

Microbial communities associated with wheat, canola and lentil seeds produced in Saskatchewan

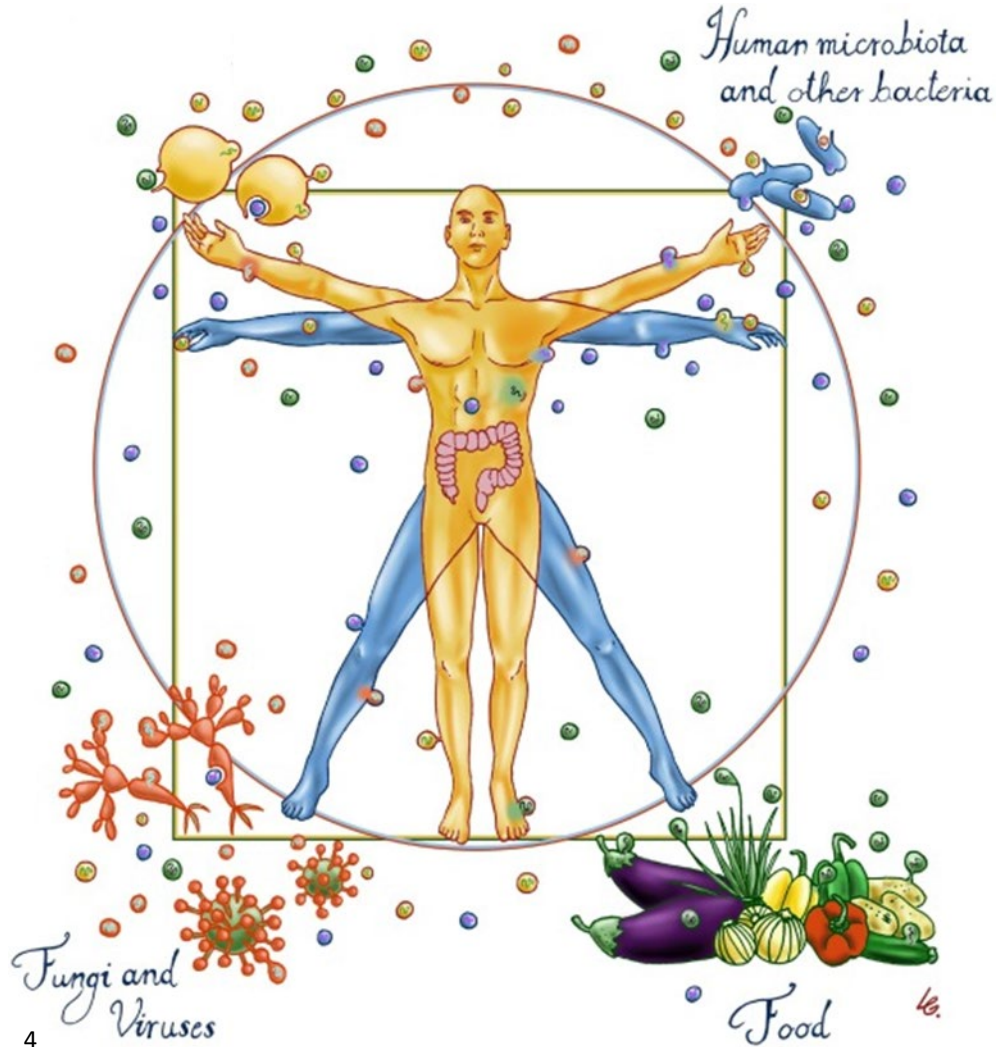
Z.P. Morales, B.L. Helgason & J.J. Germida

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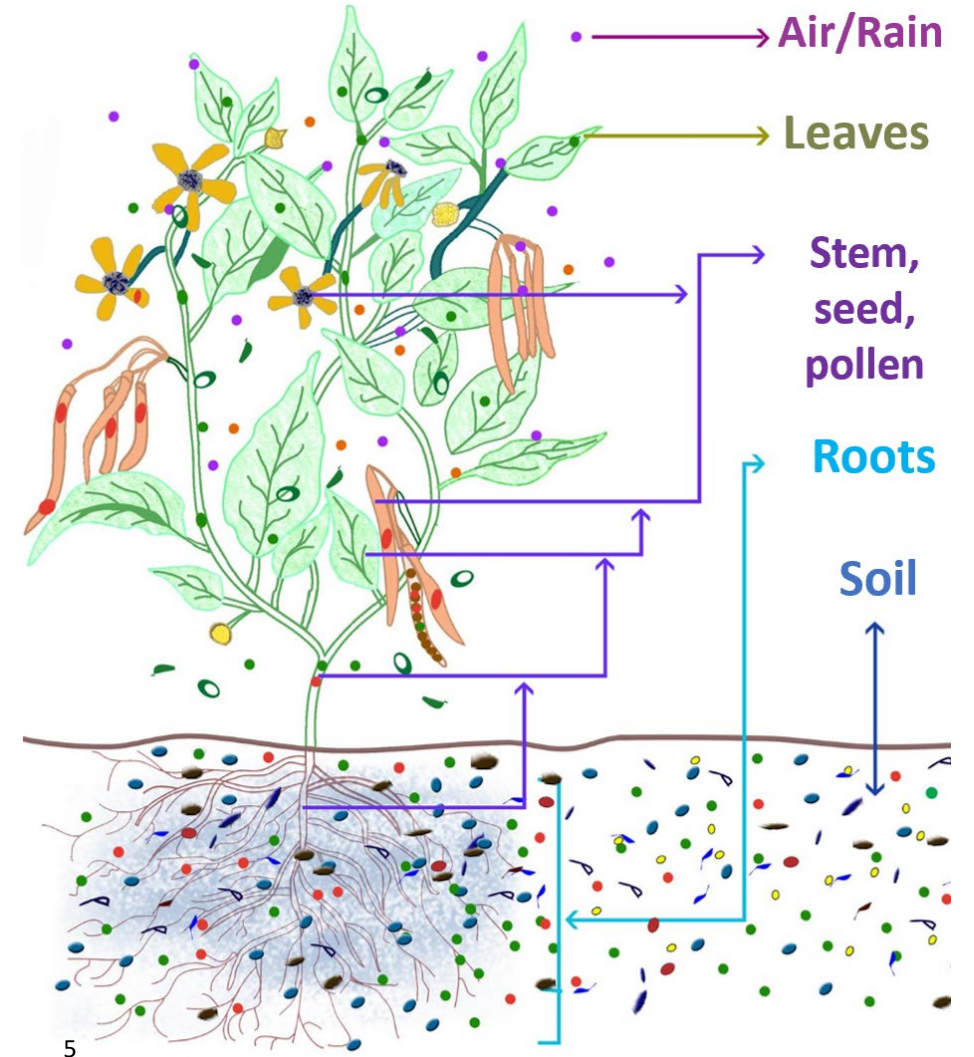
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Introduction



