

1 Soil Microbial Composition and Structure Allow Assessment of Biological Product

2 Effectiveness and Crop Yield Prediction

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21 **ABSTRACT**

22 Understanding the effectiveness and potential mechanism of action of agricultural biological  
23 products under different soil profiles and crops will allow more precise product  
24 recommendations based on local conditions and will ultimately result in increased crop yield.

25 This study aimed to use bulk and rhizosphere soil's microbial composition and structure to  
26 evaluate the effect of a *Bacillus amyloliquefaciens* strain QST713 inoculant on potatoes, and to  
27 explore its relationship with crop yield. We implemented NGS and bioinformatics approaches to  
28 assess the bacterial and fungal biodiversity in 185 soil samples, distributed over four different  
29 time points -from planting to harvest- from three different geographical regions in the United  
30 States.

31 In addition to variety, phenological stage of the potato plant and geography being important  
32 factors defining the microbiome composition and structure, the microbial inoculant applied as a  
33 treatment also had a significant effect. However, treatment preserved the native communities  
34 without causing a detectable long-lasting effect on the alpha- and beta-diversity patterns after  
35 harvest. Specific taxonomic groups, and most interestingly the structure of the fungal and  
36 bacterial communities (measured using co-occurrence and co-exclusion networks), changed after  
37 inoculation. Additionally, using information about the application of the microbial inoculant and  
38 considering microbiome composition and structure data we were able to train a Random Forest  
39 model to estimate if a bulk or rhizosphere soil sample came from a low or high yield block with  
40 relatively high accuracy, concluding that the structure of fungal communities is a better estimator  
41 of potato yield than the structure of bacterial communities.

42

43 **IMPORTANCE** The manuscript's results reinforce the notion that each crop variety on each  
44 location recruits a unique microbial community and that these communities are modulated by the  
45 vegetative growth stage of the plant. Moreover, inoculation of a *Bacillus amyloliquefaciens*  
46 strain QST713-based product on potatoes also changed specific taxonomic groups and, most  
47 interestingly, the structure of local fungal and bacterial networks in bulk and rhizosphere soil.  
48 The data obtained, coming from in-field assays performed in three different geographical  
49 locations, allowed training a predictive model to estimate the yield of a certain block, identifying  
50 microbiome variables -especially those related to microbial community structure- with a higher  
51 predictive power than the variety and geography of the block. The methods described here can be  
52 replicated to fit new models predicting yield in any other crop, and to evaluate the effect of any  
53 Ag-input product in the composition and structure of the soil microbiome.

54

## 55 **INTRODUCTION**

56 Potato, the stem tuber vegetable produced by *Solanum tuberosum*, is the crop with the highest  
57 yield out of the five most important agricultural crops in the world (rice, wheat, soybeans, maize  
58 and potatoes). Although global production of potatoes in 2012 reached 364,808,768 MT, it has  
59 been calculated that actual yield corresponds to only about 10 to 75% of potential yield (1).  
60 Improving global agricultural crop production in a sustainable way is paramount given the  
61 current prospects for world population increase (2).  
62 Potato yield has been directly correlated with edaphological and climate variation (3-5), with  
63 management practices (6) and with potato cultivar (7). Interestingly, the same biogeographical  
64 patterns have been identified as the main drivers of microbial community composition in potato  
65 plants (8-15), reinforcing the key role of soil microbiology in potato crop productivity (16).

66 Thus, in agro-ecosystems, the enhancement and sustainability of productivity can be assessed by  
67 means of the soil microbiome. Additionally, the Natural Resources Conservation Service of the  
68 US Department of Agriculture (17) links soil quality with the concept of soil health,  
69 acknowledging the relevance of soil microorganisms to drive soil functionality.

70 In this context, the use of substances, microorganisms, or mixtures thereof, known as plant  
71 biostimulants, is among the latest practices for sustainable food and energy production (18).  
72 Biological products are claimed to promote plant health and quality and recycling crop residues  
73 with low environmental impact (19, 20). Not surprisingly, the market for agricultural biological  
74 products is recording a CAGR of over 10% since 2017, and it is expected to reach a market size  
75 of over four billion dollars by 2025 (21). Rajabi-Hamedani and collaborators (22) argue that this  
76 growth is a consequence of the need to increase the efficiency of agrochemical inputs, to reduce  
77 crop damage caused by abiotic stress, and to reduce the environmental impact of production  
78 systems.

79 Most agricultural biological products based on microorganisms are expected to pertain to the  
80 functional group of Plant Growth Promoter species, so a direct impact in plant health (23, 24)  
81 and yield (25) is assumed. Different direct mechanisms involved in yield promotion have been  
82 demonstrated in certain bacterial strains, including: i) improving growth of tomato plants, by  
83 increasing root hairs development in a phytohormone-mediated process using an *Azospirillum*  
84 *brasilense* strain (26) or by increasing the tolerance to abiotic stresses through the action of an  
85 ACC deaminase produced by a *Burkholderia unamae* strain (27); ii) increasing plant growth by  
86 enhanced nutrient (P) acquisition in cucumber and tomato plants using a *Bacillus* sp. strain (28);  
87 iii) enhancing nodule formation by a two species consortia of *Pseudomonas putida* plus  
88 *Rhizobioum* sp. in beans (29); or by improving grain yield in rice by increasing panicle number

89 through the use of an *Azospirillum amazonense* strain (30). In addition, some microbial strains  
90 have also shown an indirect effect in soil and plant health, as tools for *in situ* microbiome  
91 engineering, promoting the development of other beneficial microbial species, improving the  
92 resistance of the microbiome to the invasion of plant pathogens, and increasing the natural  
93 resistance of the plant against diseases (31).

94 Instead of assuming a simple, unidirectional and direct effect of a certain microbial strain in the  
95 physiology and development of plants, agricultural biological products face challenges with  
96 consistent field performance. Different strains and species can have different functional  
97 performance under specific environmental and ecological conditions (32). For this reason,  
98 biological products' claims need to describe ecological and functional performance and not only  
99 be based on composition of matter (33).

100 In this work we aimed to contribute to global sustainability of the agricultural lands by  
101 demonstrating that assessment of bulk and rhizosphere soil microbial composition and structure  
102 can be practical tools to substantiate agricultural biological product claims, and at the same time  
103 they provide a toolkit for growers to assess and achieve increased yield and sustainability of their  
104 management practices. Applying “-omics” technologies we explored the subtle side effects of the  
105 microbial inoculant *Bacillus amyloliquefaciens* strain QST713, in the surrounding rhizosphere  
106 and bulk soil microbiota of potatoes, and its potential connection with the yield observed. We  
107 followed the recommendations of Ricci and collaborators (33) for field trials in one crop, in  
108 order to demonstrate that this product has *bona fide* effects. We were particularly interested in  
109 comparing the microbiome profile associated with treated vs. untreated samples over time and  
110 across diverse locations, to determine whether or not a common mechanism of action was at  
111 play. Both, the changes in the microorganism composition of samples across time as well as the

112 evolution of the structure of the bacterial and fungal communities were assessed. Additionally,  
113 making use of potential correlations among microbiome profiles, product use and crop yield we  
114 built a yield prediction model as a first step towards guaranteeing growers the level of  
115 effectiveness of a product under different management and environmental conditions (weather,  
116 soil microbiome, soil type, crop variety, etc). Our observations conclude that individual  
117 microorganism abundances as well as the structure of the fungal and bacterial communities  
118 change slightly but significantly after application of the inoculant and that these changes can be  
119 associated with the unique yield response at each biogeographical location.

