INTRODUCTION

Increased global consumption of chemical fertilizers is of economic and environmental concern (Shaviv & Mikkelsen, 1993). In addition to the increasing cost, chemical fertilizers have been known to alter biological, chemical, and physical fertility of soil over time (Diacono & Montemurro, 2010). In recent years, the search for a chemical fertilizer substitutes is of increased importance and urgency, particularly for wheat and corn production as both are important sources of food grains. One promising alternative is the use
of plant growth-promoting rhizobacteria (PGPR) as biofertilizers (Bhattacharyya & Jha, 2012). Bacteria inoculants have proven potential as replacements form some level of chemical fertilizers and these products are less expensive and their use decreases greenhouse gas emissions compared to chemical fertilizers (Adesemoye, Torbert, & Klopper, 2009; Borris, 2011; Hungria, Nogueria, & Araujo, 2013).

Numerous studies have examined the growth-promoting effects of bacterial inoculants on crops through various mechanisms such as nitrogen fixation (Bhardwaj, Ansari, Sahoo, & Tuteja, 2014; Govindarajan, Balandreau, Kwon, Weon, & Lakshminarasimhan, 2008), phosphorous solubilization (Srinivasan, Alagawadi, Yandigeri, Meena, & Saxena, 2012), potassium solubilization (Liu, Lian, & Dong, 2012), zinc solubilization (Al Abboud, Ghany, & Alawlaqi, 2013), IAA synthesis (Verma, Yadav, Tiwari, & Singh, 2010), and defense from pathogens (Hermosa, Viterbo, Chet, & Monte, 2012; Mahanty et al., 2017).

Less extensively investigated are the lasting effects of applying bacterial inoculants on the indigenous soil microbial diversity. Some studies suggest that the primary factor influencing rhizosphere soil biodiversity are plant growth stages; as was observed in soybean (Sun et al., 2017), forage corn (Piromyou et al., 2011), and basil (Bhartia, Barnawala, Wasnika, Tewari, & Kalra, 2016). A study on rhizosphere community in barley, however, showed only transient effects of the inoculated bacteria culture, lasting for 3 weeks after inoculation (Buddrus-Schiemann, Schmid, Schreiner, Welzl, & Hartmann, 2010). In addition to the varying results, these effects have not been widely studied on rhizosphere soil of wheat crop. Furthermore, little research has been done on whether the application of bacterial inoculations can alter the microbial community profile of an average-yielding soil to resemble that of soil that produces significantly higher crop yields. Altering the microbial profile of an average-yielding soil to resemble that of high-yielding soil allows crop growers to target specific areas of their field for higher yields and save resources (Kröber et al., 2014; Sun et al., 2017; Zhang, Sun, et al., 2010).

In this study, we examined the soil microbial community after bacterial inoculation and wheat plant growth in a controlled growth room environment. We delivered bacteria in Ca-alginate beads to prolong the survival of the bacteria formulations by preventing the influences of biotic and abiotic stressor in the soil. After growing wheat in various treated and untreated soils, we compared plant biometrics, soil chemistry, and bacterial 16s RNA T-RFLP profiles from soil of a high-yielding site to those of an average-yielding site, and average-yielding site soil treated with bacterial formulations. The aims of this study were (a) to test whether certain bacterial isolates can promote growth of wheat like soil from a high-yielding site and if so, (b) to analyze the effect of bacterial inoculation on native soil microbial diversity for any resemblances to bacterial diversity in soil from high-yielding site (Islam, Glenney, & Lazarovits, 2015). The results collected will facilitate the implementation of bacterial inoculants for microbial transformation of low productive soils.

| Sample ID | N (ppm) | P (ppm) | K (ppm) | Ca (ppm) | Mg (ppm) | Na (ppm) | Al (ppm) | Fe (ppm) | Mn (ppm) | Cu (ppm) | Zn (ppm) | B (ppm) | CEC (meq/100g) |
|-----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------------|
| G Bulk    | 3.7     | 49      | 32      | 317     | 163     | 110     | 119     | 18      | 116     | 16      | 0.3     | 0.3     | 6.22            |
| H Bulk    | 4.5     | 42      | 91      | 160     | 150     | 112     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| G         | 4.5     | 42      | 91      | 160     | 150     | 112     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| H         | 4.5     | 42      | 91      | 160     | 150     | 112     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| F-2       | 6.0     | 40      | 114     | 2040    | 240     | 110     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| F-4       | 5.9     | 50      | 140     | 2040    | 240     | 110     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| F-5       | 5.8     | 40      | 114     | 2040    | 240     | 110     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| F-7       | 5.8     | 40      | 114     | 2040    | 240     | 110     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| F-8       | 5.9     | 50      | 140     | 2040    | 240     | 110     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| F-13      | 5.9     | 50      | 140     | 2040    | 240     | 110     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| F-15      | 5.8     | 40      | 114     | 2040    | 240     | 110     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
| F-17      | 5.7     | 40      | 114     | 2040    | 240     | 110     | 172     | 15      | 110     | 19      | 0.3     | 0.3     | 6.4             |
RESULTS

2.1 Soil physical and chemical characteristics

Chemical profiles of the bulk soil used for this experiment indicated that the soil from the high productive (G-site) field had low OM, N, P, K, Mg, Ca, Na, pH, CEC, Zn, Fe, and Al content compared to the low productive (H-site) field. Soil chemical profiling from the experimentation soil after growing wheat remain in the same trend except for increased P content (Table 1) in G-site soil. Mn and pH levels of the bulk and the experimental G-site soil are higher than the H-site soil. There was no striking formulation induced differences in chemical profiles of H-site soil (Table 1). In both G- and H-site soil, there are some plant growth induced changes in the nutritional composition.