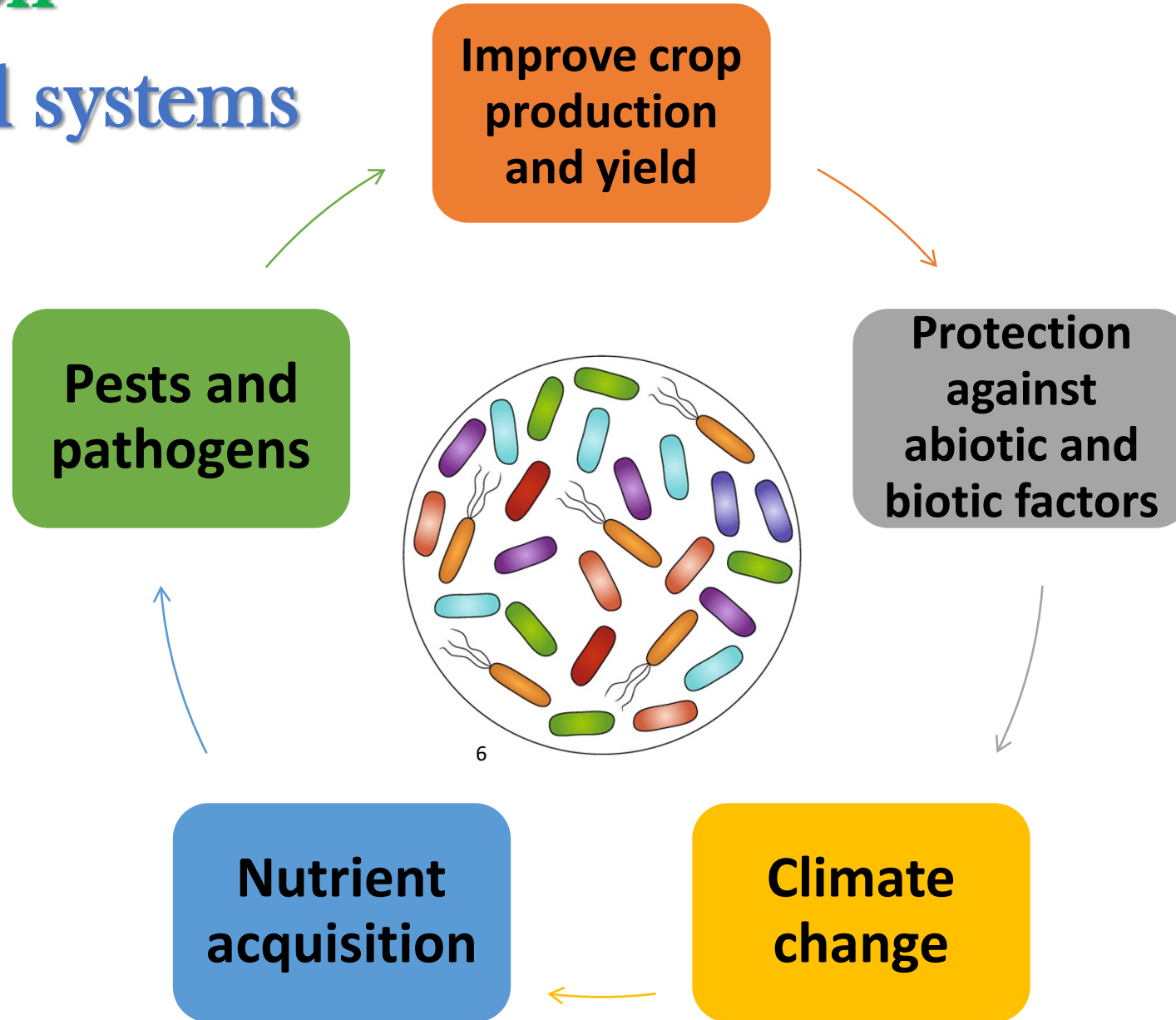
Trends in Microbiology



⁴Celluzzi, A., & Masotti, A. (2016). How our other genome controls our epi-genome. *Trends in microbiology*, 24(10), 777-787. Gopal, M., & Gupta, A. (2016). ⁵ Microbiome selection could spur next-generation plant breeding strategies. *Frontiers in microbiology*, 7, 1971.

