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1 **Seed tuber imprinting shapes the next-generation potato microbiome**

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7 # Abstract

8 Potato seed tubers are colonized and inhabited by soil-borne microbes, some of which can positively
9 or negatively impact the performance of the emerging daughter plant in the next season. In this
10 study, we investigated the intergenerational inheritance of microbiota from seed tubers to next-
11 season daughter plants by amplicon sequencing of bacterial and fungal microbiota associated with
12 tubers and roots of two seed potato genotypes produced in six different fields. We observed that
13 field of production and potato genotype significantly affected the seed tuber microbiome
14 composition and that these differences persisted during winter storage of the seed tubers. When
15 seed tubers from different production fields were planted in a single trial field, the microbiomes of
16 daughter tubers and roots of the emerging plants could still be distinguished according to the field of
17 origin of the seed tuber. Remarkably, we found little evidence of direct vertical inheritance of field-
18 unique microbes from the seed tuber to the daughter tubers or roots. Hence, we hypothesize that
19 this intergenerational "memory" is imprinted in the seed tuber, resulting in differential microbiome
20 assembly strategies depending on the field of production of the seed tuber.

21 # Introduction

22 The microbial community associated with a plant, referred to as the plant microbiome, can
23 significantly influence plant performance. The complex plant microbiome includes microbes that are
24 plant pathogens but also plant beneficial microbes that support plant growth by mobilizing scarce
25 nutrients from the soil or protect the plant against pathogens [1, 2, 3]. The plant microbiome
26 significantly expands the genomic potential of its host and is often referred to as the host's "second
27 genome" [1, 2, 4, 5, 6, 7].
28
29 Potato is the 3rd most important crop for human consumption, with an annual global harvest of
30 approximately 375 million tons. Additionally, it is a key crop that is essential for global food security
31 and a source of raw materials for industry (www.fao.org, 2017). Potato is a space-efficient crop,
32 yielding five times more consumable weight per hectare than rice and wheat. As global demand for
33 potato increases, the UN-FAO identified it as a crop with great potential to become a game changer
34 for global food security [8, 9].
35
36 Potatoes are commonly propagated vegetatively by transplanting seed tubers from one field to the
37 next [10]. As potato tubers develop underground, they closely interact with the dense and diverse
38 microbial communities in soil [11]. Studies demonstrated that the potato tuber microbiome can have a
39 profound impact on plant health and productivity [12, 13]. Potato is sensitive to a wide range of plant
40 pathogens [14, 15], but it also hosts beneficial microbes that can promote plant growth [12, 13, 16, 17, 18].
41
42 A batch of seed potatoes is of high vitality if it manifests in a large canopy and exhibits homogeneous
43 growth in the early stages of its development. Seed tubers of the same potato genotype that were
44 produced in different production fields can display significant differences in their vitality, resulting in
45 differences in growth of the emerging potato plants [19, 20, 21]. This may be caused by local

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46 environmental factors in the fields of production that confer changes in tuber physiology, but also
47 the seed tuber microbiome likely impacts the vitality of the outgrowing potato crop. Many potato
48 pathogens can be seed tuber-borne [15, 22]. Field experiments in which seed tubers were treated with
49 beneficial bacteria show that the applied microbes colonize the roots of plants that develop from the
50 treated tubers [23, 24]. Such findings suggest that seed tubers can be an important inoculum source of
51 microbes for the potato plants that emerge from them and that potato plants may inherit at least
52 part of their microbiome from the seed tuber. However, there is limited information available on
53 tuber-borne transmission of microbes from one potato generation to the next. To gain insight into
54 intergenerational inheritance of the potato microbiome, we investigated whether the field of
55 production of potato seed tubers has an impact on the microbiomes of tubers and roots of plants
56 emerging from these seed tubers when planted together in a single trial field.

57
Results

58
Effect of potato genotype, production field, and storage on the tuber microbiome

59 In the autumn of 2018, seed tubers of two potato varieties, *Colomba* (hereafter Variety A) and
60 *Innovator* (hereafter Variety B), were harvested from 3 fields of production for Variety A and 3
61 different fields for Variety B (Fig. 1a; Fig. S1a-b). To investigate the influence of plant genotype and
62 field of production on the tuber-associated microbiome, we isolated microbial DNA from 4 replicate
63 samples per field, each replicate containing peels of 6 tubers. Subsequently, we sequenced 16S rRNA
64 gene and ITS amplicons to profile the bacterial and fungal communities, respectively. Principal
65 coordinates analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA),
66 revealed that both the bacterial and the fungal microbiome on the tuber is determined primarily by
67 the field in which the potato was produced (Fig. 1b-d; Fig. S2a-c; Table S1; Table S2). The production
68 field significantly ($P = 0.001$) affected the tuber peel microbiome and accounted for up to 64% of the
69 variation in the bacterial community ($R^2 = 0.64$, Table S1) and 55% of the variation in the fungal
70 community ($R^2 = 0.55$, Table S2). In addition, the potato variety significantly ($P = 0.001$) affected
71 tuber microbiome composition, explaining 18% ($R^2 = 0.18$) and 17% ($R^2 = 0.17$) of the variation in
72 bacterial and fungal community composition, respectively (Fig. 1b-d; Fig. S2a-c; Table S1; Table S2).

