

Pulse-Wheat Rotation: Pulse Crops Effects on Wheat Endophytic Bacteria, and Feedback on Wheat Yield

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Key Words: rotation, pulse, endophytic bacteria, wheat yield.

Abstract

Crop rotation is a common cultivation strategy and the legacy of a previous crop on biotic soil properties could feedback on the productivity of the following crop due to the mutual influence existing between plants and their biological environment. Polymerase chain reaction and 454 GS FLX pyrosequencing of amplicons were used in this study to determine the effects of genotype and termination time of previous pulse crops on the productivity and endophytic bacterial community colonizing the roots of durum wheat. In 2008-09, when chickpea plants (late maturing plant) were terminated as early as yellow pea (In July), all three genotypes of chickpea passed a soil biota to durum wheat that formed endophytic bacterial community which was similar to that formed following yellow pea, an early maturing plant. These four endophytic bacterial communities were different from those formed in wheat roots following late-terminated chickpea crops. Additionally, late terminated pulse crops (September) led to the formation of endophytic bacterial communities highly dominated by Firmicutes. These communities were less diverse than those formed after early-terminated pulse crops, which were dominated by Actinobacteria. High durum wheat yield was associated with the production of fewer heads m⁻² and with the abundance of endophytic Actinobacteria and Acidobacteria. In contrast, low grain yield was associated with the abundance of endophytic Firmicutes. The effect of termination time was weak in 2009-2010, which was probably overridden by the extremely high amount of precipitation received during this period. The results demonstrate an important biotic legacy of crop termination time in agro-ecosystems, by influencing the endophytic bacterial colonization on the following crop. Environmental conditions appear as a key factor for the expression of this effect of crop termination time in agroecosystems.

Introduction

Crop rotations have supported human societies through history (3), as far back as the Roman empire (24). In particular, pulse-cereal rotations were traditionally used for their positive influence on soil biological quality and plant health as they can break disease cycles (24). Plants have evolved with the capacity to modify their soil microbial environment and create positive feedback on the productivity of plant community (8, 49).

Plant roots strongly influence soil microorganisms, providing them with niches and nutrients (11). Some bacteria associated with plant roots are capable of living inside the plant tissue without causing plant disease (48). Although bacterial endophytes occur at low population densities in roots (46), they may stimulate plant growth (45). In wheat (*Triticum aestivum* L.), bacterial endophytes from different phyla were reported (13, 22). The effect of bacterial endophytes on

wheat growth is poorly understood due to limited experimental methods (32), and it is unknown how they are selected by wheat roots in semiarid agro-ecosystems.

Chickpea (*Cicer arietinum* L.) is planted in arid and semiarid regions worldwide (27, 33, 38) often in rotation with wheat. Chickpea has an indeterminate growth habit (2). It can use water from deeper soil layers and grow vegetatively well into fall (16) in contrast to pea which matures as early as July. Previous research has reported that plants at different growing stages select rhizobacterial communities (11) with different metabolisms (4), and different symbiotic relationships with their host plants (44). We hypothesized that the later termination of chickpea could select a rhizobacterial community with reduced growth promoting abilities, resulting in lower productivity of durum wheat grown after chickpea than after pea. The objective of this study was to (1) describe the effects of termination time of previous pulse crops on the bacterial endophyte community colonizing the roots of durum wheat grown the following year, and (2) to explore the relationship between the structure of endophytic bacterial communities and wheat yield under field conditions.

Materials and methods

Experimental design and treatment application

Table 1 Description of the genotype / termination time treatments applied at rotation phase-1 to measure their effects on the durum crop grown in rotation phase-2

| Treatment | Rotation phase-1 | | | Rotation phase-2 |
|-----------|------------------|------------------|----------------|------------------|
| | Pulse genotype | Termination time | | |
| Y | Yellow Pea | Early July | - | Durum wheat |
| VE | CDC Vanguard | Early July | / | Durum wheat |
| LE | CDC Luna | Early July | / | Durum wheat |
| FE | CDC Frontier | Early July | / | Durum wheat |
| VL | CDC Vanguard | / | Late September | Durum wheat |
| LL | CDC Luna | / | Late September | Durum wheat |
| FL | CDC Frontier | / | Late September | Durum wheat |

A field experiment was set out in a randomized complete block design with four blocks at the South Farm of the Semiarid Prairie Agricultural Research Centre (SPARC) in Swift Current, SK, Canada (latitude 50° 18' N; longitude 107° 41' W). The experiment was conducted in 2 m × 8 m plots in 2008-2009, and repeated in 2009-2010. Seven preceding pulse crop treatments were applied at stage-1 of a 2-year rotation with durum wheat (*Triticum turgidum* var *durum* Desf.) cultivar AC Avonlea. They were: an early maturing yellow pea crop (cultivar CDC Handel [Y]), three chickpea cultivars terminated as early as the yellow pea by mowing (CDC Vanguard [VE], CDC Luna [LE], CDC Frontier [FE]), and the same three chickpea cultivars terminated late

(CDC Vanguard [VL], CDC Luna [LL], CDC Frontier [FL]) i.e., when they reached full maturity (Table 1). Any regrowth of the early-terminated chickpea plants was removed by continued mowing. Durum wheat was planted at stage-2 of the rotation at a seeding rate of 113 kg / ha. Durum wheat was fertilized with 43 kg • ha⁻¹ of P (11-51-0) and 111 kg • ha⁻¹ of N (46-0-0). Roundup WeatherMAX® was applied at 815 ml • ha⁻¹ on May 5th and Achieve® Liquid Gold was applied at 490 ml • ha⁻¹ on June 3rd for weed control.

Root sampling

Durum wheat root samples were taken at flag-leaf stage. Five plants were randomly taken from five locations in each plot using a shovel. Shoots were detached, and roots were placed in plastic bags and kept at 4°C for a few hours until processing. Root samples were washed under running tap water and cut into 1-cm fragments. A representative subsample (2 g fresh weight) from each plot was placed in a 1.5-ml plastic tube, surface sterilized for 1 min in 70% ethanol mixed with 30% hydrogen peroxide (1:1), and rinsed several times with sterile distilled water. Cleaned root samples were oven dried at 50 °C for 24 h and finely ground in a bead miller (Retsch, MM301).

Characterization of bacterial endophyte communities by 454 pyrosequencing

Raw DNA was extracted from ground root samples using a DNeasy Plant Mini Kit (QIAGEN group, Toronto, ON, Canada) following the manufacturer's protocol. After 10 times dilution, DNA was subjected to polymerase chain reaction (PCR) using primers 968f / 1041b amplifying bacterial 16S-rDNA universal gene fragments (51). 454 Life Science's A or B sequencing adaptors were fused to the 5' end of forward or reverse primers, and unique barcode sequences were added between the A adaptor and the forward primer in order to trace the sources of sequences after multiplex sequencing of amplicons. Platinum® PCR SuperMix (Cat. No. 11306-016, Invitrogen™) was used for PCR. Thermal cycling was conducted in an Veriti™ 96-well fast Thermal Cycler (Applied Biosystems) with the following conditions: 4 min initial denaturation at 94 °C; 30 cycles of 45 s denaturation at 94 °C, 45 s annealing at 56 °C and 1 min elongation at 72 °C; and a 15 min final elongation at 72 °C.

All PCR products were purified on agarose gel. Briefly, PCR products were run in 1% agarose gel under 65 V for 1 h, then, gel pieces containing visible bands of target size were cut off with a sterile blade, put into a sterile centrifuge tube with 30 µl TE buffer (1×dilution), vortexed for 1 min and placed at 4 °C over night for extraction. The concentration of purified PCR products in TE buffer was measured with a Nano Drop-1000 spectrophotometer (Thermo scientific®). The concentration of each sample was adjusted to 30 ng µl⁻¹. PCR products carrying different MIDs were placed in a sterile 1.5 ml plastic tube in pools of 16, and submitted for pyrosequencing at Génome Québec (Montréal, Québec, Canada).