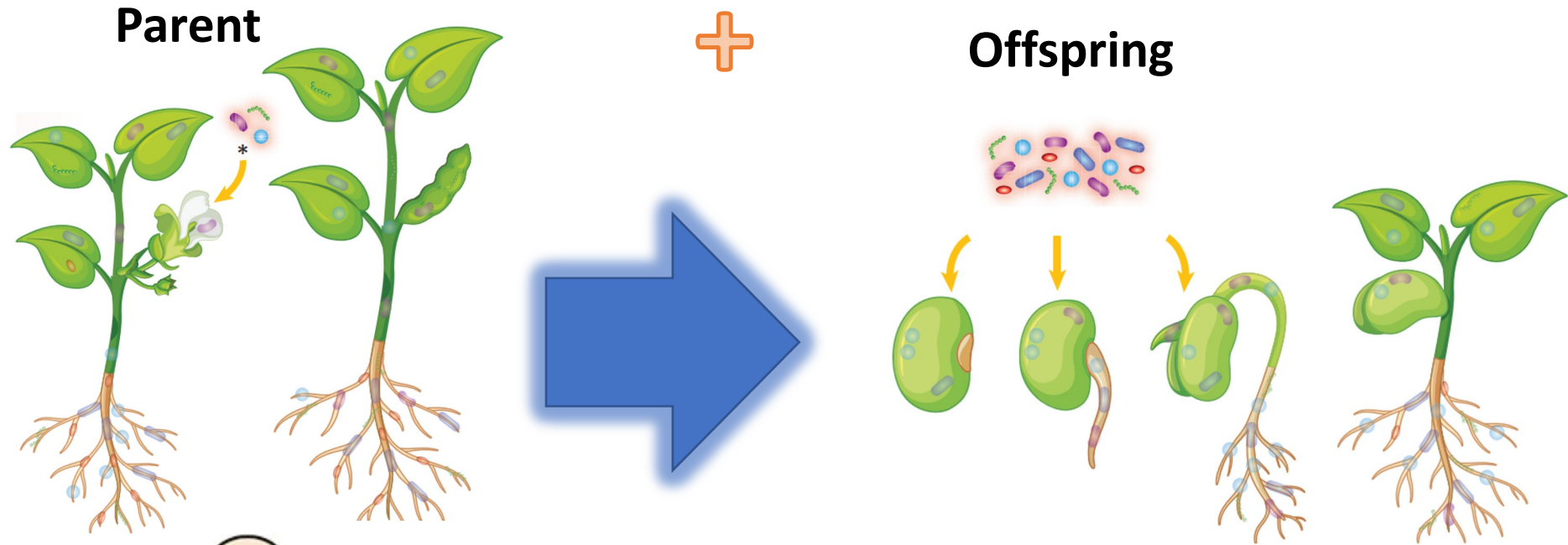
Introduction

Agricultural systems

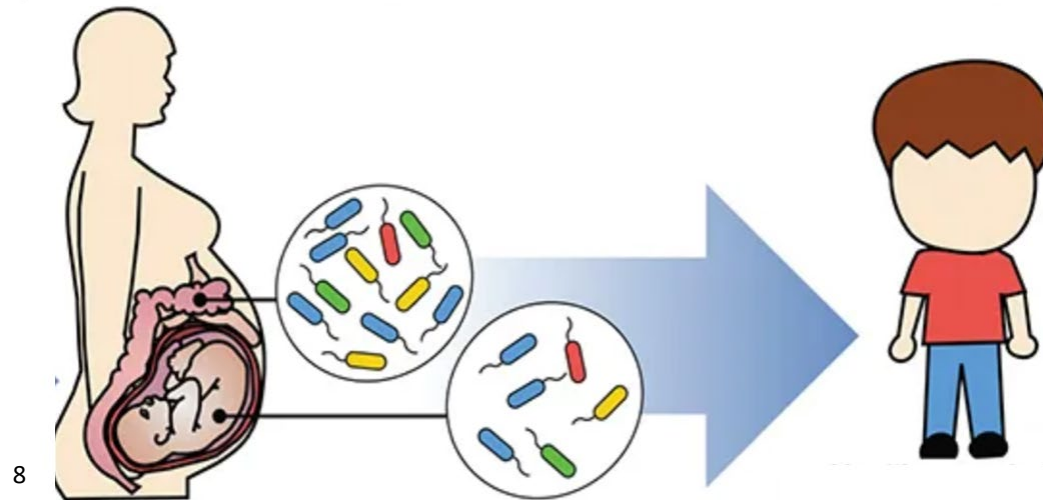


Introduction

ENVIRONMENT



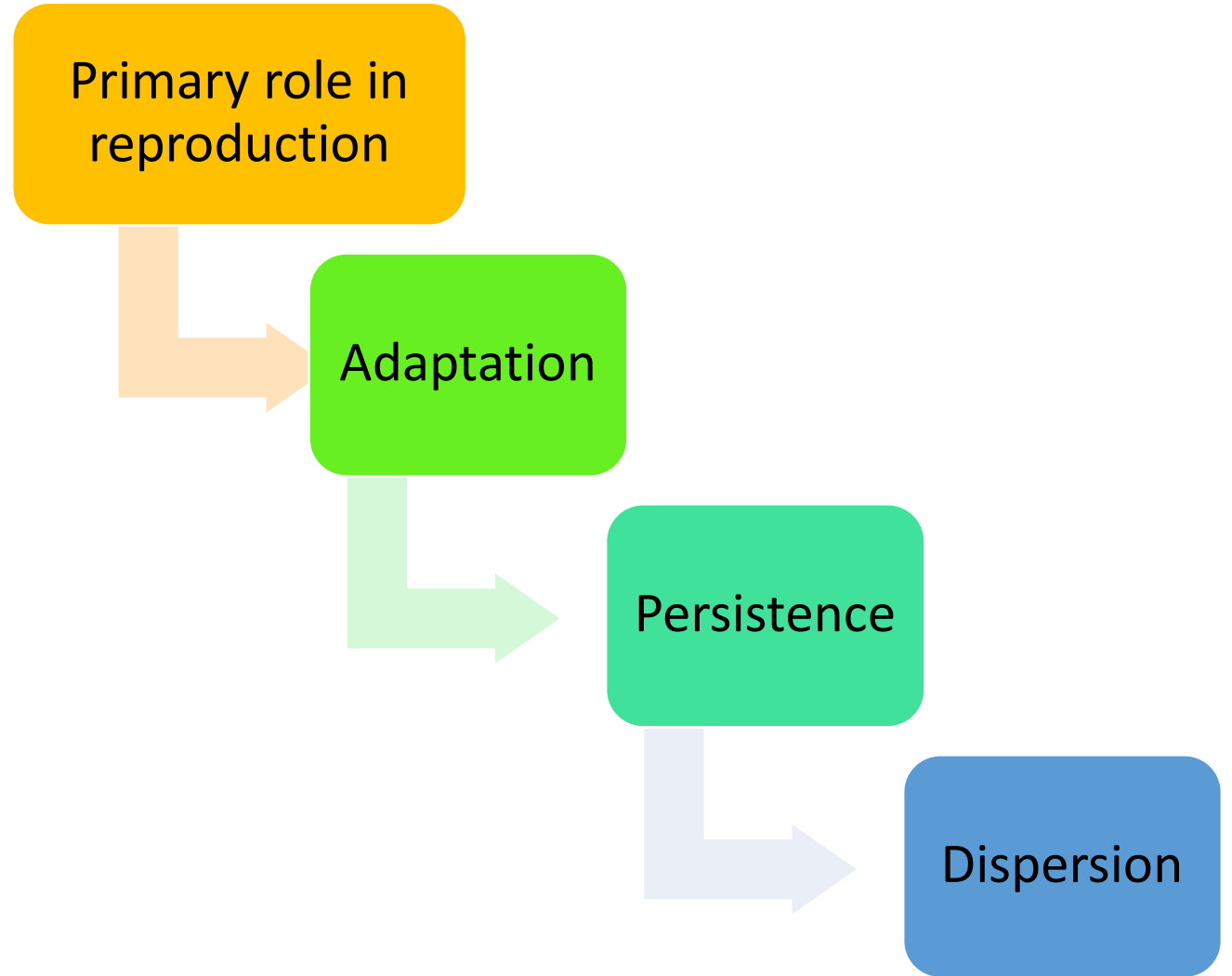
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Introduction

Seeds



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Introduction

Wheat



2018

14.7 million tonnes



Canola



10.9 million tonnes



Lentil



1.9 million tonnes



13



14



15



16

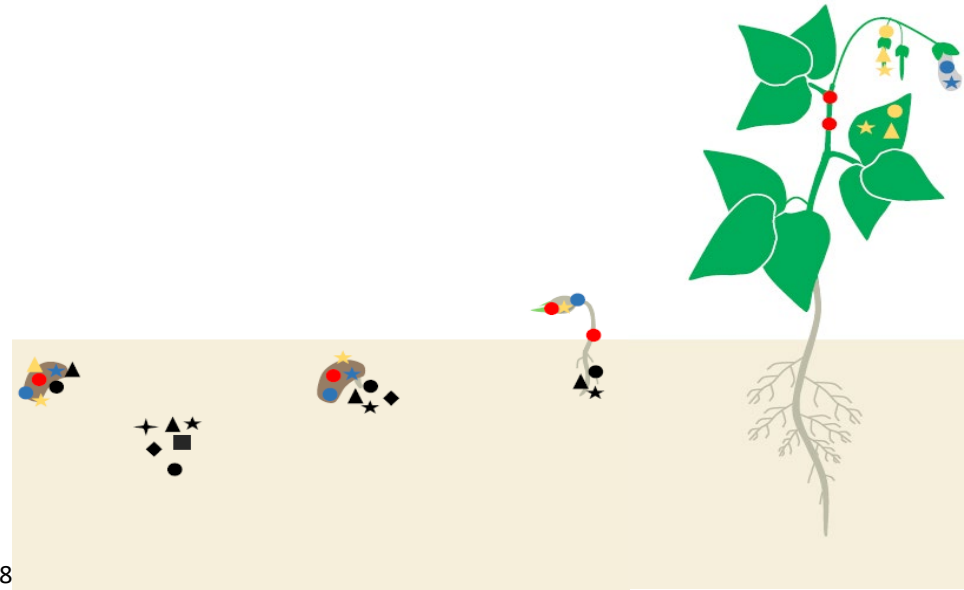


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(Statistics Canada, 2019)

¹⁰<http://www.clipartkid.com/wheatfield-cliparts/>. ¹¹<https://bilbyandbear.wordpress.com/tag/canola-fields/>. ¹²<http://www.thecombineforum.com/forums/22-pictures-blogs/20444-spraying-lentils.html>. ¹³<http://www.international.gc.ca/investors-investisseurs/cities-villes/west-ouest/saskatchewan.aspx?lang=eng>. ¹⁴<http://uregina.ca/PUBS/About%20Logo.htm>. ¹⁵<http://canadiangovernmentexecutive.ca/canada-has-more-doctors-than-ever-before/>. ¹⁶<http://www.foodinsight.org/?page=3>. ¹⁷<https://www.flickr.com/photos/laurenmannig/2979574719/>.