2.2 Wheat biometrics

Plant biometric parameters such as shoot length, chlorophyll content, dry root, and shoot weight were recorded while terminating the experiment at 30 days after planting. All the treatments increased the shoot length significantly compared to controls (H, C) except F4. Treatments F13 and F17 performed as best as G (the positive control) (Figure 1a). Chlorophyll content of the plants from the treatments such as F2, F4, F8, F13, F15, and F17 are more compared to control and were equivalent to the positive control. But none of them were significantly different either from positive or negative control (Figure 1b). Treatment F15 had the highest root weight compared to all others, but it was not significant compared to G, F13, F17. None of the treatments outperformed the negative control (C) including the positive control (G) (Figure 1c). Most treatments yielded higher shoot weight than negative controls. Treatments F15 and F17 performed significantly better than negative controls, F7 and F8. But the positive control was significantly better than all others (Figure 1d).

2.3 TRFLP, microbial richness and diversity

16S rRNA gene was amplified from extracted DNA and T-RFLP analysis was conducted using fluorescently labeled both forward and reverse primers. The mean TRF profiles of each treatment were shown in Figure 2. There are some significant differences in the TRF profiles of positive (G), negatives (H, C) controls, and in the
treatments applied H-site soils. T-RFLP profiles were analyzed for richness and diversity index using both binary (total counts of TRFs) and intensity (total TRFs intensity) data. G-site soil and some of the treatments (F13, F17) showed higher richness in terms of both binary and intensity profiles compared to control and other treatments (H, C, F5 and F7). The negative control and the control (H and C) had the lowest richness and diversity among all the treatments (Table 2). PCA analysis using the TRF data from both the forward and reverse primer separated the treatments. Some treatments (F17, F13, F15) looked like G, some (F2, F4, F8) remained like as controls and three other treatments changed completely (F9, F5, F7), the microbial profile neither like G nor H (Figure 3). The PC1 and PC2 coordinates for the position of each treatment as eigen vectors are shown in Table 3. There were about 250 TRFs from each treatment together from both primers detected and used in this study. Only about 46 TRFs from 63F and 96 TRFs from 1389R primer existed as keystone TRFs involved in this separation (Table 4).

2.4 | Relation between soil fertility, plant biometrics, and soil microbial community

Key stones of 142 TRFs (46 from 63F and 96 from 1389R) were chosen based on the PCA biplot output (Figure 3) and were tested with soil fertility and biometric parameters using PCA biplot (Figure 4). All the biometric parameters showed close association with G-site soil and F17, differential influence of various keystone TRFs have been noticed with separation of different fields (Figure 4). This PCA further narrowed down the number of keystones (to 53) involved in further separation of the fields. Eigenvectors of the first two principle components for the factors analyzed in this study are summarized in Table 5. Pearson correlation analysis has been carried out to explain the relationship between the key TRFs and the soil fertility and biometric parameters (Table 6). The probable bacterial identity of the TRF’s has been listed in Table 7. 63F TRFs such as 312 (Enterobacter asburiae), 284 (Delftia acidovorans), 304 (Caulobacter spp), 75 (Kosakonia cowanii), 48 (Sphingomonas sanguinis), 187 (Stenotrophomonas rhizophila), 295 (S. sanguinis), and the 1389R TRFs such as 478 (Burkholderia cepacia), 476 (Bacillus megaterium), 294 (Exiguobacterium acetylicum), 531 (Unidentified), 503 (Unidentified), 539 (Unidentified), 299 (Pseudomonas sp.), 182 (Paenibacillus sp.), 158 (Paenibacillus polymyxa), 317 (P. antarcticus), 530 (Unidentified), and 145 (Pantoea sp.), showed strong correlation to most of the soil and plant parameters (Table 6). Among the soil parameters examined much more significant negative correlations of TRFs were found with Mn (18/28), pH (14/26), OM (12/26), and the significant positive correlations were found with Ca (1/25), CEC (1/25), and K (7/22). Thus, the influence of these soil properties on bacterial community would be larger than those of other soil properties examined (Table 6).
3 | DISCUSSION