120

## 121 **RESULTS**

122 In this work, we assessed bacterial and fungal communities of bulk and rhizosphere soil (soil  
123 health) of potato cultivars from three different regions of the United States (Sutton and Grant  
124 (Idaho), and White Pigeon (Michigan)). Our aim was to understand the effect of a microbial  
125 inoculant (*B. amyloliquefaciens* strain QST713) in the rhizosphere microbiota and its final legacy  
126 in the bulk soil microbiota after harvest. We were also trying to identify potential microbiome  
127 biomarkers associated with samples with low or high yields. A total of 185 samples from treated  
128 and untreated plots at each location were collected over four time points, from planting (T0) to  
129 harvest (T3), focusing on the early changes occurring after one (T1) and two (T2) months from  
130 planting, where T0 and T3 are bulk soil samples, and T1 and T2 are rhizosphere soil samples.  
131 Figure 1 shows that, in two of the three locations assayed the use of the inoculant had a  
132 significant effect on increasing the crop yield (Grant p-value  $8.66 \times 10^{-10}$ , and Sutton p-value  
133  $7.67 \times 10^{-7}$ ), without any detectable effect in the third location (White Pigeon p-value 0.31) which  
134 had, indeed, a much higher yield in both control and treatment samples.

135  
136 **Variety, phenological stage and geography drive the microbiome composition of bulk and**  
137 **rhizosphere soil of potato crops.** Figure 2 shows a clear population dynamic occurring from T0  
138 (before planting) to T1 and T2 samples (one and two months after planting, respectively) in all  
139 locations. Figure 2A shows that in terms of beta-diversity of bacterial populations, variety  
140 ( $R^2=0.286$ ), phenological stage ( $R^2=0.286$ ) and location ( $R^2=0.042$ ) had significant effects, with  
141 the treatment ( $R^2=0.004$ ) having a minor non-significant effect. However, for fungal populations  
142 (Figure 2C), variety dominates as the main driver of the beta-diversity patterns ( $R^2=0.299$ ), with  
143 phenological state having a much lower impact ( $R^2=0.084$ ) than in bacterial populations, and  
144 location ( $R^2=0.067$ ) and treatment ( $R^2=0.007$ ) having similar impacts to that in bacterial  
145 populations. Additionally, for fungal populations, all covariates had significant effects (full  
146 PERMANOVA data in Table S1). As shown in Figures 2A and 2C, White Pigeon is significantly  
147 different from Grant and Sutton; this can be easily explained by the geographical distance  
148 between locations, which correlates well with the Aitchison distances of samples in the PCoA  
149 analysis. There are also different edaphological and weather conditions at each of these  
150 locations, and a different crop variety in White Pigeon as compared to Sutton and Grant, all of  
151 which are major drivers of the soil microbial populations as previously observed by Rasche (10)  
152 and İnceoğlu (14) in potato soils. The significant differences between microbial community  
153 compositions before and after planting can be clearly seen at Figures 2A and 2C, where, despite  
154 the large differences between locations, T1 and T2 samples clustered in all the three locations,  
155 away from their respective T0, especially in the case of bacterial populations. Similar  
156 observations have been reported in maize (34), rice (35) and potato cultivars (13), and in forest  
157 soils (36).

158 Regarding alpha-diversity (Figures 2B and 2D), there is a clear impact of planting in reducing  
159 the diversity of bacterial and fungal populations, as shown for both OTUs richness and Shannon  
160 (H') index values from T0 to T1. This trend can be extended until time T2 in most cases -with  
161 the exception of the Shannon index for bacterial populations at White Pigeon and for fungal  
162 populations at Grant and Sutton- indicating that the phenological stage of the plant is one of the  
163 main drivers of changes at the alpha-diversity level in both bacterial and fungal populations.  
164 Additionally, comparing control versus treated samples at the same time point, we observed  
165 significant changes in Grant at T1 for bacterial richness and Shannon index as well as fungal  
166 Shannon index (Table S2). Interestingly, Grant was the site with the largest yield increase  
167 response due to treatment. When soil samples were again analyzed after harvest (T3) in Grant  
168 and Sutton locations, we observed that in spite of the marked microbial succession patterns  
169 found from T0 to T2, there was no significant changes in alpha-diversity between the microbial  
170 communities found in the soil before planting (T0) and after harvesting (T3) (Table S3);  
171 therefore, the plant's associated soil microbiota seems to have cycled back to its original state.  
172 At the taxonomy level, despite clear population dynamic patterns from T0 to T2 sampling times  
173 in all the three locations and in both treated and untreated samples, samples from all three  
174 locations and times shared some of the most abundant genera for both bacterial and fungal  
175 communities (Figure S1). Figure S1 shows the top bacterial genera identified across samples in  
176 this study (core microbial species). Of these, five (*Arthrobacter*, *Pseudomonas*, *Sphingomonas*,  
177 *Streptomyces* and *Rhizobium*) also appeared in the soil bacteria survey performed by İnceoğlu  
178 (13) on potato fields. Among the top fungal genera shared across samples in our study (core  
179 fungal species) we found *Cryptococcus*, *Mortierella*, and *Alternaria*.



180 Thus, as previously reported (37) in tomato cultivars using a *B. subtilis* strain, and in soybean  
181 (38) and lettuce (39) cultivars using different strains of *B. amyloliquefaciens*, here we didn't  
182 detect a durable impact of the treatment on the bulk soil microbial communities in terms of major  
183 taxa (Figure S1), and alpha- and beta-diversity (Figure 2), but instead we observed a clear  
184 temporal -cyclical- dynamics which differentiates bulk soil (T0 and T3) and rhizosphere soil (T1  
185 and T2) samples (Figure 2).