Introduction



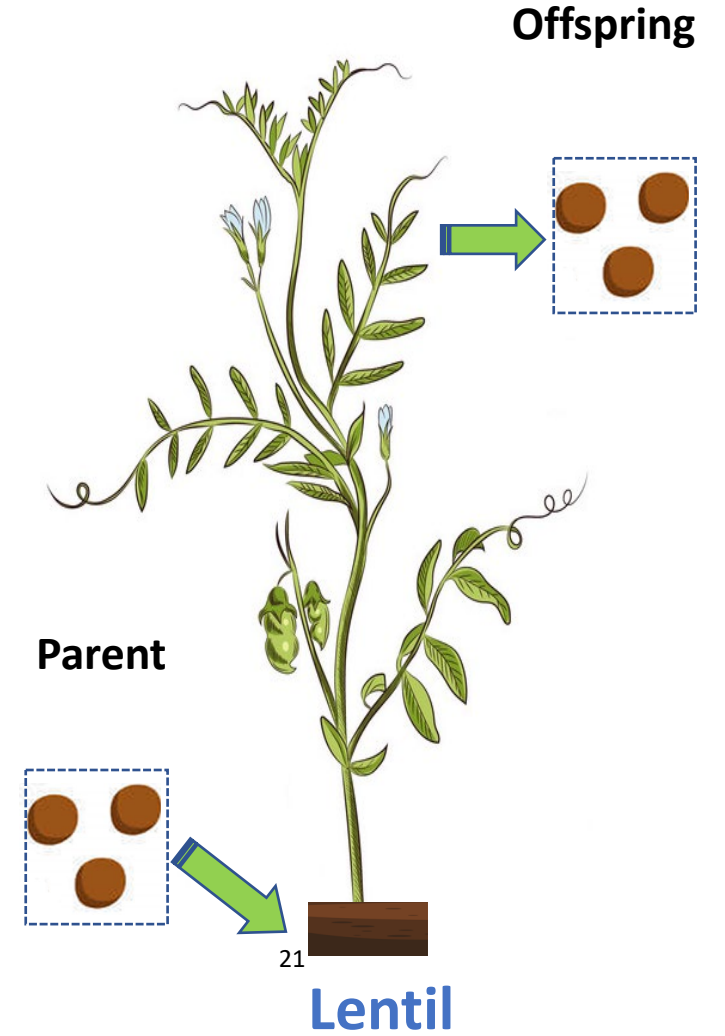
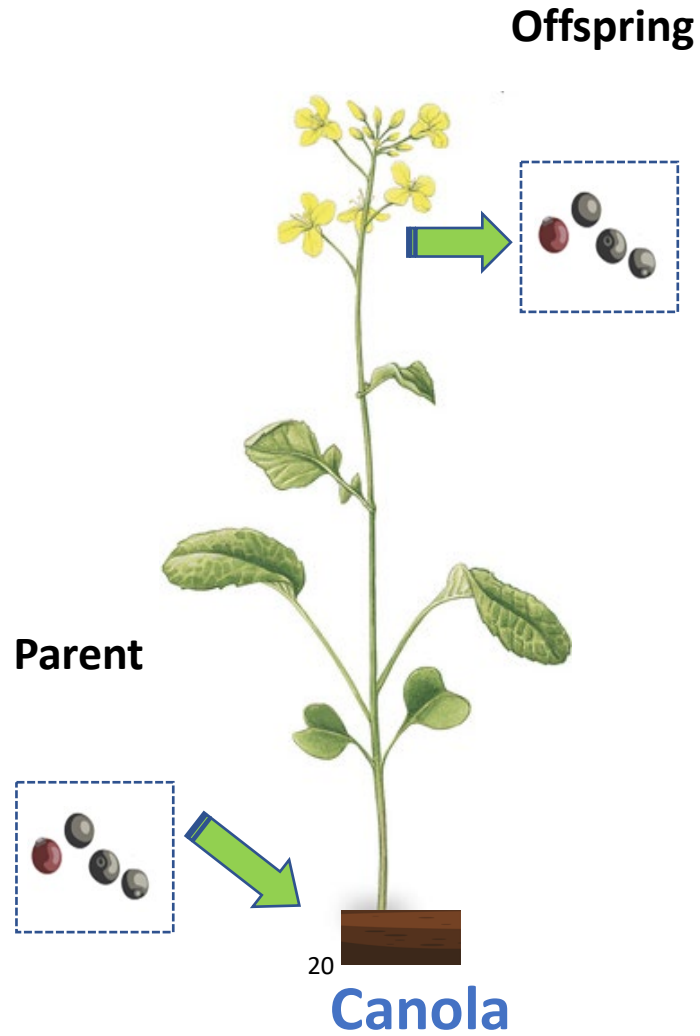
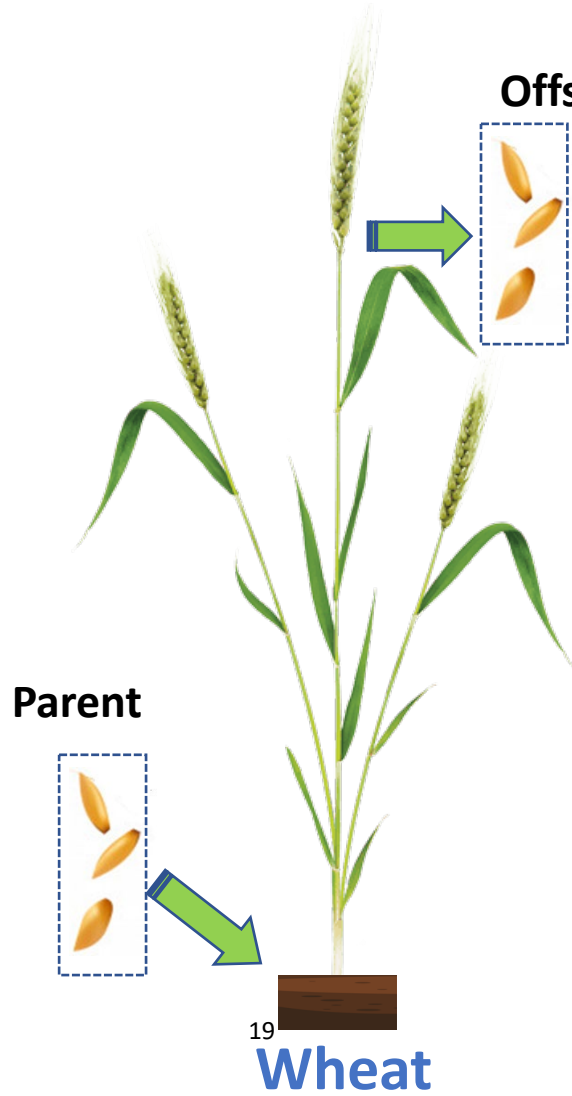
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Better strategies for manipulating the plant microbiome through breeding, inoculation and crop production practices.

- ✓ We hypothesized that the crop, genotype and environment would influence the seed microbiome assemblage.
- ✓ Seeds harvested from different field locations and years would exhibit more variability in microbial community composition.