Recent studies have established potentials of PGPR to alter the indigenous soil microbial community when applied as bacterial inoculants for increased plant growth (Bhartia et al., 2016; Sun et al., 2017; Wernitznig et al., 2014). Less is known specifically about whether PGPR can shift the microbial profile of soils, producing average yields towards that of high-yielding soil. In the present study, we treated soil from an average-yielding site with bacteria isolated from soil from a high-yielding site delivered in Ca-alginate beads for their competitive survival, as many studies previously reported that the survival of directly introduced bacteria in the

| Treatments | Total counts of TRFs (Binary) | Total TRFs intensity |
|------------|-------------------------------|----------------------|
|            | Richness (S) | Diversity Index | Richness (S) | Diversity Index |
| G          | 124 | 176 | 300 | 4.820 | 5.170 | 9.991 | 903.965 | 490.182 | 1,394.147 | 3.293 | 4.606 | 7.899 |
| H          | 79  | 151 | 230 | 4.369 | 5.017 | 9.387 | 579.857 | 325.741 | 905.598 | 2.979 | 4.513 | 7.492 |
| C          | 108 | 146 | 254 | 4.682 | 4.984 | 9.666 | 672.585 | 392.330 | 1,064.915 | 3.211 | 4.290 | 7.501 |
| F-2        | 98  | 175 | 273 | 4.585 | 5.165 | 9.750 | 934.432 | 530.717 | 1,465.149 | 3.140 | 4.605 | 7.745 |
| F-4        | 119 | 170 | 289 | 4.779 | 5.136 | 9.915 | 859.691 | 538.934 | 1,398.625 | 3.400 | 4.500 | 7.900 |
| F-5        | 95  | 155 | 250 | 4.554 | 5.043 | 9.597 | 755.766 | 420.665 | 1,176.431 | 3.100 | 4.514 | 7.614 |
| F-7        | 95  | 155 | 250 | 4.554 | 5.043 | 9.597 | 638.239 | 356.573 | 994.812 | 3.109 | 4.543 | 7.652 |
| F-8        | 118 | 165 | 283 | 4.771 | 5.106 | 9.877 | 720.224 | 435.508 | 1,155.732 | 3.293 | 4.547 | 7.840 |
| F-9        | 112 | 165 | 277 | 4.718 | 5.106 | 9.824 | 898.078 | 495.388 | 1,393.466 | 3.189 | 4.502 | 7.691 |
| F-13       | 150 | 177 | 327 | 5.011 | 5.176 | 10.187 | 1,101.811 | 530.550 | 1,632.361 | 3.513 | 4.572 | 8.085 |
| F-15       | 121 | 162 | 283 | 4.796 | 5.088 | 9.883 | 864.250 | 403.503 | 1,267.753 | 3.292 | 4.615 | 7.907 |
| F-17       | 142 | 189 | 331 | 4.956 | 5.242 | 10.198 | 1,187.166 | 537.560 | 1,724.726 | 3.381 | 4.651 | 8.032 |

**TABLE 2** Microbial diversity and richness index based on the 16S RNA based TRF profiling

**FIGURE 3** PCA ggbiplot—Principle components of the mean TRFLP profile of different treatments
native environment reduced drastically over the time because of their direct exposure to the biotic and abiotic stress factors of the introduced soil and suggested immobilization methods to protect the introduced bacteria (Gómez-Silván et al., 2010; Jain, Saxena, & Sharma, 2014). Using wheat as the bioindicator, we measured the growth-promoting effects of the bacterial isolates and analyzed the soil bacterial community profiles using 16s rRNA T-RFLP analysis. We found that although soil chemical and physical parameters remained unchanged between negative control and treatments, formulations F17 (P. polymyxa), F13 (E. acetylicum), and F15 (K. cowanii), promoted wheat plant growth. Moreover, the soil bacterial community profile shifted when treated with these formulations and 142 TRFs were identified as keystone parameters involved in such community change.

Chemical analysis of the soils from the rhizosphere of wheat revealed few changes between negative controls and treatments, whereas positive control G-site soil differed greatly in chemical composition. The test soil (H-site) measured higher in OM and most of the soil nutritional parameters compared to the G-site soil, but it still produced an average yield compared to the high-yielding G-site soil. This also indicates that the plant productivity was not solely dependent on soil chemical parameters. Years of soil biological research indicates the involvement of microbial abundance; diversity and their composition play a major role in balancing the release of nutrients from the soil and its uptake by the plants (Van Der Heijden, Bardgett, & Straalen, 2008; Schnitzer et al., 2011). Previous research from our lab indicates that the microbial profile of average productive soil was completely different from that of the high productive site soil (unpublished A & L research data). The lack of change in chemical compositions between treatments and negative control suggests that the promoted growth seen in the treated wheat plants are not a result of changes in soil chemistry, but other factors such as direct biological interactions between PGPR and the plant. By contrast, Trabelsi, Mengoni, Ben Ammar, and Mhamdi (2011) found changes in soil chemistry in their experiment with Phaseolus vulgaris during grain harvest. Specifically, they found changes in ammonium, nitrate, and phosphorus levels of bacteria inoculated plots compared to control and N-fertilized plots. The difference in results may be attributed by the difference in the stage of the crop in which the soil samples were collected. Soil in our experiment was collected when the wheat crops were at the late seedling/early tillering stage, which may not be enough time for the bacteria to establish significant effect on soil chemistry. Even though, there was not any noticeable difference in the soil chemical profile with the introduced microbes, the individual TRFs showed significant (p < 0.01) correlations either positive or negative with number of different plant biometric and soil chemical parameters (Table 6). The impact of microbial communities on soil chemical parameters and vice versa has previously been reported by Suzuki, Kunito, Aono, Liu, and Oyaizu (2005) and Sun et al. (2017). PCA (42.5% of variables explained) of all the soil chemical, keystone microbial TRFs from the initial PCA analysis and plant biometric parameters indicated that the uniqueness of each parameter in driving the separations of treatments (Figure 4) as opposed to the influence of only specific keystones on the soil fertility (Suzuki et al. (2005).