186

187 **Elements of microbiome composition and structure can be effectively modulated by use of**  
188 **a *B. amyloliquefaciens*-based soil applied biological product.** To dissect the specific effect of  
189 the biological product over the microbial composition across time at each location, we compared  
190 the fold change of each OTU in the treatment group from T0 to T1 (and from T0 to T2) vs. the  
191 fold change in the control group at the same time intervals per location (Table S4). Out of 17,241  
192 unique bacterial OTUs in the samples of the study, 16 changed significantly from T0 to T1 (none  
193 in Grant, one in Sutton, and 15 in White Pigeon), and 100 from T0 to T2 (16 in Grant, 79 in  
194 Sutton, and five in White Pigeon). These OTUs belong to 73 genera, of which, 13 changed  
195 significantly in at least two locations: *Bacillus*, *Bradyrhizobium*, *Clostridium*, *Novosphingobium*,  
196 *Rhodoplanes*, *Sphingomonas*, *Sphingopyxis*, and *Woodsholea* in Grant and Sutton; *Agromyces*,  
197 *Flavobacterium*, *Pedobacter*, and *Sporosarcina* in Sutton and White Pigeon; and  
198 *Stenotrophomonas* in Grant and White Pigeon. For fungi, out of 1,702 unique OTUs, ten OTUs  
199 changed significantly from T0 to T1 (none in Grant, eight in Sutton and two in White Pigeon),  
200 and 32 from T0 to T2 (none in Grant, 32 in Sutton, and none in White Pigeon). These OTUs  
201 belong to 30 genera, of which, one changed significantly in at least two locations: *Cryptococcus*  
202 in Sutton and White Pigeon. Thus, despite variety, phenological stage and location having a

203 larger effect than treatment in the composition of microorganism populations, the inoculant still  
204 generated common detectable abundance changes in at least two of the three locations for several  
205 taxonomic groups, some of which have known functionally relevant roles (*Bacillus*,  
206 *Bradyrhizobium*, *Flavobacterium*, *Pedobacter*, *Sphingomonas*, and *Stenotrophomonas*).

207

208 In order to get a deeper understanding of how the structure of the bacterial and fungal  
209 communities, and therefore the ecological relationships among microorganisms, impacts the  
210 effect of the bacterial inoculant, we studied the co-occurrence and co-exclusion patterns between  
211 pairs of OTUs in each sample of the trial. As some of us reported in a recent work (40), by  
212 studying the network properties of local communities inferred from the co-occurrences and co-  
213 exclusion patterns of a reference metacommunity it is possible to estimate ecological emergent  
214 properties (i.e. niche specialization, level of competition) of interest for the understanding of  
215 microbiome functioning. We first built metacommunities based on all samples of the trial. As an  
216 initial filter, for bacteria, we retained OTUs that were detected in at least 30% of the entire  
217 dataset, and 90% for fungal communities. This is due to the disproportionate number of unique  
218 OTUs detected in 16S vs. ITS soil sequencing. To keep the overall size of the data manageable  
219 we limited the number of selected OTUs to 4,000 with a maximum of 10 million possible  
220 significant pairs. We also filtered out OTU pairs that were not significantly ( $p < 0.05$ ) enriched  
221 (co-occurrence) or depleted (co-exclusion). This resulted in metacommunity networks consisting  
222 of 3,339 nodes for bacteria (19.4% of the total 17,241 bacterial OTUs) and 447 nodes for fungi  
223 (26.3% of the total 1,702 fungal OTUs), which on average captured 92.11% of the bacterial  
224 abundance and 98.62% of the fungal abundance of the samples in the study. We then explored  
225 the structure of local microbiome communities, based on just the nodes present in each

226 individual sample, aiming to detect changes in network properties that are associated with the  
227 application of the biological product at a specific location over time. Specifically, for the co-  
228 exclusion and co-occurrence bacterial networks, we calculated the modularity (a measure of the  
229 strength of partitioning of a network into modules) and transitivity (measure of the degree to  
230 which nodes in a network cluster together) as well as the proportion of co-exclusions and co-  
231 occurrences present in the local network compared to the total number of possible combinations  
232 among all OTUs in the sample.

233 Figures 3A and 3B show the evolution from T0 through T2 of four of the six local network  
234 properties studied across locations, for bacterial and fungal populations, respectively. Figure 3C  
235 lists those changes that have been significant (see Table S5 for full data) in time -from T0 to T1,  
236 and from T0 to T2- in treated vs. untreated blocks. In Grant there is a significant decrease in  
237 fungal co-occurrence transitivity and bacterial co-occurrence proportion from T0 to T1 in the  
238 treated samples when compared to untreated ones. In agreement with the observations of Ortiz-  
239 Alvarez (40) on their extensive survey of vineyard soils, it seems that any human intervention in  
240 a crop alters the structure of microbial communities of the soil, and a decreased transitivity on  
241 the fungal co-occurrence network seems to be a common indicator of these types of alterations.  
242 In the above-mentioned work, some of us argued that low clustered communities (those with low  
243 transitivity scores) can be associated with highly competitive environments with a high degree of  
244 niche specialization, which are among the most relevant properties of an ecosystem when trying  
245 to understand its functionality and its response to human interventions and land-use changes  
246 (41). It is also interesting to see a lagged effect (at T2) of the treatment in modifying some  
247 network properties of the bacterial communities in both Grant and Sutton. In Grant, the bacterial  
248 co-occurrence proportion increases from T0 to T2 (in contrast to the decrease from T0 to T1),

249 and at the same time the transitivity of the bacterial co-occurrence network increases. In Sutton,  
250 both the bacterial co-occurrence proportion as well as the bacterial co-exclusion proportion  
251 increased from T0 to T2. Thus, when attending to the microbiome structure changes caused by  
252 the treatment in Grant and Sutton, which were the locations where treatment had a significant  
253 effect over yield, we can highlight significant treatment-mediated effects over the fungal and  
254 bacterial community networks that decreased from T0 to T1, and then increased in T2.

255 Interestingly, and contrary to what was observed in Grant and Sutton, in White Pigeon, the  
256 location where treatment didn't have a significant effect over yield and that had a different  
257 variety of potato, there was an increase in the bacterial co-exclusion modularity from T0 to T1.

258

259 **Elements of microbiome composition and structure allow prediction of potato yield.** We

260 fitted a Random Forest model aiming to predict if a rhizosphere or bulk soil sample comes from  
261 a block with a yield  $\leq 30\text{t/ha}$  or  $> 30\text{t/ha}$ , based on its microbiome composition and structure

262 using multivariate compositional data (Principal Components from a beta-diversity ordination)

263 and local network properties. We measured yield data in 20 treated and 20 untreated plots from

264 the three geographical locations, and for each we utilized all samples available over times T0, T1

265 and T2. In total 112 samples were used for this task split into a training set of 84 samples and a

266 test set of 28 samples. The result of this model showed a predictive accuracy of 78.6% (Figure

267 4A) and identified four variables (two network properties and two compositional) as the most

268 important predictors of yield (Figure 4B), even with a higher importance than a variable we used

269 to encompass the effects of geography and variety that are not accounted for by the microbial

270 composition and structure. Surprisingly, the structure of fungal communities (i.e. fungal co-

271 occurrence transitivity and co-exclusion proportion), showed a much higher predictive value than

272 the structure of bacterial communities (Figure 4B, see Table S6 for full data on the importance of  
273 variables to the yield prediction model). We observed an inverse correlation between the co-  
274 occurrence transitivity of bulk and rhizosphere soil fungal communities and the yield found in  
275 the potato cultivars. This is a particularly important observation for understanding the effect of  
276 the *B. amyloliquefaciens*-based biological product assayed here in shaping the structure of fungal  
277 communities as a potential mechanism of action when increasing the yield. As shown in Figure  
278 3B and Table S5, in going from T0 to T1 the increase in fungal co-occurrence transitivity in  
279 Grant is greater in the control samples than the treated ones, and this difference is significant (p-  
280 value=0.007). In Sutton -where a smaller but significant effect of the treatment increasing yield  
281 was also found (Figure 1)- in going from T0 to T1 there was a smaller increase in fungal co-  
282 occurrence network transitivity in the treated samples when compared to the control ones (albeit  
283 the difference is not significant, p-value=0.086). In White Pigeon instead, where the treatment  
284 did not have an effect over yield and where there was a different potato variety, there is a  
285 decrease in fungal co-occurrence network transitivity in going from T0 to T1 in treated samples,  
286 and even a more marked decrease in control samples.