Methods

Parents → **Different sources (Location, harvesting year)**
Offspring → **One field 2016, Saskatoon**



Methods



P²IRC Plant Phenotyping and Imaging Research Centre

Seed samples from 5 lines:

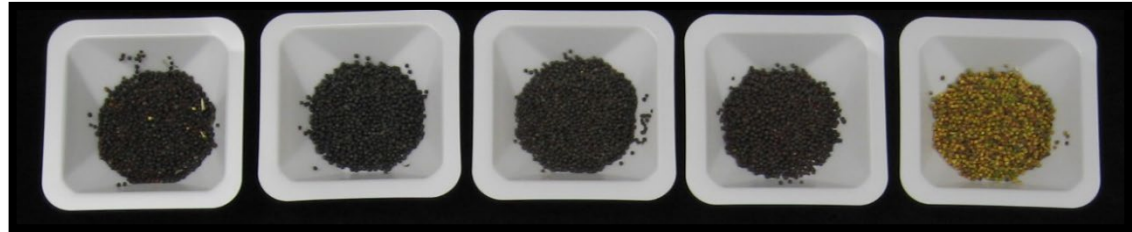
Wheat

AAC Penhold AC Barrie Frontana Red fife Sumai 3



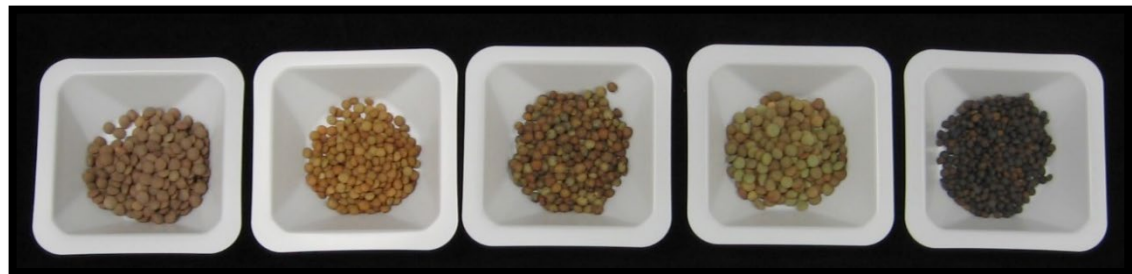
NAM 0 NAM 13 NAM 17 NAM 37 NAM 72

Canola



CDC KR-1 CDC Asterix CDC Marble CDC QG-3 Schwarze L.

Lentil

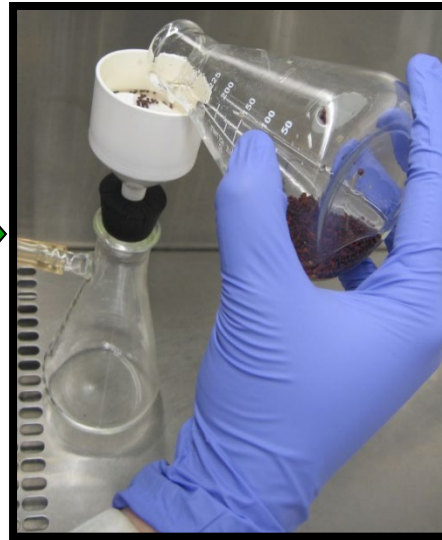
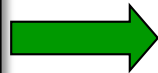


Methods

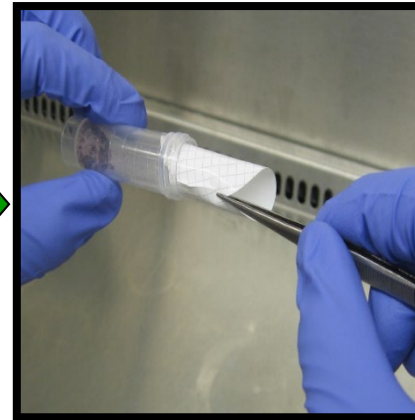
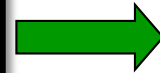
DNA Extraction



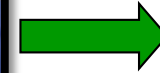
Seed sample +wash solution



Wash solution filtration

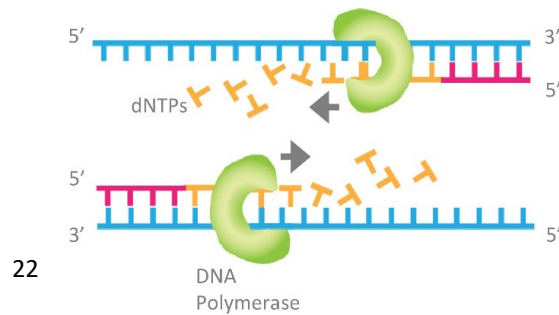


DNA extraction using the DNeasy® PowerWater® kit



Methods

Bacterial and Fungal genes, Sequencing



PCR



MiSeq Illumina

Sequence data processing, taxonomic assignment and analysis



Results

Crop type accounted for **30%** and **47%** of bacterial and fungal variance, while generation explained **10%** and **15%**, respectively.

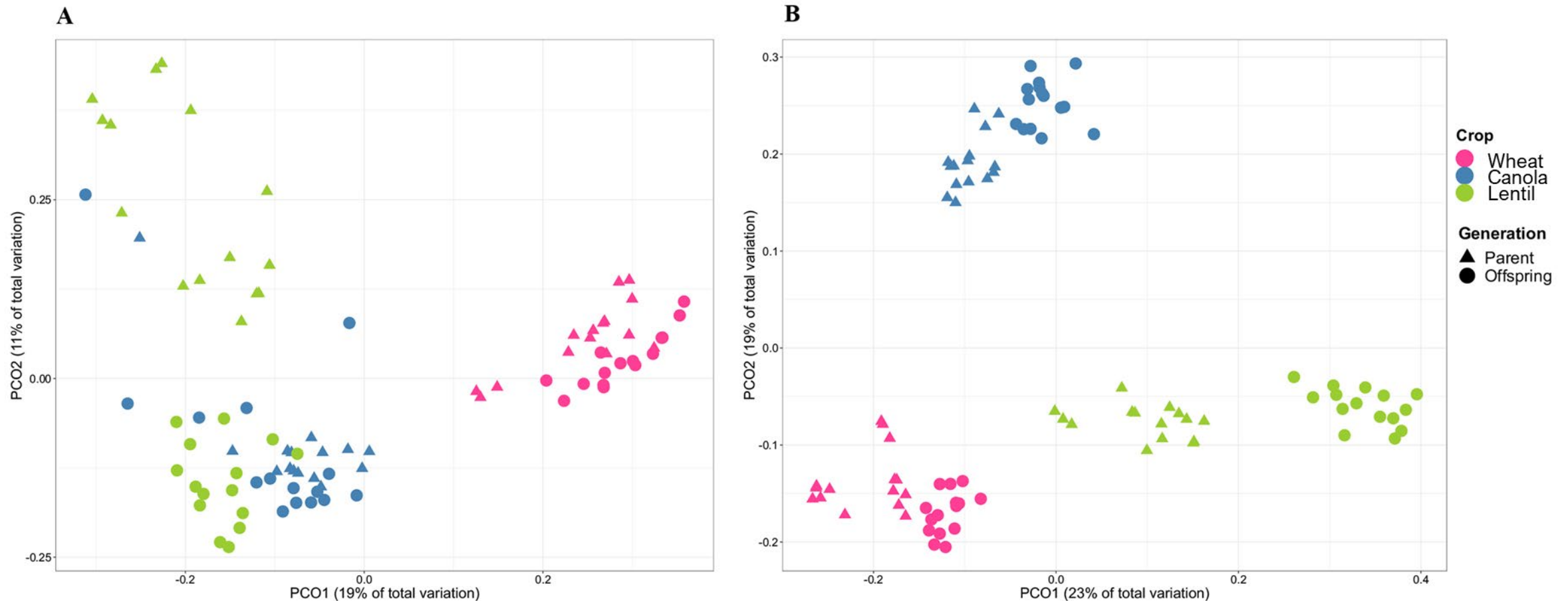


Figure 1. Principal coordinate analysis (PCoA) based on Bray-Curtis index of (A) bacterial and (B) fungal ASVs.