In the present study, wheat seeds applied with beads containing formulations F13 (E. acetylicum), F15 (K. cowanii), or F17 (P. polymyxa) showed an increase either significant or insignificant in all parameters measured compared to negative controls. Likewise, Anuroopa and Bagyaraj (2017) characterized E. acetylicum and P. polymyxa has having the ability to increase shoot length, stem girth, biovolume index, and biomass of Withania somnifera after seed treatments. The growth-promoting effects of E. acetylicum on wheat has been suggested to be due to reducing the incidence of Rhizoctonia solani associated root disease (Barnett, Roget, & Ryder, 2006). Similarly, P. polymyxa has

### Table 3

List of PCA coordinates of different treatments, separated based on the TRF profiles

| Treatments | PC1   | PC2   |
|------------|-------|-------|
| G          | −0.2145 | 0.4565 |
| H          | −0.3165 | 0.0777 |
| C          | −0.3111 | 0.0377 |
| F-2        | −0.3003 | 0.0496 |
| F-4        | −0.2787 | 0.0055 |
| F-5        | −0.3445 | −0.4919 |
| F-7        | −0.3445 | −0.4919 |
| F-8        | −0.2789 | 0.0283 |
| F-9        | −0.3099 | −0.0874 |
| F-13       | −0.2166 | 0.2379 |
| F-15       | −0.2902 | 0.2152 |
| F-17       | −0.2164 | 0.4318 |

### Table 4

Summary of key principle component TRFs of Figure 3

| TRFs | PC1 | PC2 |
|------|-----|-----|
| 39   | −1.050 | 0.120 |
| 338  | −1.081 | 0.027 |
| 39   | −0.182 | 0.048 |
| 145  | −1.360 | 0.033 |
| 153  | −1.050 | 0.120 |
| 195  | −0.160 | 0.027 |
| 535  | −1.081 | 0.027 |
| 656  | −0.460 | 0.054 |
| F   | −1.360 | 0.033 |

*There are about 46 TRFs (2, 31, 33, 34, 39, 47, 48, 75, 76, 77, 78, 111, 113, 117, 121, 128, 129, 139, 150, 161, 163, 187, 200, 202, 240, 243, 274, 281, 284, 286, 292, 293, 294, 295, 297, 298, 299, 304, 308, 312, 338, 340, 431, 432, 528, 703, 1022) from 67F and 96 (10, 15, 17, 22, 25, 29, 32, 38, 40, 48, 50, 53, 69, 71, 72, 75, 76, 83, 112, 133, 115, 117, 128, 141, 145, 153, 156, 158, 161, 164, 169, 173, 176, 177, 179, 185, 194, 195, 197, 198, 200, 203, 238, 294, 299, 303, 311, 317, 319, 320, 322, 326, 328, 331, 337, 339, 342, 400, 402, 432, 468, 469, 472, 476, 479, 481, 486, 488, 503, 507, 518, 522, 524, 530, 531, 533, 535, 539, 626, 628, 630, 645, 646, 651, 656, 751, 753, 810, 815, 1002, 1005, 1019, 1032, 1049, 1055, 1065) from 1392 primers are found to be present in the same position as you see in the Figure 3.
been shown to promote plant growth by suppressing the pathogenesis of Phytophthora and Pythium species on Arabidopsis thaliana as well as IAA production (Timmusk, West, Gow, & Huffstutler, 2009).

T-RFLP analysis has been shown to differentiate soil microbial communities using the 16s RNA gene (Suzuki et al., 2005). In this study, positive control G, and treatments F17 and F13 showed higher richness in binary and intensity profiles compared to negative control and other treatments. Similar results were obtained by Trabelsi et al. (2011) where richness of bulk soil bacterial communities increased after the growing of P. vulgaris inoculated with Ensifer mellotii and Rhizobium gallicum. In contrast, bacterial inoculation also decreases the diversity of native bacterial community (Zhang, Tang, Chen, & Zheng, 2010), but has been unchanged in the studies conducted by Sun et al., (2017). We also found an increase in the richness and the diversity of microbial communities in other treatments such as F2, F4, and F9, but those treatments were not found to be driving the diversity in the same direction as the positive control in terms of plant biomass and the community composition of native bacteria. Taken together, these studies explain that the richness and diversity towards goodness and badness are not always unidirectional.