Head number, grain yield and grain protein of durum wheat

At physiological maturity, one 1-m² size quadrat of durum plants was randomly selected from each plot, and all the heads of the plant in the quadrat were counted. At full maturity, durum wheat was harvested and the seeds from each plot were cleaned to remove debris, oven dried, and dry weights were recorded. A 350-g seed sample from each plot was used for grain protein determination using an Infratec 1229 Grain Analyzer (Foss Tecator, AB)

Bioinformatic and data analysis

All sequence data were edited to remove primers, MID, and adaptor sequence fragments, using Mothur V.1.15.0 (47). All sequences used in this study shared at least 97% similarity with known sequences (52), based on the Silva database (<http://www.arb-silva.de/>). Rarefaction analysis, classification of dominant phyla and heat map analysis were also conducted using Mothur V.1.15.0. Effects of experimental treatments on the Chao 1 and ACE richness estimating indicators were determined using Mothur V.1.15.0. The effect on durum wheat yield was tested by ANOVA in SYSTAT 12.0, and the significant differences between treatments were tested with Fisher-LSD test at the 5% level. The significance of termination time effect on the proportion of dominant bacterial phyla in wheat roots was assessed by MultiResponse Permutation Procedure (MRPP) in PC-ORD. Effects of treatments on crop-related response variables were detected by Multivariate analysis of variance (MANOVA) of SYSTAT 12.0. The relationship between crop-related response variables (durum wheat heads m⁻², percentage of grain protein, grain yield) and bacterial community structure, described as the number of OTUs measured in each bacterial phyla, was assessed by redundancy analysis (RDA) (9) and plotted using R (41) with the package Vegan 1.15-4 (15).

Results

Effect of pulse termination time on bacterial communities in durum wheat roots

Overall, the bacterial endophyte richness in the roots of durum wheat was higher in 2009 than in 2010. ANOVA detected a significant effect of preceding pulse crops on bacterial community richness in the roots of durum wheat in 2009 (Table 2). Endophytic bacteria richness was lower in the roots of durum wheat following late-terminated pulse crops in 2009 compared with early-terminated pulses. The richness of bacterial endophyte community following pea did not differ from that following the early-terminated chickpea crops. However, in 2010 the preceding pulse crops did not influence the richness of the bacterial endophyte community in the succeeding durum wheat.

Table 2 Effects of different preceding pulse crops on the richness of the bacterial endophyte community of durum wheat roots in 2009

| Preceding pulse crops | Chao 1‡ | ACE |
|-----------------------|-------------------|-------------------|
| Y† | 721 ^{ab} | 1022 ^a |
| LE | 687 ^b | 869 ^{ab} |
| VE | 748 ^{ab} | 783 ^b |
| FE | 950 ^a | 1229 ^a |
| LL | 277 ^c | 374 ^c |
| VL | 155 ^c | 223 ^c |
| FL | 217 ^c | 329 ^c |
| P value | <0.0001 | 0.001 |

†See Table 1 for the treatment descriptions.

‡Means associated with different letters within a column are significantly different at the 5 % probability level according to ANOVA protected LSD; $n = 4$.

Heat map analysis of the structure of the endophytic bacterial community, assessed as the abundance of OTUs distributed in different phyla, also revealed differences between termination times (Fig. 1). In 2009, high similarity was found in communities inhabiting durum wheat roots following early-terminated pulse crops as shown by high Yue & Clayton Theta similarity coefficient (light color). Similarity was also high in communities following late-terminated pulse crops. But little similarity was found between bacterial endophyte communities following early-, and late-terminated pulses. These results suggest that different pulse termination times at phase-1 of a crop rotation affects the composition of durum wheat bacterial endophyte communities. MRPP analysis conducted on the three dominant bacterial phyla of these communities, Actinobacteria, Firmicutes and Proteobacteria, confirmed the high significance ($P < 0.001$) of two termination times on the bacterial endophyte community structure of durum wheat roots. Higher proportions of Actinobacteria and Proteobacteria were found in durum wheat roots after the early-termination of the pulse, while Firmicutes dominated after a late-terminated pulse crop (Fig. 2). The Actinobacteria, which comprised 42% - 65% of total identified OTUs in durum wheat roots following an early-terminated pulse crop, made up less than 5% of total identified OTUs following a late-terminated crop, where Firmicutes was dominant and accounted for more than 80% of total identified OTUs.

Durum wheat yield

MANOVA showed the effect of termination time on durum wheat grain yield in 2009 was significant ($P < 0.001$). Grain yield of durum wheat was lower after a pulse crop terminated late than after an early-terminated pulse crop (Fig. 3). RDA results showed that bacterial endophyte community structure was correlated ($P = 0.001$) with durum wheat grain yield and with the number of heads per m^2 (Fig. 4). A negative relation between grain yield and abundance of heads suggests an early influence of bacterial endophytes on tiller formation leading to a lower number of reproductive stems bearing larger spikes, where early pulse termination increased durum wheat yield. The abundance of Firmicutes, the phylum dominating in durum roots after late pulse crop termination, was negatively related with wheat yield, and positively related with the number of heads per m^2 , suggesting a role for some bacterial endophytes in the modification of durum wheat plant development. The percentage of protein in the grain was unrelated to bacterial endophyte colonization of durum wheat roots (Fig. 4).

Discussion

The feedback mechanisms of soil communities on plant growth (8, 50) generates significant interest in plant ecology. How plants influence their community through their effects on the soil biota is important in explaining the dynamics of plant community composition (26, 40, 43) and the process of plant invasiveness (28, 42). The concept of soil community feedback has also been applied to field crops (19).

The results of the present study showed the contribution of soil community feedback effects to crop rotations. It is very difficult to disentangle the different influences making up a “rotation effect” (Kirkegaard et al. 2008). This is particularly true in the case of the rotation effect of chickpea. As compared with other pulse crops such as yellow pea, chickpea is much less beneficial to the productivity of a following wheat crop. Whereas differences can be attributed to different water use and biological nitrogen fixation legacy in the different pulse crops (34, 35), a large part of the difference remains unexplained (10). Varied composition of cereal

rhizobacterial communities were found in different pulse-cereal rotation series (1), suggested that changed pulse crops impact the rhizobacterial community of a following cereal crop differently.

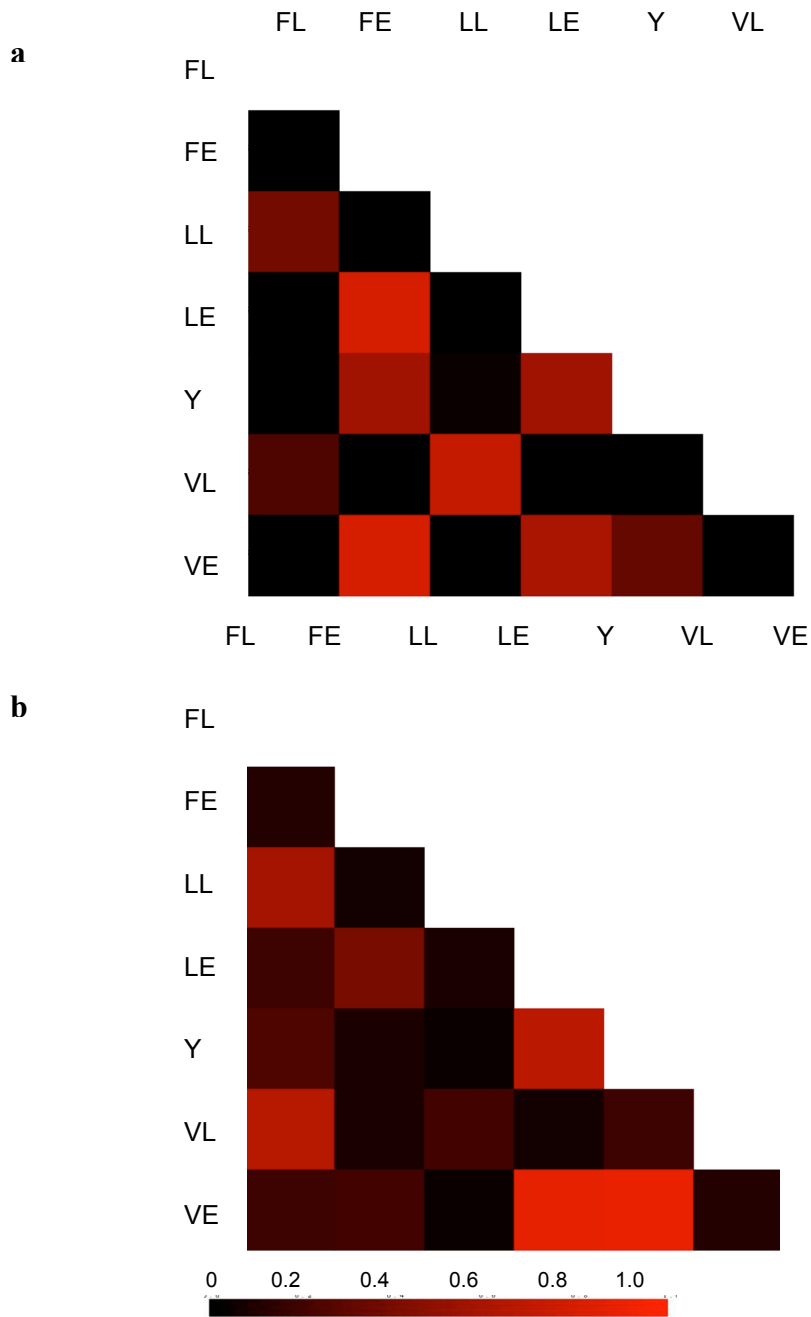


Fig. 1 Heatmap analysis based on Yue & Clayton Theta similarity coefficient calculated based on the bacterial OTU profile found in the roots of durum wheat, as influenced by the termination time and genotype of a previous pulse crop for (a) durum wheat in 2009; and (b) durum wheat in 2010. Abbreviated treatment names are as defined in Table 1.