Results

Bacteria

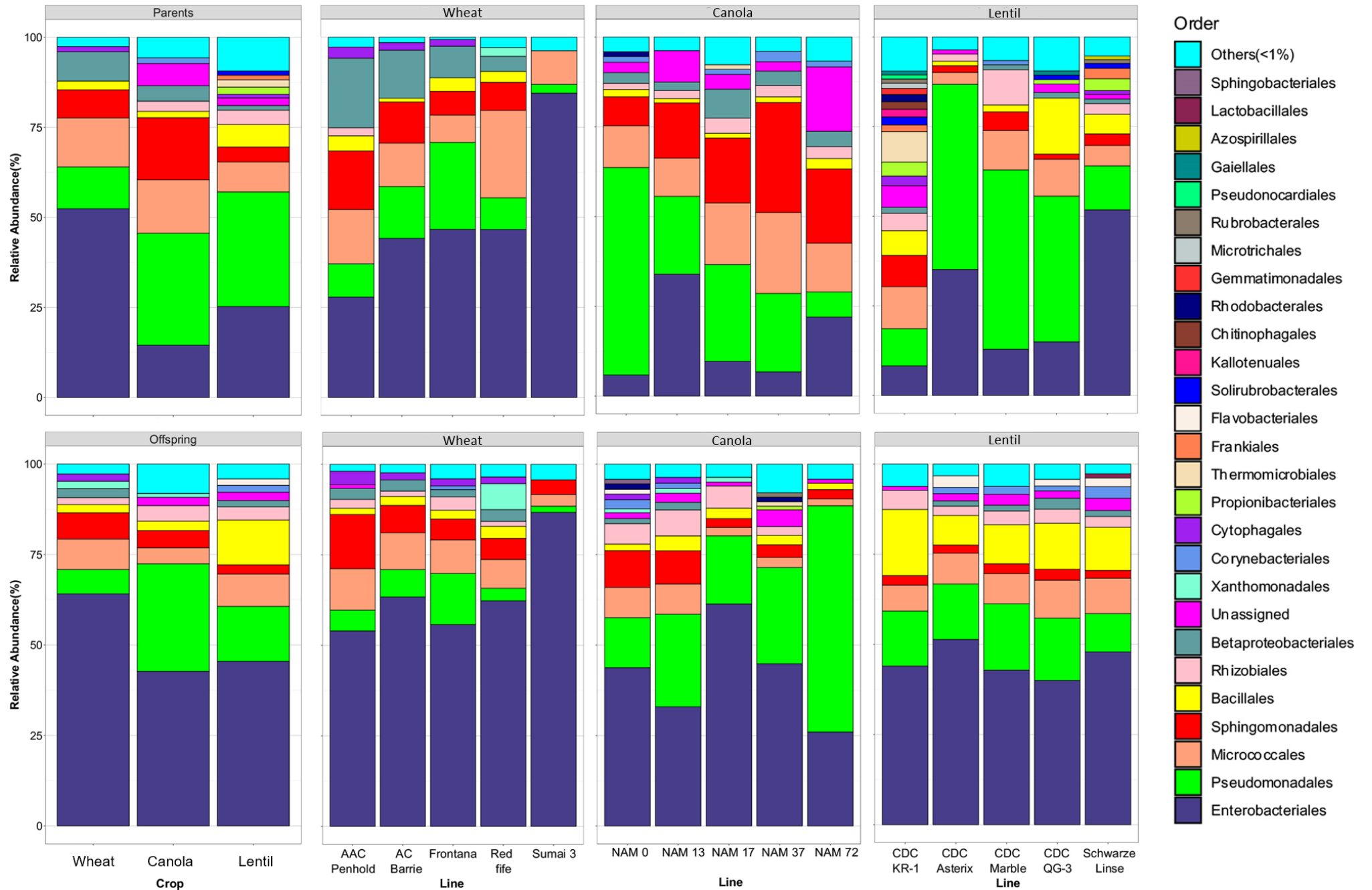


Figure 2. Relative abundance of the dominant bacterial order

Results Fungi

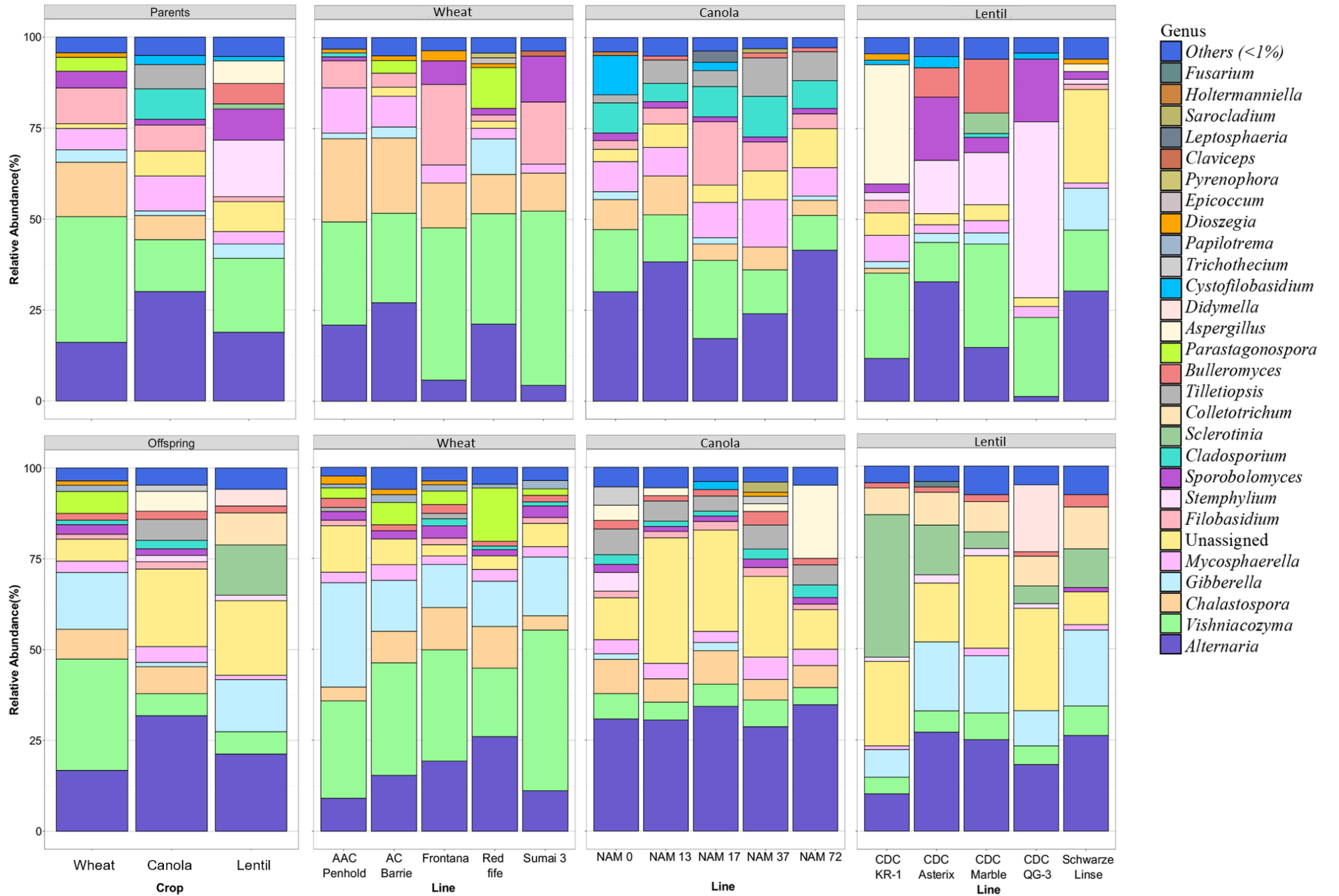


Figure 3. Relative abundance of the dominant fungal genera

Results

Core Taxa: Found in every sample and replicate examined

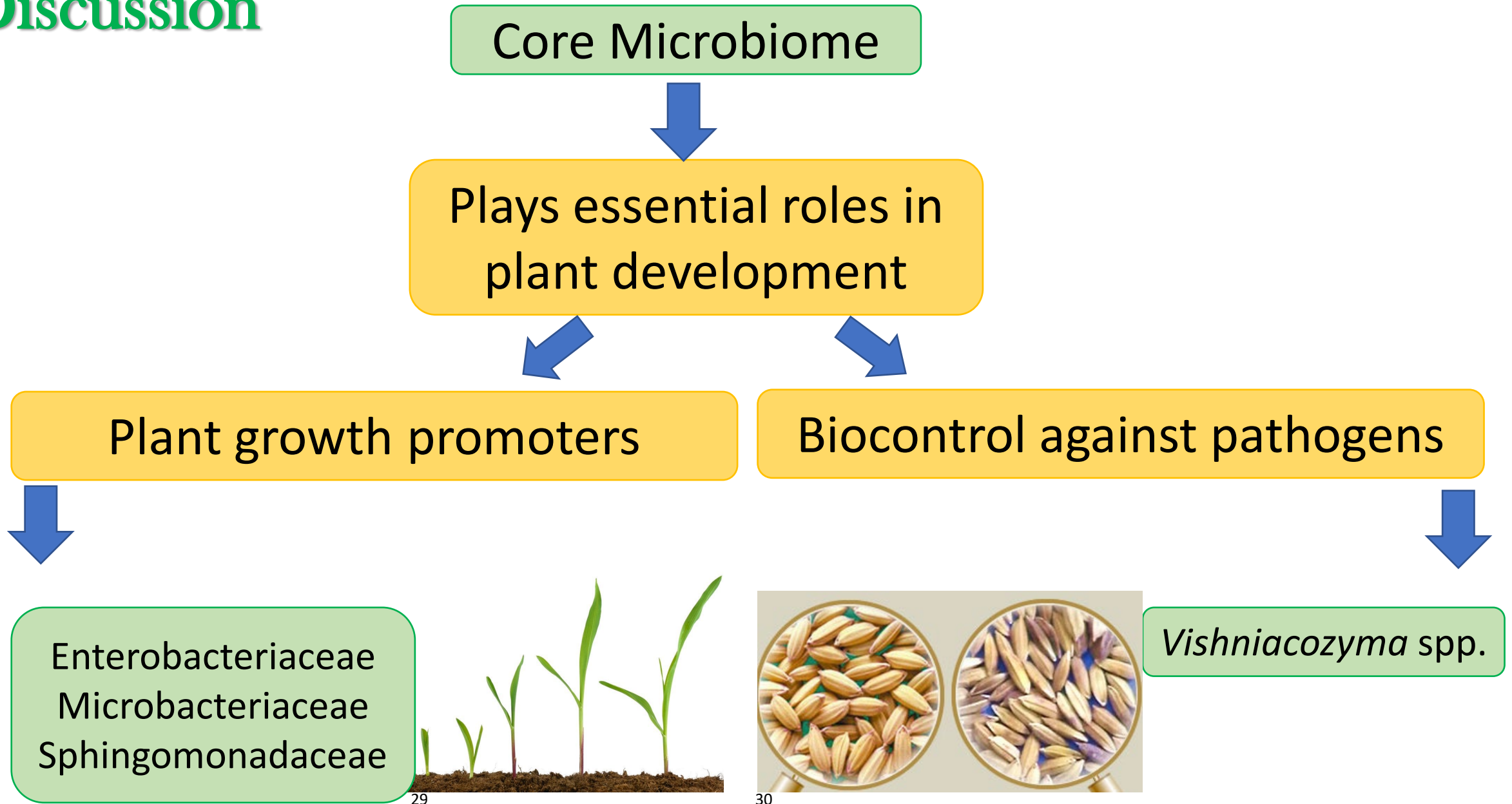
Bacteria

Enterobacteriaceae
Microbacteriaceae
Sphingomonadaceae
Nocardiaceae (*Rhodococcus* sp.)