PCA analysis allowed visualization of the position of bacterial profiles of F17, F13, and F15 closer to G and away from H or C. The difference in community profiles between F17, F13, and F15 treatment inoculations and negative controls may be a result of nutritional competition, antagonism and mutual interactions between the indigenous bacteria and the inoculated bacteria (Zhang et al., 2013). Those treatments that did not produce profiles different from negative controls may not have been able to affect the dominant preexisting bacteria. Sun et al. (2017) were also able to identify T-RFs that were unaffected by inoculation with organic-phosphorus-mineralizing bacteria, suggesting that those dominant bacteria species have the capacity to resist...
external changes. In addition to the bacterial interactions with competing species and the soil environment, the changes in T-RFLP profiles, or the lack thereof, between treatments and negative controls may be attributed to bacteria-plant interactions. Plants favor the interactions of certain bacteria under specific conditions, thereby selecting the type of benefits they receive (Costa et al., 2014).

4 | CONCLUSION

The present study indicates the potential of transforming the low productive soils with selective key microbes from high productive soils, which is an emerging need to improve the soil health in a sustainable way and to tackle with climate change situation. Among the nine bacterial formulations tested, *P. polymyxa*, *K. cowanii*, and *E. acetylicum* showed their potential in positive community shift and associated soil productivity. Further research with long-term studies under field conditions with various carrier formulation, fertilizer rates, and tillage effects will add value to the current research.

5 | EXPERIMENTAL PROCEDURES

5.1 | Background of the soils used in this study

Two neighboring farmers’ fields located in Dunnville, Ontario, Canada showed years of productivity differences despite planting with seeds of the same genetic potential. They differ slightly with agronomic practices; the high productive G-site soil receives less crop input and minimal till compared to H-site soil. The location coordinates of the high productive farms are 42° 56′ 06.28″ N and 79° 41′ 00.92″ W and the low productive farms are 42° 56′ 45.11″ N and 79° 33′ 02.06″ W. The productivity of high productive G-site soil appeared to be double (Corn yield more than 320 bushels/ac) that of low productive H-site soil (Corn yield around 150 bushels/ac). The G-site soil is identified as Sandy loam and the H-site soil is clay loam. The chemical properties of the soil have been stated in Table 1. Both site soils appeared to have many differences in the soil physical, chemical, and biological properties (Islam et al., 2015; A and L unpublished data). Interestingly, the low productive farm site appeared to have higher nutritional profile, organic matter (OM) content, and slightly higher pH compared

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**TABLE 5** Eigenvectors of the first two principle components for the factors analyzed in this study

| Parameters          | PC1   | PC2   | PC1   | PC2   | PC1   | PC2   |
|---------------------|-------|-------|-------|-------|-------|-------|
| Plant Biometrics    |       |       |       |       |       |       |
| Shoot Length (cm)   | 1.628 | -1.548| 243   | -0.713| 337   | -0.411|
| Chlorophyll Content | 2.869 | 0.023 | 274   | 2.455 | 402   | 2.4   |
| Root Weight (g)     | 1.203 | -0.074| 281   | -0.751| 469   | 2.58  |
| Shoot Weight (g)    | 3.119 | -0.401| 284   | -1.852| 476   | -2.584|
| Soil parameters     |       |       |       |       |       |       |
| Organic Matter      | -2.944| 1.1   | 295   | -1.566| 493   | -2.099|
| Nitrogen (N) ppm    | -0.96 | -1.27 | 298   | -1.506| 503   | -1.964|
| Phosphorus (P) ppm  | 1.667 | 0.555 | 304   | -1.728| 507   | -1.526|
| Potassium (K) ppm   | -2.771| 0.693 | 308   | 1.852 | 518   | -0.686|
| Calcium (Ca) ppm    | -2.376| 1.034 | 312   | -2.214| 530   | -1.474|
| Magnesium (Mg) ppm  | -2.023| 0.903 | TRFs-1389R | 531   | -2.293|
| Sodium (Na) ppm     | -2.012| 1.51  | 39    | 2.829 | 533   | -0.434|
| Zinc (Zn) ppm       | -2.307| 1.689 | 53    | -0.687| 539   | -1.739|
| Manganese (Mn) ppm  | 3.358 | 0.614 | 112   | 2.011 | 626   | 2.255 |
| Iron (Fe) ppm       | -2.057| 1.278 | 145   | -1.627| 628   | -1.75  |
| Copper (Cu) ppm     | -0.927| -0.161| 158   | -1.734| 645   | -0.818|
| Aluminum (Al) ppm   | -1.371| 1.115 | 164   | 2.327 | 753   | 2.465 |
| pH                  | 3.395 | 0.559 | 169   | -1.512| 815   | 2.683 |
| CEC                 | -2.162| 0.423 | 176   | 1.712 | 1.005 | -0.256|
| TRFs-63F            | 54    | 2.303 | 317   | -1.834| 749   | 2.524 |
| TRFs-63F            | 48    | 0.935 | 320   | 1.718 | 182   | -2.429|
| TRFs-63F            | 75    | -0.525| 326   | -2.211| 478   | -2.791|