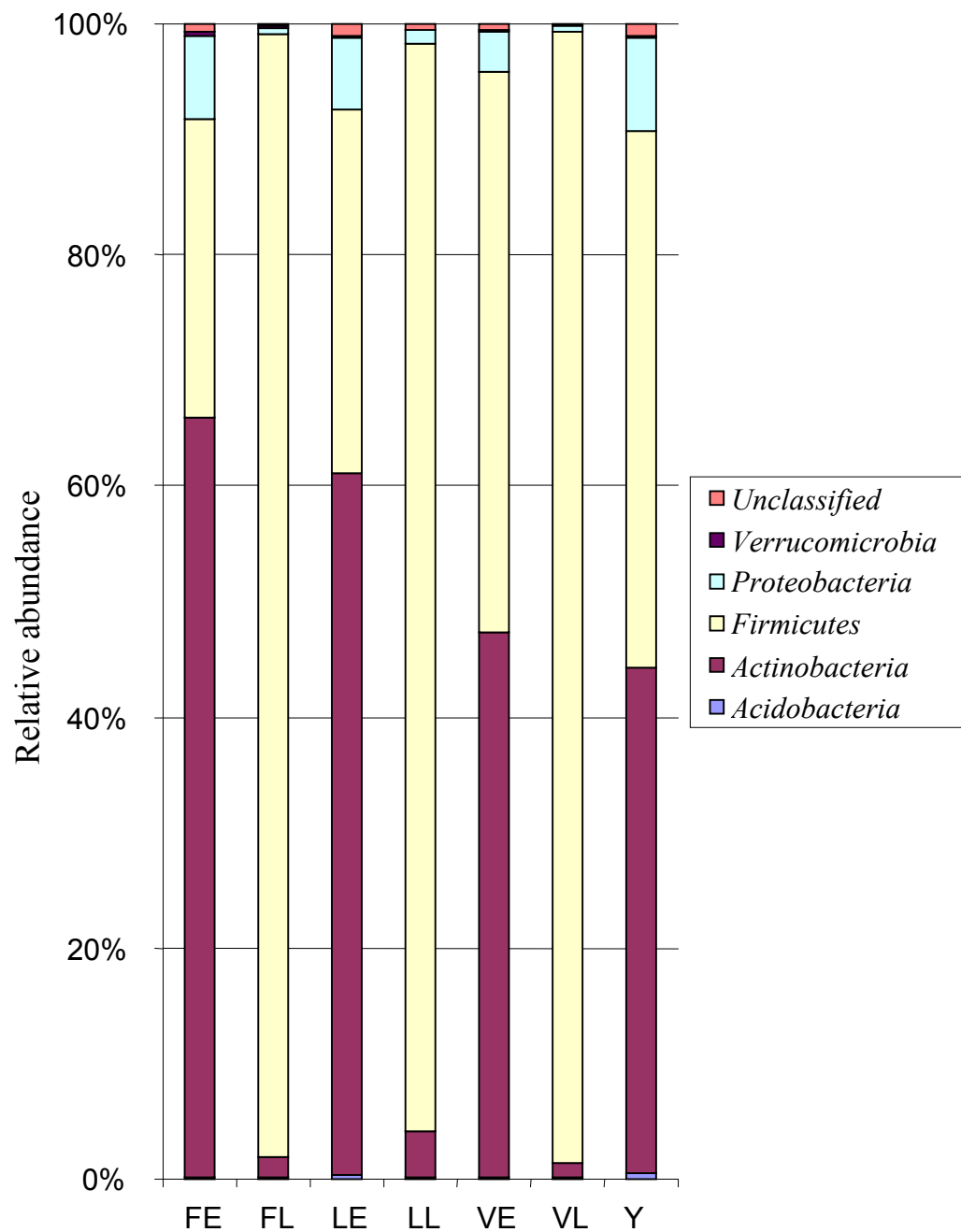


Fig. 2 Relative abundance of sequences belonging to each endophytic bacterial phyla found in the roots of durum wheat grown in 2009, as influenced by the time of termination of a previous pulse crops. Abbreviated treatment names are as defined in Table 1.

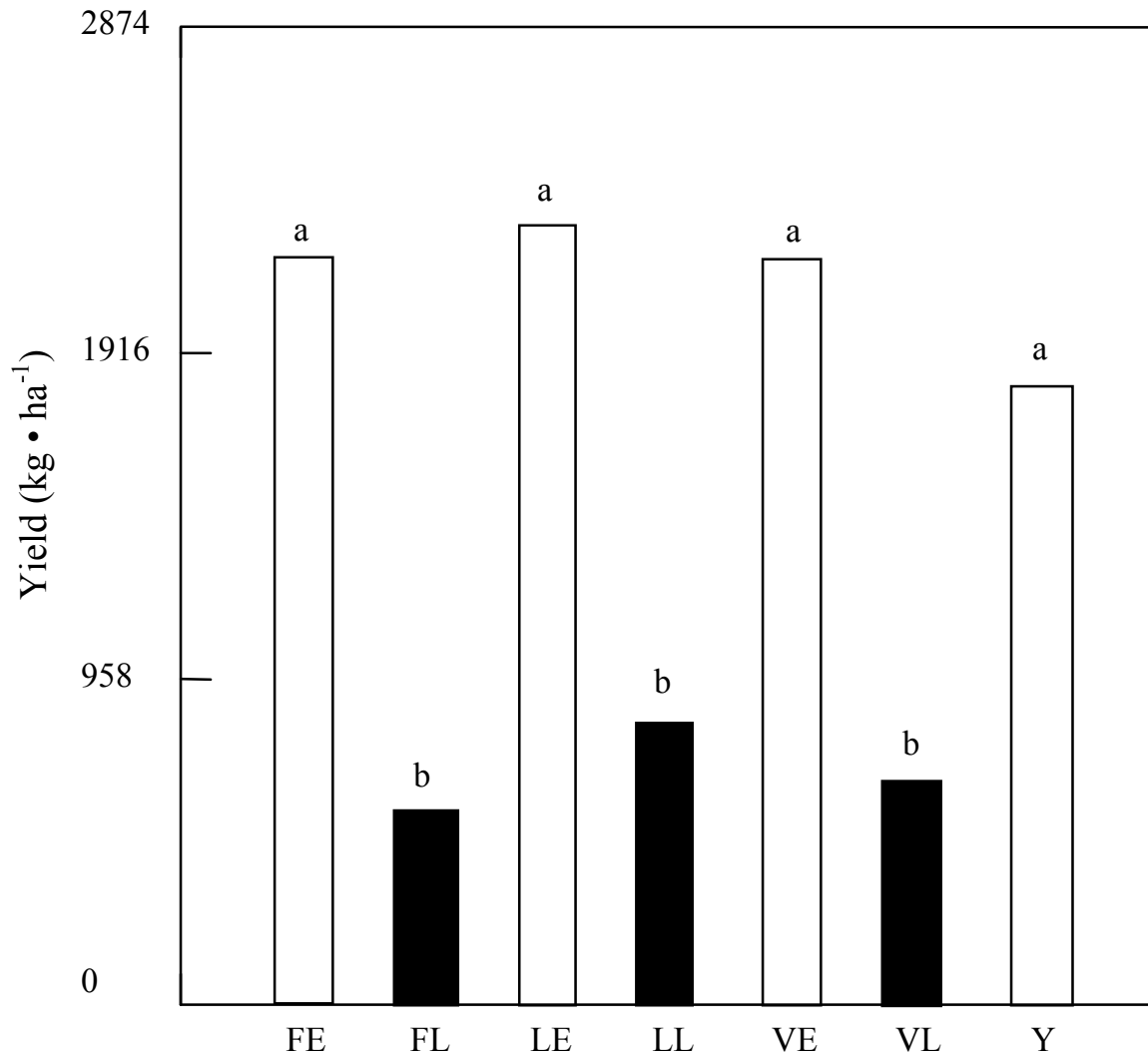


Fig. 3 Durum wheat yield measured in 2009, as influenced by the termination time and genotype of a pulse crop grown in 2008. ($P < 0.001$, $n = 4$). Abbreviated treatment names are as defined in Table 1.