287 The other two compositional variables (PC3 and PC1) contributing to the predictive power of the  
288 model fitted can be explored by looking at the taxonomy of the OTUs in each showing a  
289 significant correlation with the yield. It is necessary to keep in mind that the predictive power of  
290 PC3 and PC1 variables, as principal components of a multivariate analysis, came from the  
291 interaction patterns among the OTUs and not from their individual behavior. However, we can  
292 highlight the presence, for instance, of the fungal biocontrol agent *Trichoderma* sp. (42) as the  
293 OTU with the highest positive correlation with yield in PC3 (Figure S2).

294 As described in the methods section, since yield is constant for all samples within a plot, we  
295 converted yield to a categorical variable ( $\leq 30\text{t/ha}$ ,  $> 30\text{t/ha}$ ). The distribution of the yield data  
296 was bimodal, and thus it seemed logical to divide the categories on a zero probability density  
297 point for the bimodal distribution. However, in order to assess if this decision may have had an  
298 impact in the features identified as important by the yield predictive model presented here, we  
299 investigated the models resulting from splitting the yield data into three ( $\leq 26\text{t/ha}$ ,  $> 26\text{t/ha}$  to  $\leq$   
300  $35\text{t/ha}$ ,  $> 35\text{t/ha}$ ) or four ( $\leq 20\text{t/ha}$ ,  $>20\text{t/ha}$  to  $\leq 26\text{t/ha}$ ;  $> 26\text{t/ha}$  to  $\leq 35\text{t/ha}$  ,  $> 35\text{t/ha}$ )  
301 categories. As can be seen from Table S6, fungal co-occurrence transitivity and fungal co-  
302 exclusion proportion always had higher importance than geography and variety, independent of  
303 the number of yield categories used. In the model with three yield categories the bacterial co-  
304 exclusion proportion also had higher importance than geography and variety, whereas in the  
305 model with four yield categories, fungal co-inclusion modularity and PC12 had higher  
306 importance than geography and variety. However, dividing yield into more categories resulted in  
307 decreased accuracy (64.3% when splitting into three categories and 57.1% when splitting into  
308 four categories) due to the limited training set size being divided into an increasing number of  
309 categories.

310 Van Klompenburg and collaborators (43) performed a systematic literature review to identify the  
311 most used machine learning algorithms for crop yield prediction as well as the most used  
312 features to train those algorithms. They identified that most researchers have used neural  
313 networks in their work with the most frequently used features being temperature, rainfall and soil  
314 type. Interestingly, none of the articles reviewed utilized soil microbial or fungal composition or  
315 structure as features. In recent work, Jeanne and collaborators (16) developed a model to  
316 correlate potato yield to soil bacterial diversity. They showed that their species balance index

317 related to potato yield (SBI-py) had a high correlation (0.77) with yield, whereas the Shannon  
318 diversity, Pielou diversity and Chao 1 diversity failed to correlate well with yield. Here, we built  
319 a machine learning potato yield model based on bacterial and fungal communities of rhizosphere  
320 and bulk soil and their structure, which can predict with relatively high accuracy whether a  
321 potato plot will have a yield of more or less than 30t/ha, which was the value that divided the  
322 bimodal distribution of yield in our training set. It is also worth noting that the dataset in this  
323 study included as a variable the application of a bioinoculant, thus this yield model also  
324 represents a first step towards understanding when and where biological products work. Despite  
325 the small sample size and the treatment of yield as a categorical value, independent of the  
326 number of categories used for splitting yield, we always found that the structure of fungal  
327 communities was a better estimator of potato yield than the structure of bacterial communities,  
328 which is a finding that merits further investigation.

329

## 330 **DISCUSSION**

331 The use of microbial inoculants to increase the yield of plants is a useful strategy, increasingly  
332 used in agriculture. In addition to the direct impact of the microbial inoculant in the plant, due to  
333 its unique metabolic properties, the introduction of an allochthonous strain in the microbial  
334 rhizosphere and bulk soil ecosystems may have an impact on the entire microbiome, affecting  
335 the composition and structure of the native communities. Our work demonstrates that variety  
336 being the main driver of the microbial profile of rhizosphere and bulk soils from potatoes,  
337 phenological stage of the plant and geography also have a major impact in the microbiome  
338 composition, especially in the bacterial community. Even though relegated to last position, the  
339 use of a microbial inoculant based on *B. amyloliquefaciens* QST713 -a strain isolated from the

340 soil of a Californian organic peach orchard with a demonstrated effective broad-spectrum  
341 bactericide and fungicide activity (44) through a number of different mechanisms of action (45,  
342 46)- had a significant effect over the beta-diversity of fungal communities. Looking at alpha-  
343 diversity we observed significant changes at T1 in one location (Grant) for both bacterial and  
344 fungal communities in treated plots. Given that variety, plant phenological stage and geography  
345 have such strong influence over bulk and rhizosphere soil community composition and structure,  
346 the treatment effects observed were analyzed per location as evolution between two time points  
347 when comparing treated versus untreated plots. This also means that the patterns identified here  
348 as derived from product use may be of a more correlative than deterministic nature. Nonetheless,  
349 several OTUs changed significantly from T0 to T1 and from T0 to T2 in the inoculated soils,  
350 including several functionally important members of the soil microbiota, as well as modified  
351 specific microbial network properties. Specifically, a potential link between the bioinoculant and  
352 yield whereby the bioinoculant reduces the transitivity of the co-occurrence fungal network of  
353 the rhizosphere and bulk soil where it is applied through its biofungicide activity seems fit.  
354 Importantly, we also observed that this *B. amyloliquifaciens* QST713 trial did not cause any  
355 legacy effect on the microbiome profile of the soil analyzed after harvesting, i.e. the effect of the  
356 bioinoculant is cyclical and the native microbiome returns to its original state after harvesting.  
357 We also presented here a Random Forest yield prediction model for potatoes based on a soil  
358 health assessment of its microbial composition and structure. This model is our first step towards  
359 understanding not only why, but also when and where biological products work increasing yield.  
360 In addition, the significant contribution of the local network properties on the estimation of the  
361 actual yield of a certain block reinforces the idea of the need of a more functional vision of  
362 agriculture microbiomes, as certain emergent properties can be deduced from them. In particular,



363 low clustered (low co-occurrence transitivity) fungal communities, as found here as positively  
364 contributing to the yield, are expected to be driven by a higher degree of niche specialization  
365 (40). Thus, *B. amyloliquefaciens* QST713 seems to help the soil microbiota adopt a conformation  
366 with lower fungal co-occurrence network transitivity than expected from untreated plots which is  
367 conducive to improved yield, but in a reversible manner (the fungal communities return to their  
368 original stage post-harvest).