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| Mineral          | Al ppm | Cu ppm | Mn ppm | Zn ppm | Mg ppm | N ppm | K ppm |
|------------------|--------|--------|--------|--------|--------|-------|-------|
| Aluminum (Al)    | 0.38   | 0.71   | 0.67   | 0.75   | 0.75   | 0.65  |
| Copper (Cu)      | 0.32   | 0.18   | 0.63   | 0.59   | 0.54   | 0.67  |
| Zinc (Zn)        | 0.01   | 0.68   | 0.81   | 0.81   | 0.68   | 0.68  |
| Iron (Fe)        | 0.31   | 0.52   | 0.35   | 0.38   | 0.56   | 0.48  |
| Magnesium (Mg)   | 0.01   | 0.55   | 0.27   | 0.30   | 0.55   | 0.55  |
| Chlorophyll Content | 0.33 | 0.67   | 0.64   | 0.64   | 0.64   | 0.64  |

Correlation (R) between TRFs and soil chemical and physical parameters.
| TRFs | Shoot Length (cm) | Chlorophyll Content | Shoot Weight (g) | Organic Matter (ppm) | Nitrogen (P) ppm | Phosphorus (K) ppm | Potassium (Ca) ppm | Calcium (Mg) ppm | Sodium (Na) ppm | Zinc (Zn) ppm | Manganese (Mn) ppm | Iron (Fe) ppm | Copper (Cu) ppm | Aluminum (Al) ppm | PH | CEC |
|------|------------------|---------------------|------------------|----------------------|------------------|-------------------|-------------------|------------------|----------------|----------------|------------------|---------------|----------------|------------------|----|-----|
| 476  | -0.28            | -0.61               | -0.69            | 0.84                 | 0.24             | -0.13             | 0.83              | 0.71             | 0.64           | 0.47           | 0.79            | -0.82         | 0.64           | 0.51               | 0.52 | -0.84 | 0.75 |
| 478  | -0.22            | -0.60               | -0.73            | 0.88                 | 0.30             | -0.12             | 0.92              | 0.79             | 0.73           | 0.62           | 0.85            | -0.88        | 0.66           | 0.73               | 0.51 | -0.88 | 0.82 |
| 488  | 0.48             | 0.71                | 0.73             | -0.81               | -0.31            | 0.57              | -0.66             | -0.52            | -0.47          | -0.63          | -0.57          | 0.80           | -0.38          | -0.06              | 0.81 | -0.41 |
| 503  | -0.34            | -0.40               | -0.69            | 0.61                 | -0.21            | 0.07              | 0.70              | 0.68             | 0.67           | 0.72           | 0.69            | -0.58         | 0.47           | 0.54               | 0.30 | -0.61 | 0.68 |
| 507  | -0.02            | -0.17               | -0.49            | 0.49                 | 0.33             | 0.07              | 0.45              | 0.62             | 0.36           | 0.59           | 0.50            | -0.59         | 0.35           | 0.35               | -0.49 | 0.52 |
| 518  | 0.26             | 0.19                | -0.13            | 0.28                 | 0.21             | 0.38              | 0.38              | 0.59             | 0.47           | 0.51           | 0.59            | -0.39         | 0.27           | 0.68               | 0.32 | -0.28 | 0.65 |
| 530  | -0.25            | -0.29               | -0.51            | 0.61                 | 0.26             | -0.16             | 0.47              | 0.63             | 0.43           | 0.48           | 0.68            | -0.69         | 0.47           | 0.26               | 0.46 | -0.61 | 0.55 |
| 531  | -0.02            | -0.42               | -0.56            | 0.69                 | 0.44             | -0.39             | 0.80              | 0.83             | 0.82           | 0.71           | 0.55            | -0.72         | 0.64           | 0.50               | -0.69 | 0.87 |
| 533  | -0.49            | -0.24               | -0.38            | 0.20                 | -0.23            | -0.37             | 0.15              | 0.42             | 0.36           | 0.35           | 0.24            | -0.20         | 0.51           | 0.14               | 0.62 | -0.20 | 0.34 |
| 539  | -0.44            | -0.48               | -0.68            | 0.61                 | 0.09             | -0.28             | 0.52              | 0.77             | 0.54           | 0.54           | 0.64            | -0.66         | 0.73           | 0.26               | 0.74 | -0.61 | 0.64 |
| 626  | 0.59             | 0.65                | 0.56             | -0.70               | -0.13            | 0.67              | -0.44             | -0.28            | -0.30          | -0.33          | -0.46          | 0.64           | -0.24          | 0.09               | -0.09 | 0.70  | -0.25 |
| 645  | -0.26            | -0.42               | -0.39            | 0.18                 | 0.03             | -0.35             | 0.30              | 0.52             | 0.39           | 0.39           | 0.15           | -0.19         | 0.68           | 0.15               | 0.69 | -0.18 | 0.35 |
| 749  | 0.23             | 0.60                | 0.51             | -0.60               | -0.37            | 0.53              | -0.59             | -0.44            | -0.45          | -0.25          | -0.26          | 0.53           | -0.54          | -0.05              | -0.47 | 0.60  | -0.49 |
| 753  | 0.27             | 0.61                | 0.51             | -0.62               | -0.36            | 0.53              | -0.51             | -0.23            | -0.24          | -0.35          | -0.26          | 0.56           | -0.16         | -0.03              | 0.11 | 0.62  | -0.19 |
| 815  | 0.60             | 0.69                | 0.80             | -0.72               | 0.07             | 0.36              | -0.61             | -0.34            | -0.30          | -0.28          | -0.55          | 0.64           | -0.48          | -0.07              | -0.22 | 0.72  | -0.33 |
| 1,049| 0.19             | -0.27               | -0.29            | 0.52                 | 0.67             | -0.13             | 0.54              | 0.67             | 0.49           | 0.50           | 0.47           | -0.62         | 0.44           | 0.51               | 0.43 | -0.52 | 0.62 |
| 1,055| 0.31             | 0.16                | -0.07            | 0.08                 | 0.18             | 0.33              | 0.28              | 0.58             | 0.44           | 0.41           | 0.35           | -0.19         | 0.41           | 0.62               | 0.52 | -0.08 | 0.58 |