The present study showed that the influence of contrasting crop termination time associated with pea and chickpea crops on the soil microbiota largely explained the poor rotation effect of chickpea. This conclusion is supported by studies showing little immediate effect of plants on the soil microbiota in field situation (Kulmatiski and Beard 2011). Change in the soil microbiota following the establishment of a new crop was shown to proceed slowly over a few years (Hamel et al. 2005). By contrast, decomposing plant residues in soil initiates within hours, a rapid succession of microorganisms with increasing ability to decompose complex organic compounds (6, 31). Changes in the soil microbiota induced by decomposing residues may feedback on crop yield, as observed in this study. Root endophytes, which are known to influence plant growth, are a subset of the soil microbiota (45). Consequently, changes in the bacterial community structure in durum wheat roots with changes in preceding pulse crop termination time is likely

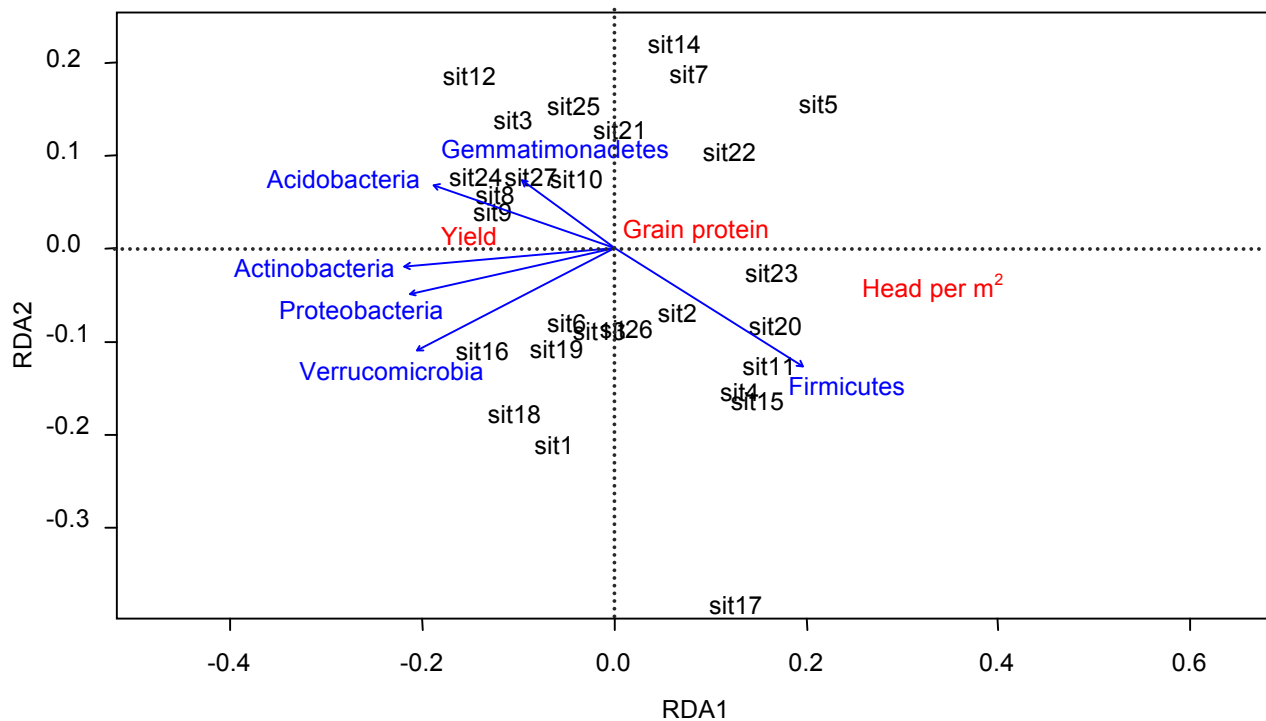


Fig. 4 Redundancy analysis (RDA) showing the relationship among identified endophytic bacterial phylum, number of wheat head (m^2), grain protein and yield of durum wheat in 2009 ($P = 0.001$, $N = 28$). Abbreviated treatment names are as defined in Table 1.

attributable to variation in the abundance of key microorganisms in the microbial pool under the influence of decomposing residues.

The abundance of Actinobacteria was related with high durum wheat yield in this study. The number of Actinomycetes, a diverse group of Gram + actinobacteria able to depolymerize recalcitrant compounds (39), often peaks in the last stage of microbial succession in decomposing plant residues (6). In our study, the abundant Actinobacteria in the seedbed of durum wheat after an early-terminated pulse crop may have resulted in abundant colonization of durum roots by endophytic Actinomycetes with biocontrol activity. Endophytic *Streptomyces* (Actinomycetes) isolated from wheat roots were found to be effective in antagonizing the pathogenic fungus *Gaeumannomyces graminis* (14), an important pathogen of wheat in Saskatchewan (7). The abundance of Actinobacteria in durum roots after early pulse termination may be the cause of the better durum wheat yield observed. Over 11% of the 116 isolates with plant growth promotion properties found in pulse crops in Saskatchewan were Actinobacteria (21).

A positive feedback of the soil microbiota on durum wheat yield, following an early-terminated pulse crop, could be derived from the abundance of certain plant growth promoting Proteobacteria or Actinobacteria, two bacterial groups associated with early pulse termination and high durum wheat yield in this study. Endophytic Proteobacteria and Actinobacteria were reported in wheat (12, 22) and related to important functions including increasing drought tolerance and nutrient uptake in host plants (5, 18, 23, 36).

Plant growth promotion ability of Firmicutes was also reported (45), but this bacterial phylum contains several plant pathogens as well. In particular, Phytoplasma, a group of specialised

Firmicutes, are obligate parasites of plant phloem tissue causing several complex disease syndromes with symptoms such as stunting, excessive branching, formation of sterile-deformed flowers, virescence, growth reduction, smaller leaves, and phyllody in many plant species (29, 37). Phytoplasma were reported as the causal agent of wheat blue dwarf disease, a very important disease of wheat in arid and semiarid areas (53). Phytoplasma infection of wheat in Saskatchewan and Manitoba led to malformed seeds and grain yield reduction (37). A stimulating influence of late chickpea termination on parasitic Firmicutes proliferation in the roots of durum wheat may have resulted in a negative feedback of the soil microbiota on durum wheat productivity. It is also possible that late chickpea termination only has a neutral influence on the following durum wheat, therefore, early-terminated pea passed a positive feedback of the microbiota to durum wheat, which created the difference in the productivity of durum wheat following chickpea and pea. Endophytic bacteria are found in many plant species (48), as plants provide diverse niches for many kinds of endophytic organisms (45). These organisms also benefit their host plants, as several plant growth promoting endophytic bacteria belonging to *Pseudomonadaceae* and *Enterobacteriaceae* were reported in several pulse crops (21). The abundance of endophytic species in host tissues can vary with time (12, 25) and it cannot be ruled out that a change in the bacterial endophyte community composition in chickpea roots from July to September, rather than the impact of crop residues decomposition, is one of the main causes of reduced productivity in the following durum wheat.

The absence of termination time effect in 2009-10 might be due to weather conditions where the extremely wet conditions reduced or masked potential treatment effects. Increased soil water content can directly affect the physiological status and symbiotic capability of soil bacteria. Soil moisture is a key factor in soil organic matter decomposition, influencing gas diffusion rate, soil temperature, soil pH, the osmotic status of soil bacterial cells, and substrate availability (17, 20). High soil water content can also impact the bacterial endophyte community in cereals by influencing plant growth, root health and rhizodeposition (30). Unusual moisture level in 2010 was probably the dominant process selecting soil bacterial communities. The strength of the process was seemingly overwhelming the plant related selective processes. The significant influence of unpredictable climate reduces our ability to precisely manage agro-ecosystems. Over all, termination time of pulse crops had a significant influence on the composition of bacterial endophyte communities living in the roots of the subsequent durum wheat in crop rotation. The modification of the microbiota by pulse rotation crops provides feedback effects influencing the productivity of the wheat crop following the pulses. The declining quality of organic residues as a substrate for soil microorganisms with time since the termination of a pulse crop most likely promoted the selection of beneficial endophytic bacteria, after an early-terminated crop. The effects of pulse crop termination times on durum wheat bacterial endophyte communities and productivity is seemingly sensitive to environmental conditions, such as soil moisture level.

Acknowledgement

We are grateful to excellent technical support of Keith Hanson, Lee Poppy, Cal McDonald, and Elijah Atuku. We are also grateful to financial support by Novozymes, Saskatchewan Pulse Growers, and Agriculture and Agri-Food Canada Matching Investment Initiative.