369 Our model trained in only three locations, including only two potato varieties, and where half of  
370 the samples were treated with the bioinoculant may be biased, for instance, in recognizing the  
371 effects of *B. amyloliquefaciens* QST713 over the soil microbiome as the main features predictive  
372 of yield. However, the fact that fungal co-occurrence network transitivity is linked with potato  
373 yield, has not been reported before and merits further study. Possible future avenues of research  
374 derived from the current work include: i) investigating whether fungal co-occurrence network  
375 transitivity continues to be an important variable in models predicting yield in a more diverse  
376 datasets than the one described here (more varieties, more locations, more samples, wider variety  
377 of edaphological and weather conditions); ii) building predictive models containing not only  
378 microbiome data, but also edaphological and climate information; iii) investigating further the  
379 link between decreased fungal co-occurrence network transitivity and nutrient metabolism in the  
380 soil; and iv) investigating further metacommunity networks or individual sample networks with  
381 low transitivity to understand the taxonomic composition of modules and explain further each of  
382 the niches identified within them; among others. Ultimately, the inverse correlation between crop  
383 yield and fungal co-occurrence transitivity identified in this study as the potential mechanism of  
384 action of *B. amyloliquefaciens* QST713 in increasing potato yield is a useful concept to design  
385 and test interventions for increasing crop yield. This finding also demonstrates that assessment of

386 soil microbial composition and structure in agricultural input trials can be practical tools to  
387 substantiate biological product claims, and that they provide a toolkit for growers to assess and  
388 achieve increased yield and sustainability of their management practices.

389

## 390 **MATERIALS AND METHODS**

391 **Field trials.** Russet Burbank potatoes were planted in Sutton and Grant locations in Idaho. Seed  
392 variety Lamoka was planted in White Pigeon, Michigan. Applications were performed at 46.770  
393 l/ha spray volume combining grower standard practice of Quadris and Admire Pro plus the  
394 biological treatment, tank mixed and applied in-furrow. Treatment consisted of a biological  
395 product containing a minimum of  $2.7 \times 10^{10}$  CFU/g of *B. amyloliquefaciens* QST713 at a dose of  
396 0.935 l/ha out of the total spray volume. Grower standard fungicide and insecticide applications  
397 were chemigated over trial area as seeded. Plots were 0.405 hectares (1 acre) each. Harvest was  
398 conducted by harvesting 2.787m<sup>2</sup> within each plot. Yield weights were evaluated and recorded in  
399 lbs and cwt/ac.

400

401 **Sample collection.** Whole plants from the field were collected and processed to obtain bulk soil  
402 and rhizosphere samples over the three regions. The field samples were processed to obtain bulk  
403 soil from all the root surfaces by vigorous shaking, and to collect rhizosphere samples we  
404 followed the protocol by Lundberg and collaborators (47) with slight modifications: roots  
405 (separated from mother tubers) were chopped into small bits and collected in a clean tube, filled  
406 with 40 ml of PBS buffer, vortexed and centrifuged to obtain a pellet. The rhizosphere pellet was  
407 stored at -80°C until genomic DNA was extracted. Samples were collected at four different time  
408 points: before planting and/or treatment (T0), one month after planting (T1), two months after

409 planting (T2) and at harvest (T3). Samples from White Pigeon were collected only for three time  
410 points: T0, T1 and T2. From each time-point, a total of 20 samples (ten treated and ten untreated)  
411 were collected, except for White Pigeon at T0 (four treated and four untreated), T1 (12 treated  
412 and nine untreated), and T2 (four treated and four untreated), and for Grant at T0 (four treated  
413 and four untreated). A total of 76 bulk soil samples (T0 and T3) and 109 rhizosphere samples  
414 (T1 and T2) were collected. Samples were collected across different locations for each field and  
415 the composite was submitted for analysis, in order to achieve a more homogenized sampling  
416 reducing the effect of microbial variability.

417

418 **Sample analysis.** After collection, samples were immediately sent for molecular analysis to  
419 Biome Makers laboratory in Sacramento, US. DNA extraction was performed with the DNeasy  
420 PowerLyzer PowerSoil Kit from Qiagen. To characterize both bacterial and fungal microbial  
421 communities associated with bulk soils and rhizosphere samples, the 16S rRNA and ITS marker  
422 regions were selected. Libraries were prepared following the two-step PCR Illumina protocol  
423 using custom primers amplifying the 16S rRNA V4 region and the ITS1 region described  
424 previously (48). Sequencing was conducted in an Illumina MiSeq instrument using pair-end  
425 sequencing (2x300bp). The bioinformatic processing of reads included the merging of forward  
426 and reverse paired reads to create robust amplicons, using Vsearch (49) with minimum overlaps  
427 of 100 nucleotides and merge read sizes between 70 and 400 nucleotides. OTU clustering was  
428 performed at 97% sequence identity, followed by quality filtering through *denovo* chimera  
429 removal using the UCHIME algorithm (50). Taxonomic annotation was performed using the  
430 SINTAX algorithm (51), which uses k-mer similarity to identify the top taxonomy candidate,  
431 after which we retained results where the species level had a score of at least 0.7 bootstrap

432 confidence. We used the SILVA database version 132 (52) and UNITE database version 7.2 (53)  
433 as taxonomic references.

434

435 **Alpha- and beta-diversity analysis.** Exploratory analyses of 16S and ITS OTU counts were  
436 conducted separately using the R package vegan (54). Alpha- and beta-diversity were analyzed  
437 using OTU counts. Alpha-diversity metrics (Shannon and richness) were calculated and plotted  
438 across all covariates available. Wilcoxon rank-sum tests were performed to compare control and  
439 treated samples within location-timepoint subgroups. For beta-diversity, Kruskal's non-metric  
440 multidimensional scaling was performed in conjunction with Aitchison distances. Relative  
441 abundances for OTUs as well as annotations at various taxonomic levels (genera, families, etc.)  
442 were used in the analyses. Permutational multivariate analysis of variance was performed on the  
443 Aitchison distance matrix, using all possible combinations of the variety, location, timepoint and  
444 treatment variables.