Microbacteriaceae (*Rathayibacter* sp.)

Fungi

Alternaria sp.
Mycosphaerella tassiana
Chalastospora gossypii
Vishniacozyma victoriae
Vishniacozyma victoriae
Vishniacozyma victoriae
Gibberella baccata
Filobasidium sp.
Vishniacozyma sp.
Filobasidium magnum
Dioszegia hungarica
Mycosphaerella tassiana

Discussion



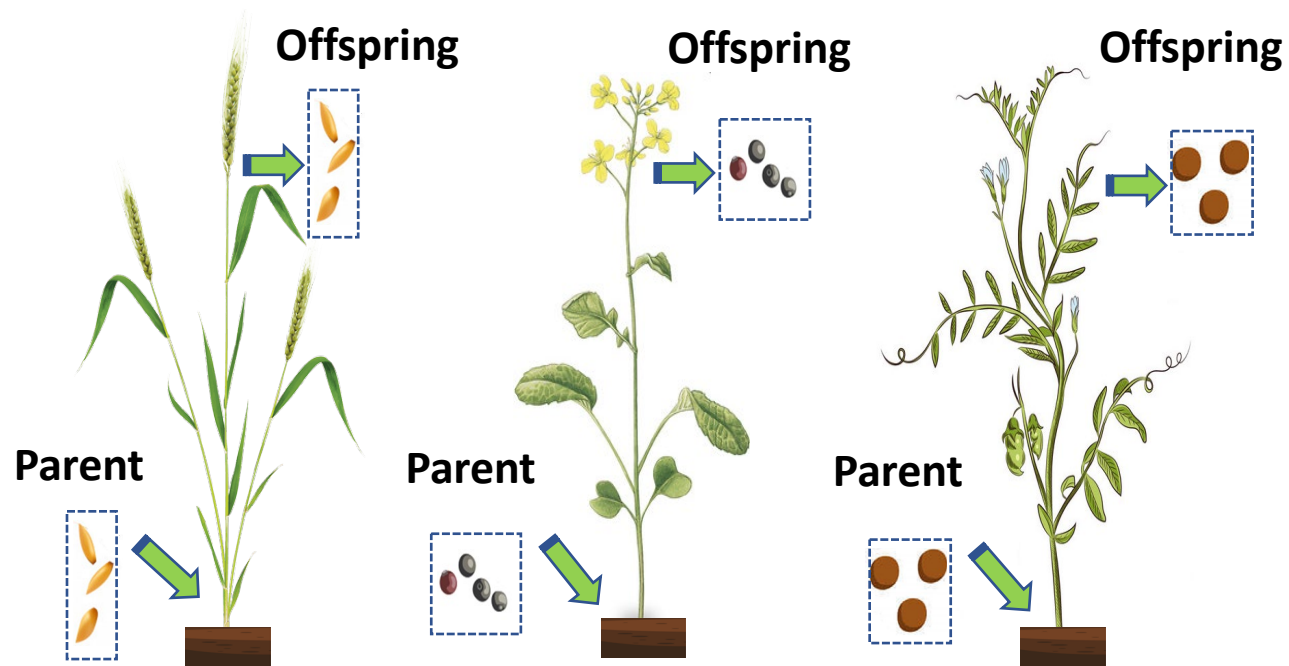
Discussion

Differences between parents and offspring

ENVIRONMENT



(P2IRC 1.3, 2016)