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Pulse-wheat rotation: pulse crops effects on wheat endophytic bacteria, and feedback on wheat yield



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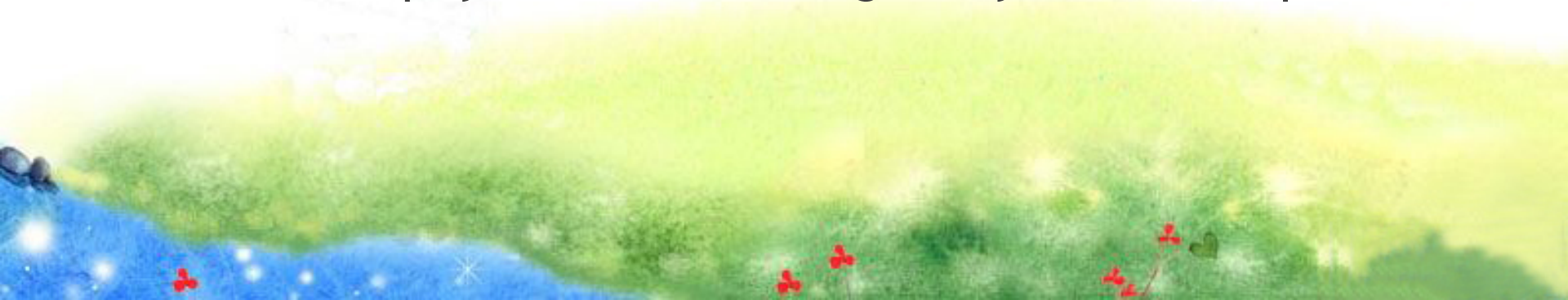


Introduction

- * As a former crop in durum-based rotation, different pulse crops bring varied benefits on durum wheat growth.
- * Study of soil water content and soil available nutrients can not fully explain why chickpea is less beneficial.



- * Microbial community was ignored.
- * Endosphere bacteria has close relationship to their host plants.
- * These endophyte bacteria bring many effects to plants.



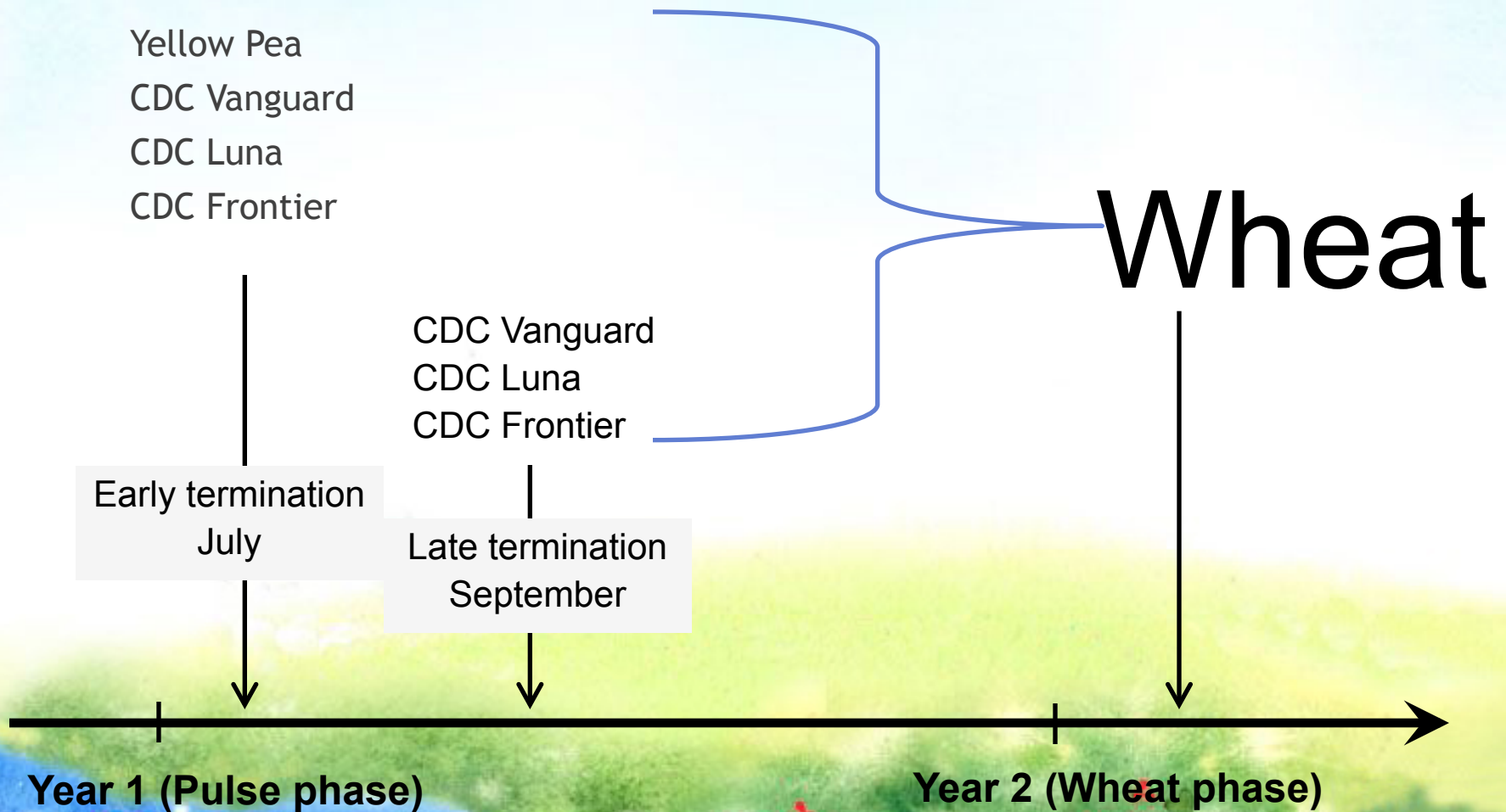
Effects of pulse crops on bacterial endophytes and yield of subsequent durum wheat in rotations

Hypothesis

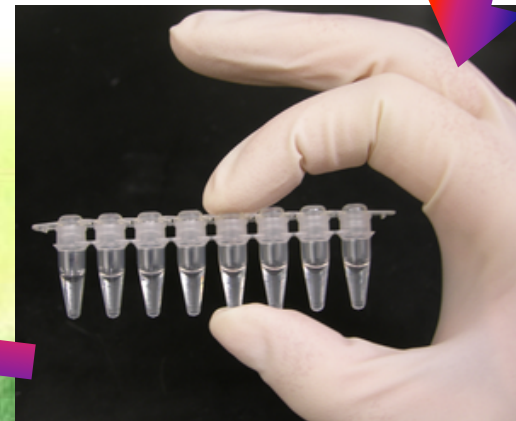
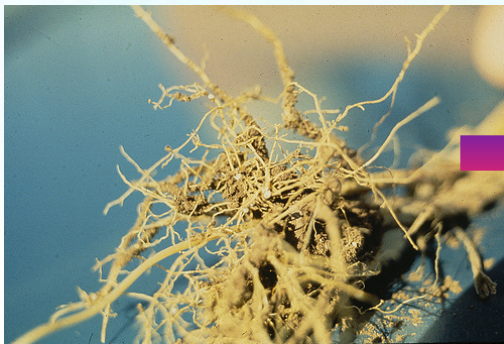
Different termination times of pulse crops affect the composition of the bacterial community of durum wheat endosphere.