445

446 **Differential abundance.** For all subsequent analyses, the zero counts in the data were replaced.  
447 Valid values for replacement were calculated under a Bayesian paradigm, assuming a Dirichlet  
448 prior. Non-zero values were then adjusted to maintain the overall composition (55). Differential  
449 abundance determination was carried out using the R package edgeR (56). For each OTU, the  
450 fold change attributable to the treatment across different times (e.g. T0 to T1) was calculated.  
451 This was done by conducting a hypothesis test separately for each location, measuring the fold  
452 change of a given OTU in the treatment group (from T0 to T1) vs. the fold change in the control  
453 group (from T0 to T1), and then repeating the test but using times T0 and T2.

454

455 **Calculation of local network properties.** Meta-community networks were built for 16S and  
456 ITS data separately using the methods described by Veech (57) and Ortiz-Álvarez (40). In a  
457 nutshell, we first built a metacommunity network of all samples: this was done by estimating the  
458 co-occurrence and co-exclusion that would occur solely by chance for all possible OTU pairs,  
459 given the data. We selected OTU pairs that occurred significantly more than expected by chance  
460 to create the co-occurrence networks. Similarly, those that occurred significantly fewer times  
461 than expected by chance constituted the co-exclusion network. Local networks (single sample-  
462 level) were calculated by subsetting the metacommunity network for OTU pairs detected in each  
463 sample and estimating a local network. The R package igraph was used to calculate network  
464 properties: modularity, transitivity and proportion of co-exclusions and co-occurrences in  
465 relation to the total number of combinations among all OTUs in a sample (58). An adequate  
466 description of the ecological meaning of the different network properties calculated in this work  
467 can be found in the review work of Proulx and collaborators (59). Network properties were  
468 compared using a linear model. Using the network property as outcome, hypothesis tests were  
469 performed to compare timepoint differences in treated vs. control samples (analogous to the  
470 approach used for investigating differential abundances).

471  
472 **Yield model.** Yield data was first explored using medians and inter-quartile ranges (IQRs).  
473 Wilcoxon rank sum tests were performed on these yield data. The OTU counts were transformed  
474 using the centered log-ratio (CLR) transformation. CLR-transformed 16S and ITS data were  
475 jointly projected onto 70 principal components. Yield was modelled as the outcome of these 70  
476 principal components, along with fungal and bacterial network properties, treatment, soil type (to  
477 distinguish between bulk and rhizosphere soils), and a variable that encompasses variety and

478 geography, using a probability forest as described by Malley (60). Since the yield is a constant  
479 variable for all time points within a plot, the yield was converted to a categorical variable ( $\leq$   
480 30t/ha,  $> 30$ t/ha). The threshold for this division, 30 tonnes, was set at a zero probability density  
481 point for the bimodal distribution of yield. We used a total of 112 samples (all T0 through T2  
482 samples in the study for which we had yield data) and split them into training ( $n = 84$ ) and test ( $n$   
483  $= 28$ ) sets. Variable importance for each variable in the model was calculated using the Gini  
484 index. As a sensitivity test, probability forests were fit for a three-way split of the yield variable,  
485 and variable importances were compared. Among the 70 principal components of the  
486 microbiome included in the model, the ones with the highest importance in the probability forest  
487 were selected for further analysis. The loadings of these principal components were clustered  
488 using an unsupervised hierarchical clustering algorithm to visualize some of the most influential  
489 OTUs' impact on these principal components.

490

491 **Data availability.** Raw files for bacterial and fungal amplicons for each sample are available in  
492 NCBI under BioProject accession code: PRJNA699261.

493

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507

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509 I.B., A.D., J.D., V.T., and A.A. conceived and designed the work; N.I. contributed to the  
510 development of the analytical pipelines, and built the data and computational infrastructure; N.I.,  
511 I.B., and D.A. performed and supervised data analysis; N.I., I.B., D.A., and A.A. wrote the  
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513

514

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684  
685 **Figures Legends**

686  
687 **Figure 1.** Yield data (t/ha) for control and treated blocks across locations. Discontinued line  
688 separates blocks into two categorical variables ( $\leq 30$ t/ha,  $> 30$ t/ha), and corresponds to one of the  
689 natural zero probability density points in the bimodal yield distribution. The box limits  
690 correspond to the 25th and 75th percentile, and the central line is the median. The whiskers are  
691 the 5th and 95th percentile. The dots represent outliers (points below 25th percentile -  $(1.5 *$   
692  $IQR)$  and above 75th percentile +  $(1.5 * IQR)$ , where IQR is the interquartile range or absolute  
693 difference between 75th and 25th percentiles.

694  
695 **Figure 2.** Beta- and alpha-diversity of bacterial and fungal populations in samples across  
696 locations and sampling times. **(A, C)** Beta-diversity (PCoA ordination) of bacterial and fungal  
697 populations. **(B, D)** Alpha-diversity (OTU Richness and Shannon ( $H'$ ) index) of bacterial and  
698 fungal populations. T0 - before planting; T1 - one month after planting; T2 - two months after  
699 planting. Boxplot limits are the same as defined in Figure 1.

700  
701 **Figure 3.** Local network properties across locations and sampling times. **(A, B)** Local network  
702 properties of bacterial and fungal populations in samples from the three locations (Grant, Sutton  
703 and White Pigeon) at three sampling times (T0 - before planting; T1 - one month after planting;

704 T2 - two months after planting). **(C)** Significant changes from T0 to T1 and from T0 to T2 in  
705 treated vs. untreated blocks.

706

707 **Figure 4.** Random Forest yield model fitted to predict blocks with yields of  $\leq 30\text{t/ha}$  or  $> 30\text{t/ha}$   
708 based on soil microbiome composition and structure data. **(A)** Confusion matrix for the Random  
709 Forest model over the test set samples. **(B)** Importance figures of the main variables contributing  
710 to the predictive power of the Random Forest model.

711

## 712 **Supplementary Figures Legends**

713

714 **Figure S1.** Taxonomic composition of soil samples across locations and sampling times. (A)  
715 Most abundant bacterial genera identified. (B) Most abundant fungal genera identified. T0 -  
716 before planting; T1 - one month after planting; T2 - two months after planting; T3 – after  
717 harvest.

718

719 **Figure S2.** Taxonomic assignment and their relationship with yield (fold change values) of the  
720 OTUs contributing to the ten most important principal components of the beta-diversity  
721 ordination generated for the yield predictive model.

722

## 723 **Supplementary Table Legends**

724

725 **Table S1.** Full PERMANOVA for bacterial and fungal beta-diversity.

726

727 **Table S2.** Wilcoxon rank-sum test for bacterial and fungal alpha-diversity of control vs. treated



728 samples.

729

730 **Table S3.** Wilcoxon rank-sum test for bacterial and fungal alpha-diversity of T0 vs. T3 samples.