*Numbers in Green are the (63F TRFs) and Blue are 1389R TRFs. The correlation was determined by Pearson correlation analysis. The letters in bold are significant @α = 0.05*
to the high productive site soil. One considerable observation for these differences may be that the growers follow varied cultivation practices such as no till (high productive farm) and conventional till (low productive farm). We brought the soil samples from both sites and studied using our pot culture experiments under growth room conditions. Surprisingly the productivity difference stayed the same after destroying the soil structure, which ruled out the factor of differing farming practices. At the same time, pasteurized G-site soil lost its higher production capacity and produced crops like that of the H-site soil. The abundance and diversity of microbial community profiles between these site soils appeared very different. Taken together, the current study has been laid on the above-mentioned background information.

5.2  |  Preparing alginate beads with selected top performing bacteria

Bacteria isolated from high-yielding areas of 13 corn fields that have shown to have the potential to promote plant growth were selected (see the list in Table 8) to be made into alginate beads. Isolates were grouped per their identity. For formulations containing multiple isolates, isolates were grown together in 150 ml LB broth shaking in a flask overnight @ 37°C, 150 rpm. Cultures were spun down and the supernatant were discarded. Bacteria pellets were resuspended in 10 ml of sterile distilled water. To the bacteria solution 2.5% glycerol and 3% alginate was added and mixed completely. Using a 50 ml burette, the bacteria-alginate mixture dripped into a beaker containing 0.1M CaCl₂ solution, forming Ca-alginate beads with bacteria. The beads were left in the CaCl₂ solution to harden for 1 hr, then rinsed with distilled water and stored in an airtight container at 4°C.

5.3  |  Bioindicator wheat growth room assay

Wheat seeds were planted in paper cups (10 replicate cups with 5 plants in each, 50 plants in total per treatment) containing soil from an average-yielding farm (H) with each bead formulation, as well as soil from a high-yielding farm (G), and beads with no inoculum as control (C). Each cup contained five wheat seeds with approximately $1.0 \times 10^9$ colony forming unit (CFU) of bacteria in 10–12 beads. Seeds were planted on 220 g of soil and topped with

| Sl. No | Key stone TRF’s | Taxonomic identity |
|-------|----------------|--------------------|
| 46    | R-628          | Saccharibacillus kuerensis |
| 47    | R-645          | Lysinibacillus sphaericus |
| 48    | R-749          | Enterobacter asburiae |
| 49    | R-753          | Unidentified |
| 50    | R-815          | Unidentified |
| 51    | R-1005         | Unidentified |
| 52    | R-1049         | Unidentified |
| 53    | R-1055         | Unidentified |

(Continues)
TABLE 8 Treatment details

| Formulation ID | Isolate Identity           | Isolate Number |
|----------------|---------------------------|----------------|
| F-2            | Azospirillum lipoferum    | 46, 118        |
| F-4            | Rhizobium leguminosarum   | 340            |
| F-5            | Burkholderia ambifaria    | 114, 171, 156  |
| F-7            | Burkholderia graminis     | 172, 107, 108  |
| F-8            | Burkholderia vietnamiensis| 147            |
| F-9            | Pseudomonas lirida        | 43, 54, 90, 167|
| F-13           | Exiguobacterium acetylicum| 313            |
| F-15           | Kosakonia cowanii         | 202            |
| F-17           | Paenibacillus polymyxa    | 255            |

Note: These formulations were tested along with positive control (G) (high productive G-site Soil with no treatment application), negative control (H) (average productive H-site Soil with no treatment application), and a control (C) (H-site soil with alginate bead without any bacterial inoculum).

80 g of soil. Cups were lightly watered and covered until seedlings emerge, then opened, watered daily for 30 days, until the termination of experiment. Chlorophyll content was measured 1 day before the termination of experiment. At harvest, biometric parameters such as shoot length, dry shoot, and root weights were recorded. Rhizosphere soil samples were collected and stored in –20°C for microbial community analysis. One pooled soil sample from each treatment was collected for chemical profiling.