Time line of field experiment



Samples collection and processing

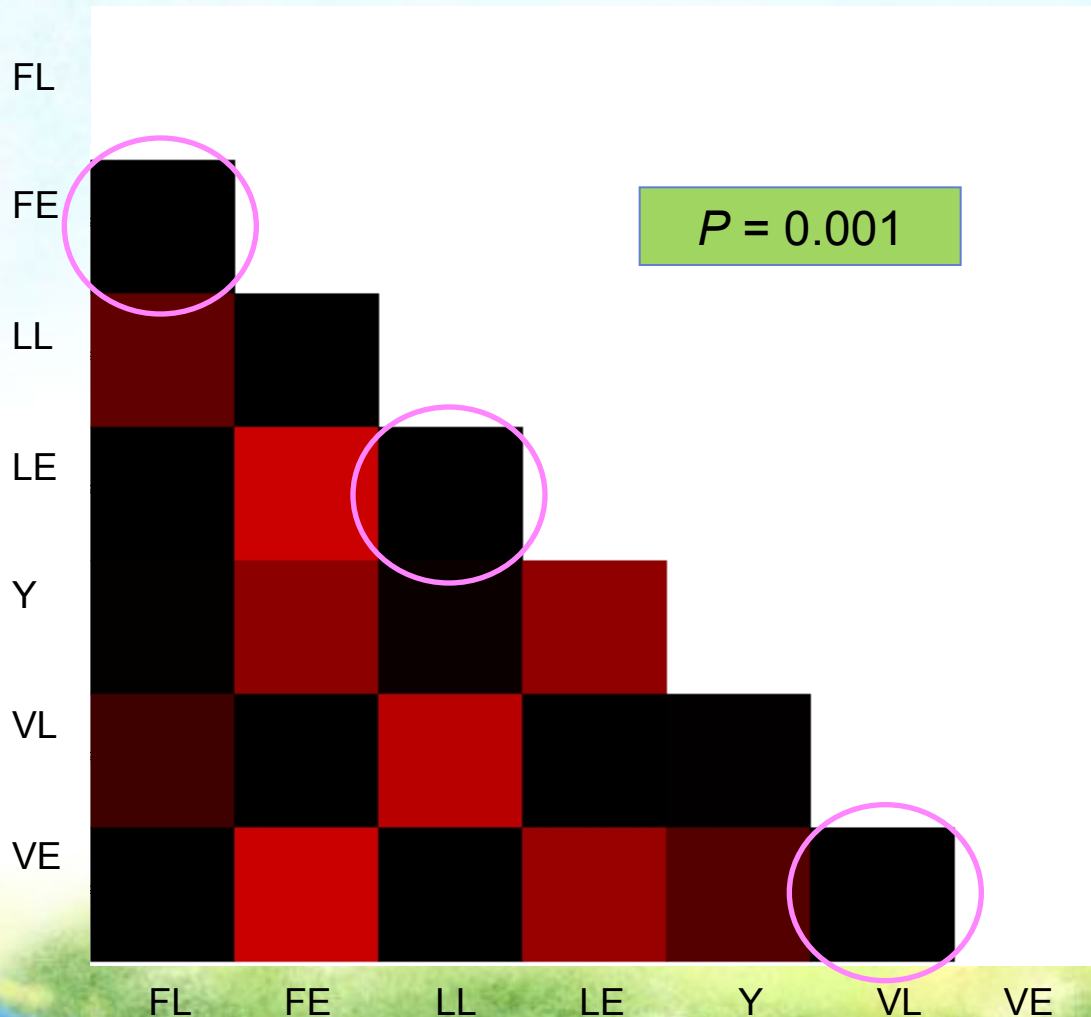


Results

ANOVA showed increased bacterial richness in durum wheat endosphere after early terminated pulses in 2009

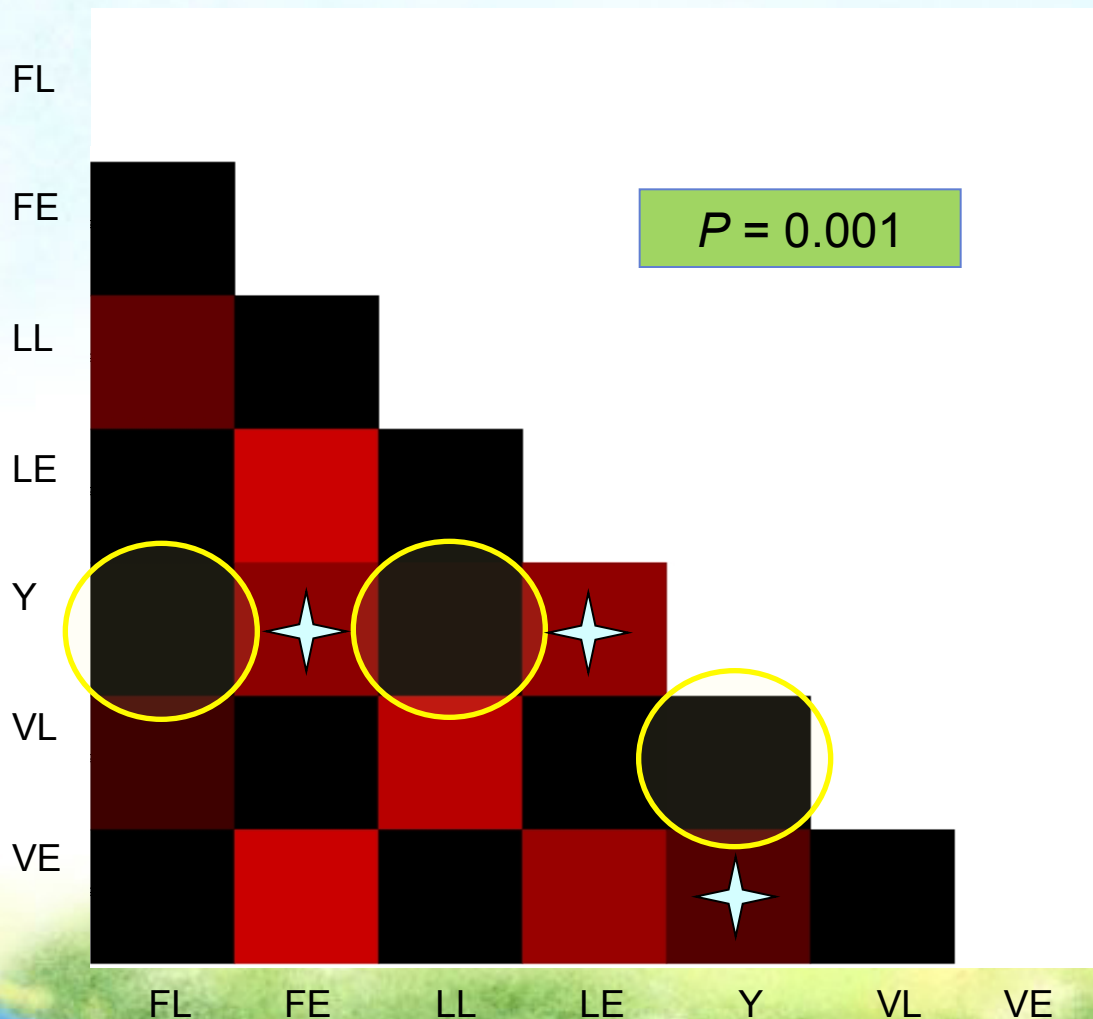
| Treatment | Chao 1 | ACE |
|--------------------------------|--------|--------|
| Yellow Pea | 721 ab | 1022 a |
| CDC Luna-Early termination | 687 b | 869 ab |
| CDC Vanguard-Early termination | 748 ab | 783 b |
| CDC Frontier-Early termination | 950 a | 1229 a |
| CDC Luna-Late termination | 277 c | 374 c |
| CDC Vanguard-Late termination | 155 c | 223 c |
| CDC Frontier-Late termination | 217 c | 329 c |
| P value | <0.001 | 0.001 |

Heat map analysis showed the effect of termination time on the bacterial community in durum wheat root endosphere