731

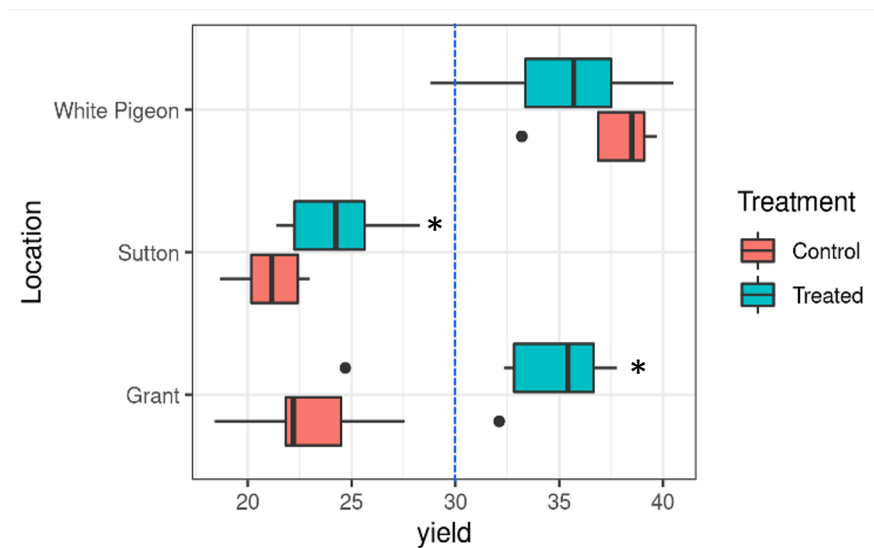
732 **Table S4.** Significant differential abundance of bacterial and fungal OTUs of control vs. treated  
733 samples at T1 vs. T0 and T2 vs. T0.

734

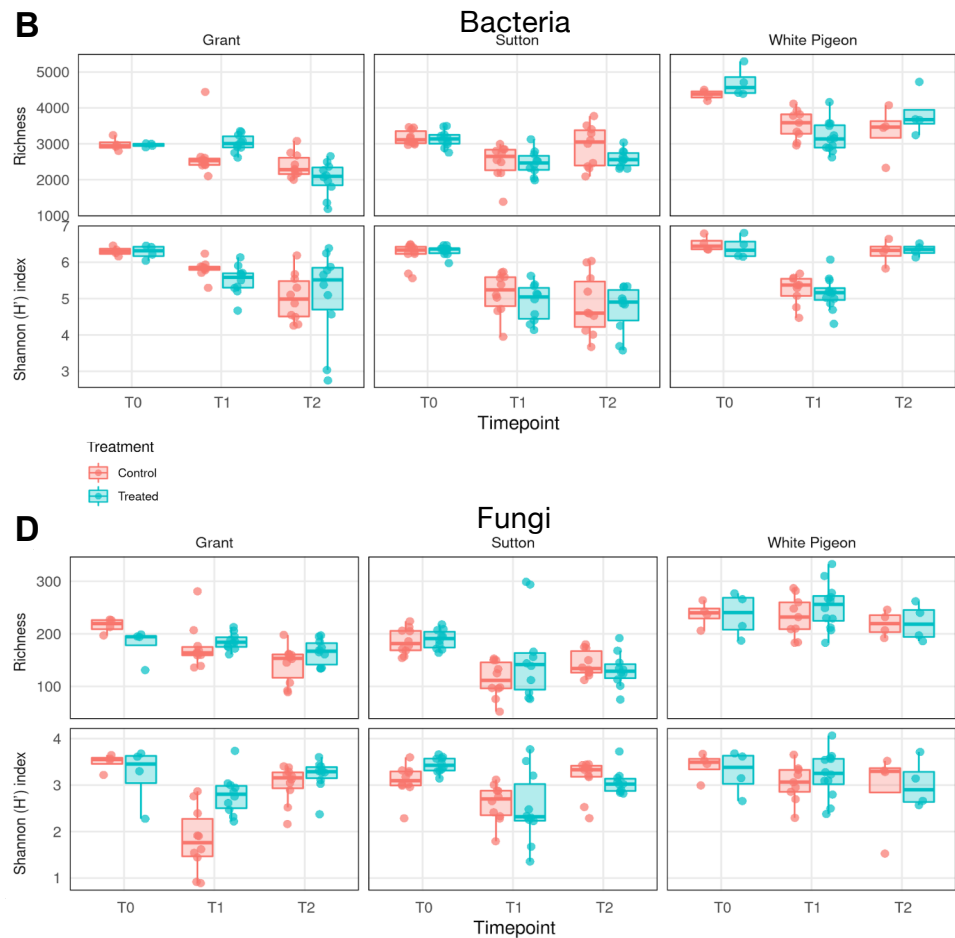
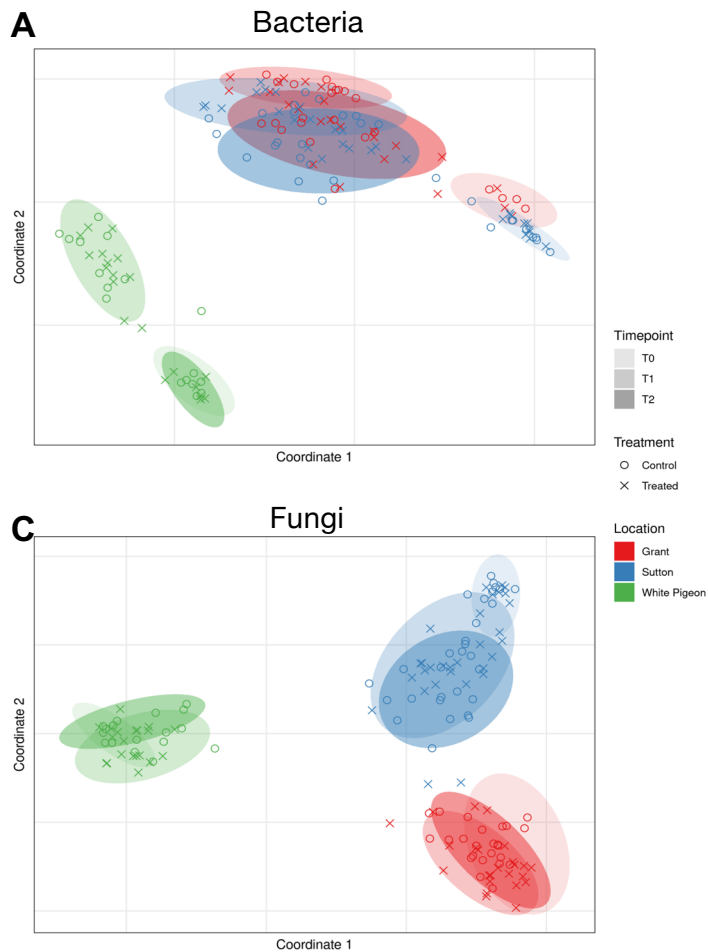
735 **Table S5.** Network property changes of bacterial and fungal communities of control vs. treated  
736 samples at T1 vs. T0 and T2 vs. T0.

