonstrated in wheat, where genotypes with a greater investment in belowground structures responded more positively to compost additions and had greater N uptake from these materials (Junaidi et al. 2018). A higher investment in root biomass may allow for greater exudation rates of low molecular weight substrates from plants roots that are critical for promoting positive rhizosphere priming processes (Pausch et al. 2016). Differences in root architecture and root plasticity have also been reported to enhance the potential for crop varieties to obtain N from decaying organic materials in soil (Andresen et al. 2016; Junaidi et al. 2018). This was not likely to be the case in this study since carrots were grown in pots where root spread was limited, however, future studies should consider exploring this mechanisms in larger pots and/or field studies, since it can also be an important factor influencing differences in NUE among crop genotypes.

While many studies have focused on quantifying changes in broad soil microbial processes such as ß-glucosidase activity to evaluate priming processes, it is also important to consider changes in specific microbial communities. For example, Fontaine et al. (2003) pointed out that many soil microbes are r-strategists that are adapted to decomposing labile and nutrient-rich substrates, and these microbes proliferate rapidly when labile C compounds such as root exudates are introduced into soil. In contrast, other soil microbes are k-strategists that are more efficient at depolymerizing and degrading more recalcitrant or specific organic substrates, and it is the competition between microbes with these two diverse strategies that actually contributes to priming processes in soil. In this study, we observed distinct differences in β diversity among soil bacteria associated with carrot plants in comparison with the unplanted control, as well as lower α diversity, providing evidence that carrot plants do recruit and support specific microbial taxa from soil (Fig. 3b). This was not surprising, given that the composition of rhizosphere microbial communities often represents a subset of microbes in bulk soil since only a subset of the soil microbial community can respond to and effectively compete for the labile carbon compounds released by plant roots. For example, Hargreaves et al. (2015) observed distinct differences in microbial communities inhabiting bulk soil and the rhizosphere of switchgrass (*Panicum virgatum* L.). Moreover, they observed greater abundances of taxa known to be active in degrading cellulose and chitin in the switchgrass rhizosphere, indicating that switchgrass plants can promote positive priming of organic materials by recruiting and supporting the growth of these specific taxa. Similarly, Turner et al. (2013) identified distinct differences in rhizosphere community structure between pea (*Pisum sativum* L.) and wheat plants along with greater concentrations of bacteria with cellulolytic activity in the rhizospheres of wheat plants, which do not host symbiotic microbes that can fix atmospheric N in nodules like pea. Consequently, we suspect that the unique microbial taxa recruited by carrot plants in this study could play a role in helping carrot plants promote soil priming processes to obtain N.

Previous studies have demonstrated that the composition of plant root exudates can vary among plant genotypes (Micallef et al. 2009), and this can in turn alter soil microbial community structure and influence organic matter decomposition rates (Zwetsloot et al. 2020). While explicitly quantifying differences

in root exudates was beyond the scope and resources available for this study, we suspect that differences in the composition of root exudates among the carrot genotypes evaluated likely contributed to differences in the soil bacterial families observed (Fig. 2; Table S5), and this could have influenced decomposition rates. For example, soil bacterial communities associated with 8503 and 3999, which both had greater soil ß-glucosidase activity (Fig. 1; Tables S1 & S2) and greater %N in their shoot tissues at harvest (Table 4), clustered together and were clearly separate from Uppercut, Sun255 and Karotan (Fig. 3b). Moreover, 8503 was the only genotype to contain abundant populations of *Micromonosporaceae*. Many species of these bacteria have been noted for their ability to degrade chitin, cellulose, lignin and pectin, and play important roles in the turnover of organic materials in soil (Trujillo et al. 2014). Thus, we suspect that these taxa could have played a role in the greater soil β-glucosidase activity observed with this genotype. Populations of *Rhodospirillaceae,* which have potential to fix atmospheric N (Madigan et al. 1984) were enriched in 3999, indicating that these bacteria could have aided in the potential for this genotype to obtain greater concentrations of N in its tissues at harvest. Microbes belonging to the *Chromatiaceae* were enriched in 8503, 3999 and Sun255. These bacteria are well known for their role in the sulfur cycle and some species such as *Nitrosococcus,* are nitrifiers. While most *Chromatiaceae* are phototrophic, there is evidence that they can colonize plant rhizospheres by switching their growth strategies to chemo-, organo-, and/or mixo-trophy (Cúcio et al. 2016). Consequently, it is possible that microbes belonging to this family also aided in the potential for these carrot genotypes to obtain critical plant nutrients in this experiment.

Interestingly, other bacterial taxa were enriched in the soils of carrot genotypes that did not appear to be as effective at promoting C and N cycling, indicating that these microbes could have been better at immobilizing and preventing these genotypes from obtaining N. For example, *Opitutaceae* were enriched in Karotan, Sun255 and Uppercut. These bacteria belong to the phylum Verrucomicrobia. The ecological role of bacteria belonging to this phylum is still not well understood, though they have been negatively correlated with soil fertility (Navarrete et al. 2015). Other studies investigating relationships between rhizosphere bacterial community structure and N

availability have noted that these microbes are a subset of microbial taxa that can tolerate low soil nutrients and persist in the rhizosphere until harvest (Bell et al. 2015). Consequently, they may be k-strategists that are better at decomposing more specific substrates. Interestingly, a recent study using ^{13}C labeling of chitin residues demonstrated that while *Opitutaceae* did not appear to degrade chitin directly, they did become enriched in ^{13}C at later time points indicating that they may have been utilizing products of the chitin degraders, such as extracellular polysaccharides (Wieczorek et al. 2019). The genotypes Karotan, Sun255 and Uppercut also had a lower relative abundance of microbes from the family *Nitrosomonadaceae.* These are ammonia oxidizers that can rapidly transform ammonia to nitrate (Thion et al. 2016).