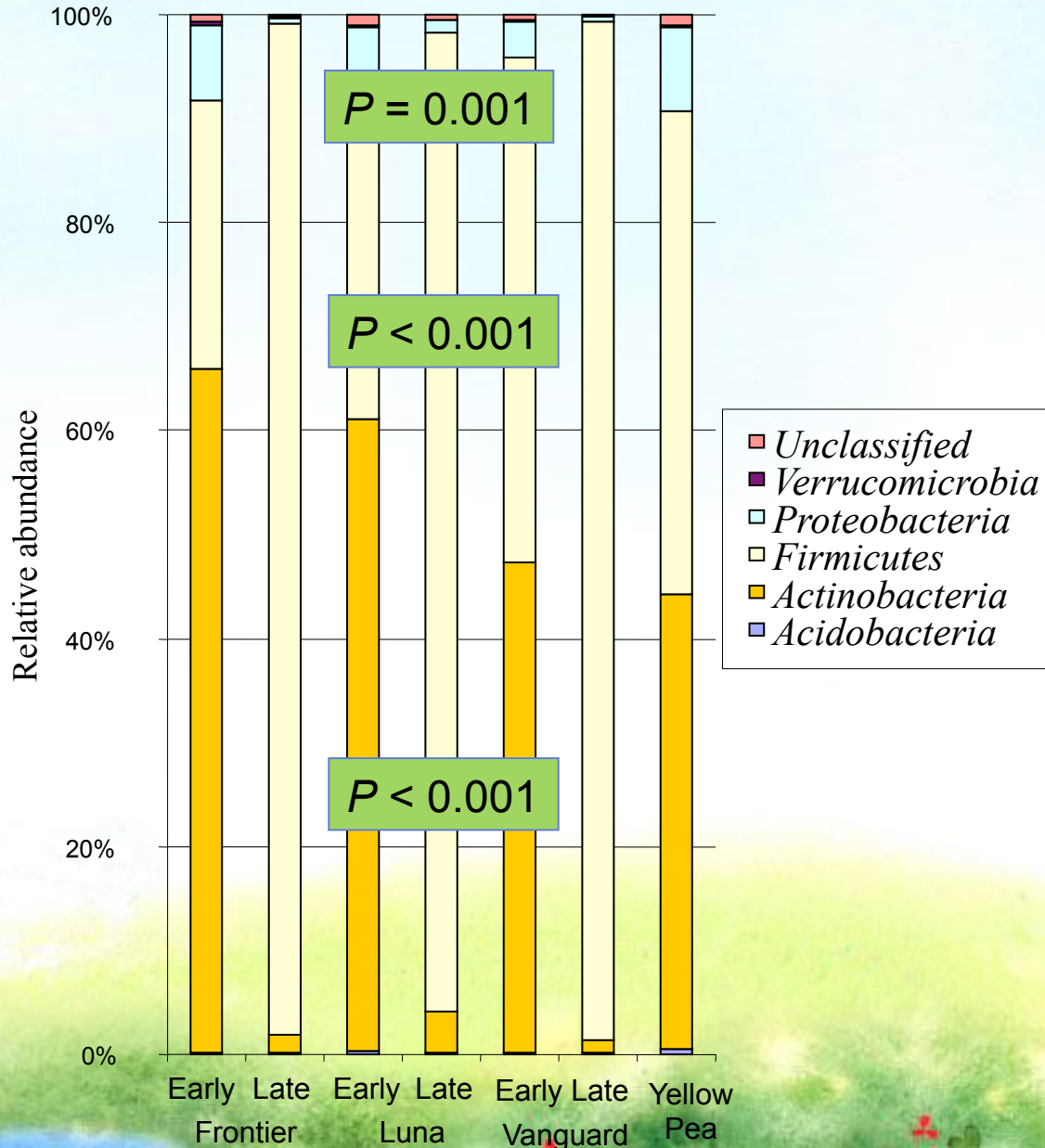
Y: Yellow Pea
LE: CDC Luna-Early termination
VE: CDC Vanguard-Early termination
FE: CDC Frontier-Early termination
LL: CDC Luna-Late termination
VL: CDC Vanguard-Late termination
FL: CDC Frontier-Late termination

Heat map analysis showed the effect of termination time on the bacterial community in durum wheat root endosphere

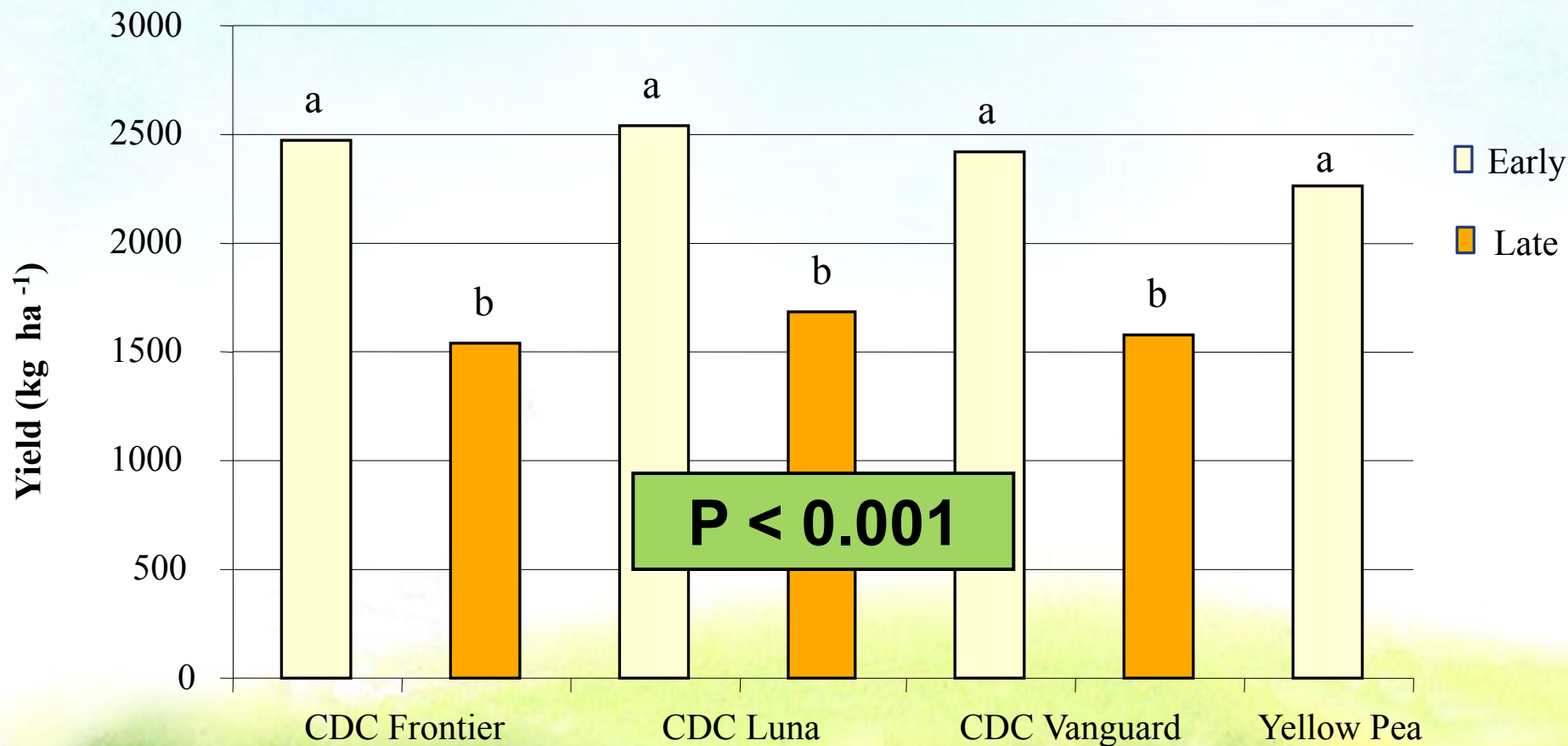


Y: Yellow Pea
LE: CDC Luna-Early termination
VE: CDC Vanguard-Early termination
FE: CDC Frontier-Early termination
LL: CDC Luna-Late termination
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FL: CDC Frontier-Late termination

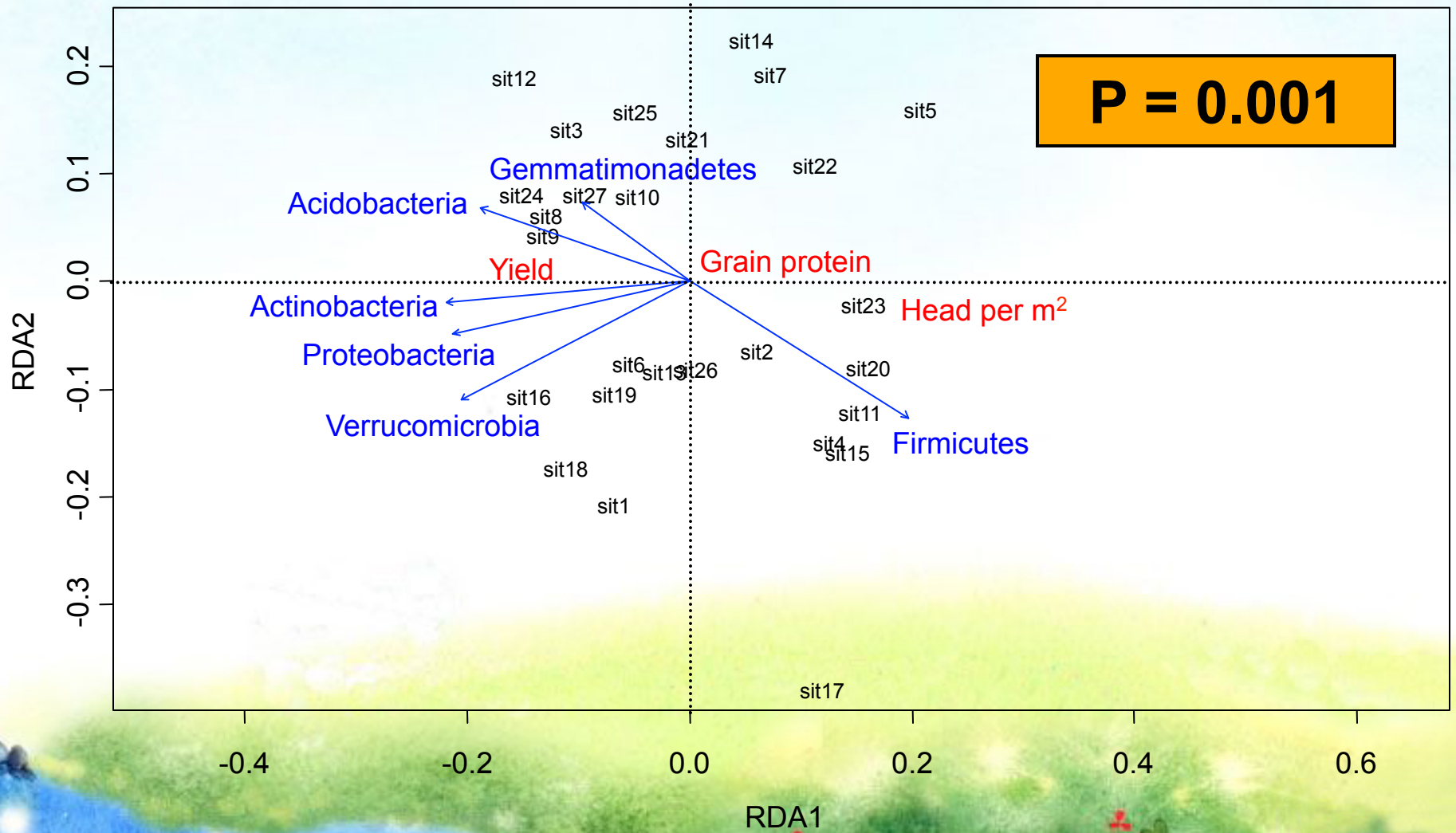
Identified bacterial phylum in durum wheat endosphere