737

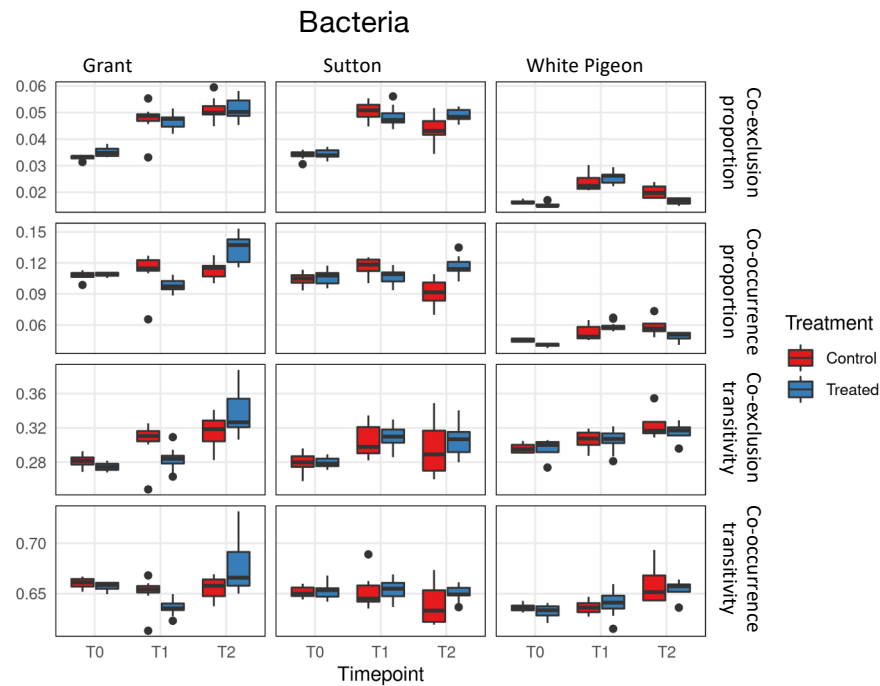
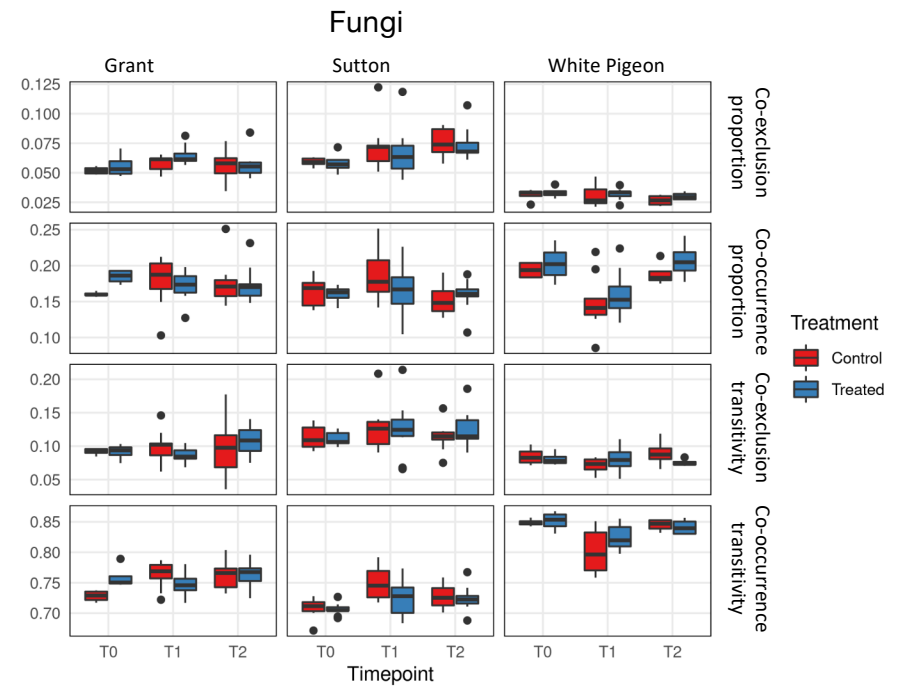
738 **Table S6.** Importance of variables in Random Forest yield predictive models.



**Figure 1.** Yield data (t/ha) for control and treated blocks across locations. Discontinued line separates blocks into two categorical variables ( $\leq 30$ t/ha,  $> 30$ t/ha), and corresponds to one of the natural zero probability density points in the bimodal yield distribution. The box limits correspond to the 25th and 75th percentile, and the central line is the median. The whiskers are the 5th and 95th percentile. The dots represent outliers (points below 25th percentile -  $(1.5 * IQR)$  and above 75th percentile +  $(1.5 * IQR)$ , where IQR is the interquartile range or absolute difference between 75th and 25th percentiles).



**Figure 2.** Beta- and alpha-diversity of bacterial and fungal populations in samples across locations and sampling times. **(A, C)** Beta-diversity (PCoA ordination) of bacterial and fungal populations. **(B, D)** Alpha-diversity (OTU Richness and Shannon ( $H'$ ) index) of bacterial and fungal populations. T0 - before planting; T1 - one month after planting; T2 - two months after planting. Boxplot limits are the same as defined in Figure 1.

**A****B****C**

Network Property	Location	Comparison	Estimate	p-value
Fungal co-occurrence transitivity	Grant	T1-T0	-0.991	0.007
Bacterial co-exclusion modularity	White Pigeon	T1-T0	2.200	0.008
Bacterial co-occurrence proportion	Grant	T1-T0	-0.544	0.049
Bacterial co-occurrence proportion	Sutton	T2-T0	0.808	$1 \times 10^{-4}$
Bacterial co-occurrence proportion	Grant	T2-T0	0.690	0.013
Bacterial co-exclusion proportion	Sutton	T2-T0	0.437	0.014
Bacterial co-occurrence transitivity	Grant	T2-T0	1.436	0.038

**Figure 3.** Local network properties across locations and sampling times. **(A, B)** Local network properties of bacterial and fungal populations in samples from the three locations (Grant, Sutton and White Pigeon) at three sampling times (T0 - before planting; T1 - one month after planting; T2 - two months after planting). **(C)** Significant changes from T0 to T1 and from T0 to T2 in treated vs. untreated blocks.

**A**

		Actual	
		≤30t/ha	>30t/ha
Predicted	≤30t/ha	16	6
	>30t/ha	0	6

**B**

Variable	Importance
Fungal co-occurrence transitivity	5.120
PC3	3.410
Fungal co-exclusion proportion	3.070
PC1	1.451
Variety + Location	1.221

**Figure 4.** Random Forest yield model fitted to predict blocks with yields of  $\leq 30\text{t/ha}$  or  $> 30\text{t/ha}$  based on soil microbiome composition and structure data. **(A)** Confusion matrix for the Random Forest model over the test set samples. **(B)** Importance figures of the main variables contributing to the predictive power of the Random Forest model.