5.4 | Soil chemical analysis

Bulk soil sample from G and H site and one pooled sample from each treatment were sent to A and L Canada Laboratories (London, ON, Canada) for standard soil chemical profiling. Briefly, the soil samples were air dried at 40°C for 48 hr. Then the chemical parameters such as Organic matter (OM), total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sulfur (S), zinc (Zn), manganese (Mn), iron (Fe), boron (B), chloride (Cl), aluminum (Al), pH, and cation exchange capacity (CEC) were measured using standard protocol (Jones, 1999; Rice, Baird, Eaton, & Clesceri, 2012) and expressed in ppm except the OM, which is a percentage value.

5.5 | Bacterial community DNA extraction and TRFLP analysis

Rhizosphere soil community DNA was extracted using the Soil DNA Isolation Kit (Norgen Biotek Corporation, Thorold, ON, Canada) following the manufacturer’s protocol with minor modifications. The composition of soil bacteria community diversity was analyzed via T-RFLP of 16S-rRNA gene using a pair of fluorescently labeled universal primers FAM-labeled forward primer 63F (CAGGCCCTAACACATGCAAGTC) (Marchesi et al., 1998) and VIC labeled reverse primer 1389R (ACGGCCGGTGTGTACACAG) (Osborn et al., 2000). Each PCR reaction mixture of 50 µl contained 2X reaction buffer (Classic+ Taq Polymerase Master Mix, Tonbo Biosciences, San Diego, CA, USA), 0.01 nmol of each primer and approximately 50 ng of template DNA and PCR grade water to make up to volume. A negative control (PCR mixture without DNA template) was included for each PCR run.

The PCR reactions were carried out in T100™ Thermal Cycler with initial denaturation temperature of 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 45 s, primer annealing at 56°C for 45 s and elongation at 72°C for 2 min followed by a final extension at 72°C for 10 min. Each PCRed product was run on 1% agarose gel containing Gelred dye, isolated, and purified using a DNA Clean and Concentrator-5 kit (Zymo Research Corporation, Irvine, CA, USA). For each sample, 15 µl of purified PCR product was digested with 0.5 µl of Hpal (New England Biolabs) restriction enzyme for 3 hr at 37°C. Digested samples were diluted to 1:3 ratio with PCR grade water and the fluorescently labeled terminal restriction fragments (TRFs) were separated by capillary electrophoresis in an automated DNA sequencer equipped with fluorescence detector (Applied Biosystem 3,730 DNA Analyzer) at the University of Guelph (Guelph, Ontario, Canada). TRF length (in base pairs) were determined in comparison with internal LIZ size standard (1,200 bp) using GeneMarker® software V2.4.0, with 10 bp size cutoff and 100 to 35,000 fluorescent unit peak intensity detection range. Intensity data were exported to XLSTAT 2013 software and compared to determine microbial community profiles. The abundance of each TRF was determined by measuring the fluorescence unit and the diversity using the binary numbers. Each biological replicate samples were analyzed in duplicates. To identify the peaks, we referred to a previously established internal bacterial TRF database. The database has been established through in-silico analysis (digested with Hpal enzyme using NEBCutter V2.0 (http://nc2.neb.com/NEBcutter2/) online tool) of the 16S rRNA gene sequences of about 1,000 bacterial strains isolated and identified from rhizosphere, root, and endosphere of corn and wheat and the data base has been further confirmed by digesting the corresponding sequences retrieved from NCBI data base.

5.6 | Statistical analysis

The means and standard errors of biometric data shown in Figure 1 were calculated using Excel 2016 and the analysis of variance (one-way ANOVA) to access the significance in difference was assessed using Tukey post hoc tests at a 5% confidence level (p < 0.05) and the means were compared using Duncan’s mean comparison. Shannon’s richness and diversity for both binary and intensity data were calculated separately using Shannon’s diversity formula in Excel (Table 2). PCA of TRFLP profiles (Figure 3) were made by implementing R ggbiplot package in RStudio (Version 1.0.143) to find out the change in microbial diversity of the fields and to list (Table 3) keystone peaks involved in field separation. The coordinates of PC1 and PC2 of each sample field, which shown as vector on the biplots, were also extracted from R (Version 3.3.3) (Table 4).
PCA plots were made again with keystone TRFs, soil parameters and the plant biometrics using ggbiolot package (Figure 4). The corresponding coordinates of each parameter point on the biplots were exported from R (Version 3.3.3) (Table 5). The correlations of TRFs against soil and plant biometric parameters were also calculated in XLSTAT (Table 6). The study was repeated twice to confirm the reproducibility of the results.

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CONFLICT OF INTERESTS
None declared.

AUTHOR CONTRIBUTIONS
S.K. Conceived and designed the study. S.K. and E.L. carried out the experiment, collected, analyzed the data, and wrote the manuscript. S.K., E.L., G.P., G.L., and S.A reviewed the manuscript. All authors read and contributed to the manuscript.

ETHICS STATEMENT
None required.

DATA AVAILABILITY STATEMENT
All data are provided in the results of the manuscript.

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