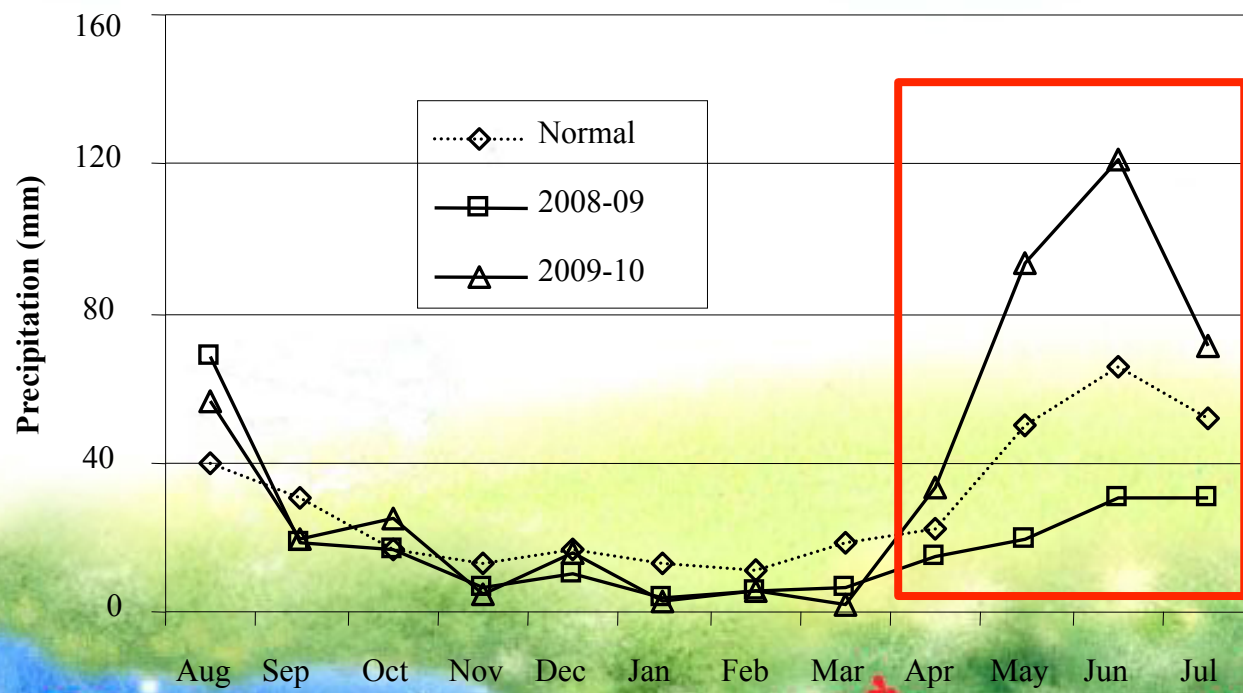
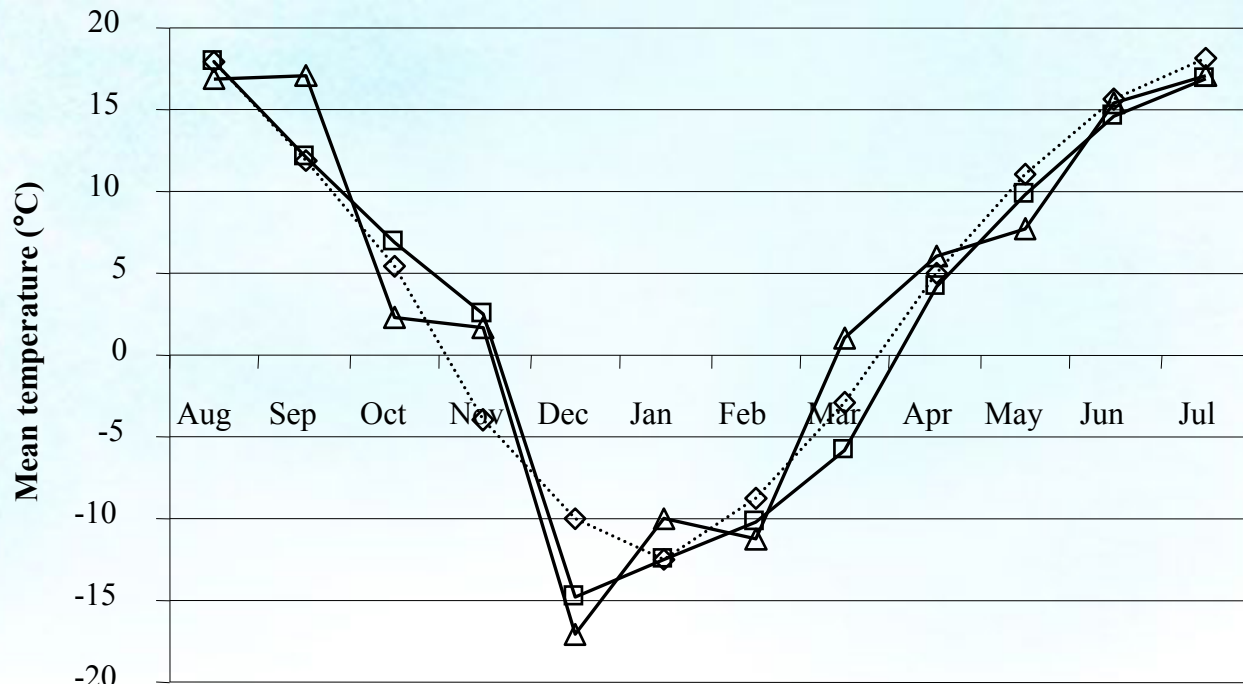
Durum wheat yield as influenced by the preceding pulse crop and the time of its termination



Redundancy analysis showed relationship between the structure of wheat root endosphere bacterial community and wheat yield



* Note: sitx: experimental plot profiles



* Temperature and precipitation recorded in 2008-2009 and 2009-2010, as compared to 30 years' average (1971-2000)

Conclusion

- * Different termination time of pulse crops is a determinant of the diversity and composition of the root endosphere bacterial community of a following durum wheat.
- * The structure of the wheat root endosphere bacterial community is related with grain yield.
- * Environmental factors, such as precipitation, affected composition of the bacterial endophyte communities in durum wheat.

Acknowledgement

- Saskatchewan Pulse Growers
 - Novozyme Biosciences
 - University of Saskatchewan
 - Agriculture and Agri-Food Canada
- Dr. Chantal Hamel
 - Dr. Vladimir Vujanovic
 - Dr. Yantai Gan
 - Keith Hanson
 - Cal McDonald



A dreamlike sky with soft, white clouds. A large, textured moon is visible on the right side. A small, stylized tree is positioned near the moon. Several white birds are flying in the sky. The overall color palette is a mix of light and dark blues, with white highlights from the clouds and birds.

Thank you

another sky another world another dreams