Consequently, having a lower relative abundance of these microbes could reduce the capacity for these genotypes to obtain nitrate relative to 8503 and 3999.

To better estimate whether there were differences in the potential for genotype-induced differences in the soil bacterial communities to cycle C and N in this study, we used PICRUSt2 to quantify differences in the potential abundance of genes involved in cellulose degradation and N metabolism (Fig. 4; Table S6). Interestingly, genes involved in *β -glucosidase* were enriched in 8503, 3999 and Uppercut, supporting our assertions that these genotypes can better stimulate decomposition of organic materials in soil. Moreover, none of the genes involved in cellulose degradation or N metabolism were enriched in Karotan, which appeared to have the lowest priming activity in this study (Hartman et al. 2018). In addition to facilitating decomposition, plants can also recruit and support microbes that mineralize and release plant available N (Rudisill et al. 2016). In this study, many genes associated with N metabolism were more associated with 3999 and 8503, providing evidence that these genotypes might be better able to recruit and support bacteria that can contribute to NUE. Finally, it is interesting to note that genes associated with cellobiose degradation were enriched in Sun255 and Uppercut, which were also enriched with microbial taxa from the family *Opitutaceae.* Since these microbes are suspected to play a role in degrading substrates produced by other microbes rather than directly degrading complex substrates on their own (Wieczorek et al. 2019), this may help explain why these genotypes were less effective in stimulating positive priming processes in

this study. However, it is important to evaluate these results with caution, since the potential presence of the gene does not always correlate with enhanced gene expression (Zheng et al. 2018).

Finally, it is important to point out that differences in soil microbial communities induced by the carrot genotypes in this study (Fig. 2; Table S5), could play other important roles in carrot production systems such as resistance to root-knot nematodes. Several studies have identified distinct microbiomes associated with resistant and susceptible cucumber and tomato genotypes (Kwak et al. 2018; Upreti and Thomas 2015; Yao and Wu 2010), and we recently discovered this phenomenon in carrot (Abdelrazek et al. 2020a, 2020b). The exact cause of the genetic resistance to root-knot nematodes in genotypes like 3999 and 8503 is not yet clear (Williamson et al. 2009), though it is possible that these genotypes are able to sense the presence of these nematodes at the root surface and strengthen their cell walls to resist invasion in class hypersensitive response. We have theorized that these genotypes may also be able to host endophytic microbes within carrot taproots that help prevent root-knot nematodes from infecting carrot plants (Abdelrazek et al. 2020a). In contrast, Sun255, Karotan and Uppercut may need to mount alternative strategies to resist attack by pathogenic nematodes, such as recruiting microbes with antagonistic activity against pathogens in their rhizosphere. Interestingly, taxa belonging to the *Oxalobacteriaceae* and *Micrococcaceae,* which were enriched in the soil of these three genotypes, are present in elevated concentrations in so-called 'disease suppressive soils' (Cretoiu et al. 2013; van der Voort et al. 2016). Consequently, it is possible that these microbes could help these genotypes fight pathogens, and this should be explored in future studies.

Conclusions

Results of this study confirm previous reports that carrots are efficient at obtaining N in soil (Thorup-Kristensen 2006; Veitch et al. 2014; Westerveld et al. 2006), and at least part of this activity could be due to bacterial-induced priming processes. The results also confirm previous reports that at least part of the composition and functional potential of soil microbial communities associated with carrot

roots are under genetic control (Abdelrazek et al. 2020a; 2020b). In addition to serving as a first line of defense against invading pathogens as previously reported, results of this study indicate that changes in bacterial community structure induced by carrot genotypes also have potential to influence nutrient use efficiency in carrot crops. Finally, carrot genotypes appear to differ in their capacity to promote decomposition of plant residues to obtain at least part of their nitrogen needs. By learning more about the genetic mechanisms regulating these relationships, it may someday be possible to leverage these beneficial plant-soil-microbial interactions in crop breeding programs (Hoagland 2015). Currently most plant breeding programs are conducted under conventional farming systems where plants are selected for high yield in the presence of inorganic fertility programs. As a result, researchers have theorized that varieties developed in conventional systems may not have the traits needed to interact with soil microbes to obtain nutrients from organic sources (Lammerts van Bueren and Struik 2017; Mwafulirwa et al. 2016). For example, it is energetically expensive for plants to provide C resources to support soil microbes, and thus high rates of inorganic N fertilizers could disrupt relationships between plants and microbes that play a role in organic matter decomposition (Heijboer et al. 2016). In contrast, selecting varieties in organic farming systems where plants are more reliant on organic materials to obtain nutrients could lead to the development of varieties that promote organic matter priming. Consequently, it is tempting to speculate that our organic breeding program has selected for genotypes that are better at obtaining nutrients from organic materials, since experimental genotypes 8503 and 3999 have been performing well in our organic field trials (Simon et al. 2017). However, future studies conducted under a diverse set of environmental conditions will be needed to confirm whether this is the case. Since fungi also play a critical role in the decomposition of organic materials in soils, future studies should also investigate genotype-induced changes in these communities as well. Regardless of the approach, researchers need to improve understanding of how plants interact with their microbiomes to address environmental challenges and improve productivity and NUE in agricultural systems (Vandenkoornhuyse et al. 2015).

Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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