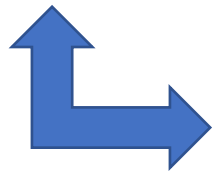


Agricultural management practices



Discussion

Offspring
Same field



Differences among crops and lines persisted



Intimate relationship host and microbes

Conclusions

- ✓ Crop, genotype and environment influence the seed microbiome assemblage in a broad range of agricultural crops.
- ✓ The presence of a core microbiota suggest that transmission, preservation, and recruitment of microorganisms are determined to some extent by the host.

Information collected in this study provides support to the use of new and better strategies for microbial persistence within seeds, leading to a more sustainable agriculture.

Acknowledgements

Soil Microbiology Lab 5E25



UNIVERSITY OF SASKATCHEWAN
College of Agriculture
and Bioresources



**Plant Phenotyping
and Imaging
Research Centre**



POTASHCORP - A FOUNDING PARTNER



Questions?



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Crop	Line	Main Characteristics
<i>T. aestivum</i>	AAC Penhold	Canadian hard red spring wheat cultivar developed at the Swift Current Research and Development Centre (SCRDC), Agriculture and Agri-Food Canada (AAFC), Swift Current, SK in 2004. This cultivar shows high yield potential and improved protein content. Resistant to leaf rust and common bunt and moderate resistant to <i>Fusarium</i> head blight and stem rust (Cuthbert et al., 2017) (Canadian Food Inspection Agency, 2019).
	AC Barrie	Canadian hard red spring wheat cultivar developed at the SCRDC, AAFC, Swift Current, SK in 1994. It combines high grain yield with high protein content, resistant to leaf and stem rust, common bunt, and loose smut (Mccaig et al., 1995).
	Frontana	Brazilian wheat cultivar released in 1940, widely used <i>Fusarium</i> head blight resistant source (Steiner et al., 2004).
	Red fife	Red spring wheat cultivar with excellent bread-making quality. Originated in Galicia region of central Europe and released in Canada in 1845 (Fu et al., 2005; Mccallum et al., 2008).
	Sumai 3	Chinese spring wheat cultivar released in 1970 developed by Suzhou Institute of Agricultural Sciences in Jiangsu province. This cultivar is recognized as one of the best sources of <i>Fusarium</i> head blight resistance in the world (Zhu et al., 2019).
<i>*B. napus</i>	NAM 0	Canadian Line, black seed, low fiber, low erucic acid, low glucosinolate content.
	NAM 13	German cultivar (Campino), black seed, high fiber, low erucic acid, low glucosinolate content.
	NAM 17	Canadian Line, black seed, low fiber, low erucic acid, low glucosinolate content.
	NAM 37	Australian cultivar (Wesroona), black seed, high fiber, low erucic acid, high glucosinolate content.
	NAM 72	Canadian Line, yellow seed, very low fiber, low erucic acid, low glucosinolate content
† <i>L. culinaris</i>	CDC KR-1	Large red cultivar developed by the Crop Development Centre (CDC) at the University of Saskatchewan, Canada and released in 2009. Exhibits gray seed coat and red cotyledon colour, moderate resistant to Ascochyta blight and Anthracnose Race 1.
	CDC Asterix	Extra small green cultivar developed by the CDC at the University of Saskatchewan, Canada and released in 2012. Exhibits green seed coat and yellow cotyledon colour, moderate resistant to Ascochyta blight and intermediate resistant to Anthracnose Race 1.
	CDC Marble	French green cultivar developed by the CDC at the University of Saskatchewan, Canada and released in 2012. Exhibits green marble seed coat and yellow cotyledon colour, moderate resistant to Ascochyta blight and intermediate resistant to Anthracnose Race 1.
	CDC QG-3	Green cotyledon cultivar developed by the CDC at the University of Saskatchewan and Saskatchewan Pulse Growers, Canada and released in 2014. Exhibits green seed coat and green cotyledon colour, intermediate resistant to Ascochyta blight and moderate resistant to Anthracnose Race 1. CDC QG-3 is an imidazolinone tolerant variety.
	Schwarze Linse	Black German cultivar developed in 1964. Exhibits black seed coat and red cotyledon colour (Shaikh et al., 2013).

Nested-association mapping (NAM) populations

Crop	Line	Harvest year	Field site	GPS coordinates
Parents				
<i>T. aestivum</i>	AAC Penhold	2015	Saskatoon, Canada	52°8'23.29"N, 106°36'49.589"W
	AC Barrie	2014	Saskatoon, Canada	52°8'49.11"N, 106°32'47.738"W
	Frontana	*	Swift Current, Canada	50°16'51.56"N, 107°45'28.63"W
	Red fife	2014	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97"W
	Sumai 3	*	Swift Current, Canada	50°16'51.56"N, 107°45'28.63"W
<i>B. napus</i>	NAM 0	2014, 2015	Mini-Cage Increase SK, Canada	52°8'1.568"N, 106°38'6.961"W
	NAM 13	2014	Hoop Tent SK, Canada	52°8'1.568"N, 106°38'6.961"W
	NAM 17	2014	Hoop Tent SK, Canada	52°8'1.568"N, 106°38'6.961"W
	NAM 37	2014	Hoop Tent SK, Canada	52°8'1.568"N, 106°38'6.961"W
	NAM 72	2014	Hoop Tent SK, Canada	52°8'1.568"N, 106°38'6.961"W
<i>L. culinaris</i>	CDC KR-1	*	Regina, Canada (Pulse Trading company)	50°27'3.013"N, 104°29'50.96"W
	CDC Asterix	2011	Saskatoon, Canada	52°9'44.56"N, 106°32'35.50"W
	CDC Marble	2012	Saskatoon, Canada	52°9'31.29"N, 106°32'35.89"W
	CDC QG-3	2012	Saskatoon, Canada	52°3'44.71"N, 106°24'47.45"W
	Schwarze Linse	2015	Saskatoon, Canada	52°3'53.86"N, 106°26'21.631"W
Offspring				
<i>T. aestivum</i>	AAC Penhold	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	AC Barrie	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	Frontana	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	Red fife	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	Sumai 3	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
<i>B. napus</i>	NAM 0	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
	NAM 13	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
	NAM 17	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
	NAM 37	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
	NAM 72	2016	Saskatoon, Canada	52°10'52.918"N, 106°30'10.587" W
<i>L. culinaris</i>	CDC KR-1	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	CDC Asterix	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	CDC Marble	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	CDC QG-3	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W
	Schwarze Linse	2016	Saskatoon, Canada	52°9'45.14"N, 106°30'57.97" W