73 It is common agricultural practice to store seed tubers over the winter prior to planting in spring. To
74 study the effects of cold storage on tuber microbiomes, the above-mentioned seed tubers had
75 remained in cold storage at 4°C in the dark for 7 months (Fig. 1a). These so-called post-storage seed
76 tubers were then processed in the same manner as the seed tuber samples, after which the bacterial
77 and fungal microbial communities were profiled by amplicon sequencing. Although there were
78 significant changes in the composition of the bacterial ($P = 0.001$) and fungal ($P = 0.019$) microbiome
79 before and after storage of the tubers (Fig. S3a and c, Table S3), post-storage seed tubers clustered
80 closely with those of the pre-storage seed tubers from the same field of production (Fig. S3b and d).
81 Notably, tubers from different fields of production maintained their distinct microbial community
82 patterns even after 7 months of cold storage (Fig. 1e-g; Fig. S2d-f; Table S1; Table S2). On post-
83 storage seed tubers, the production field accounted for up to 57% of the variation in the bacterial
84 community ($R^2 = 0.57$, Table S1) and 46% of the variation in the fungal community ($R^2 = 0.46$, Table
85 S2).

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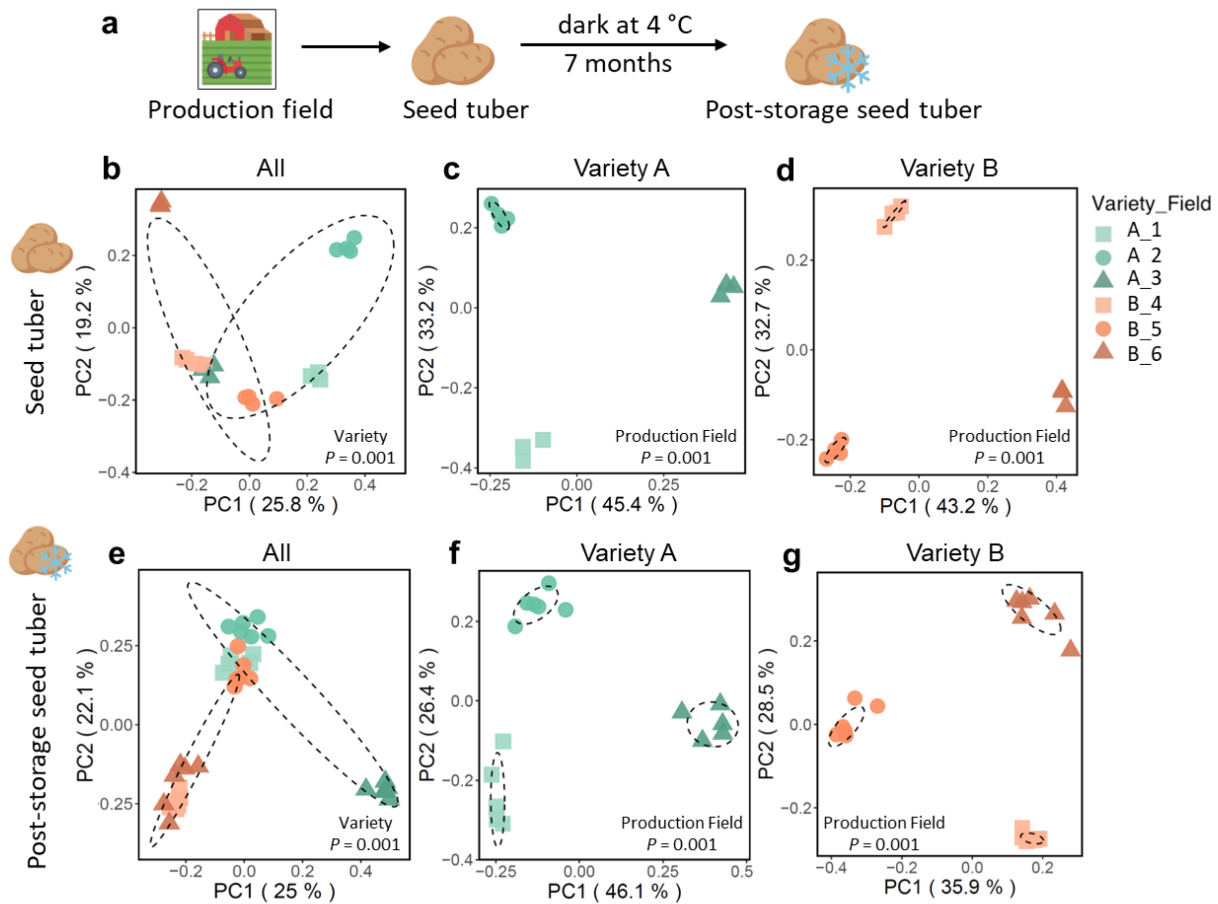

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Fig. 1 Bacterial community composition of seed tuber and post-storage seed tuber samples. a Graphic representation of the sampling strategy. Seed tubers of two potato varieties (A and B) were harvested from 3 fields of production per variety and sampled for microbiome analysis before and after a 7-month cold storage period. Principle component analysis (PCoA) of 16S amplicon sequencing data representing bacterial communities on **b)** seed tubers of Variety A and B, **c)** seed tubers of Variety A only, or **d)** seed tubers of Variety B only, **e)** post-storage seed tubers of Variety A and B, **f)** post-storage seed tubers of Variety A only, and **g)** post-storage seed tubers of Variety B only. Each symbol represents the bacterial community of one replicate potato peel sample. Each sample consists of a pool of potato peels collected from 6 seed tubers. For each variety, 4 replicate of seed tuber samples and 6 replicate of post-storage seed tuber samples were collected from each of the 3 fields of production. Green symbols represent Variety A and orange symbols represent Variety B. Different shapes within a same color represent distinct production fields. The *P* from PERMANOVA is shown in each PCoA plot. Each ellipse represents a 68% confidence region and depicts the spread of data points within each group.

87

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88 **Seed tubers, roots of emerging plants, and daughter tubers harbor distinct microbiomes**

89 Seed tubers of Variety A and B of the above-mentioned 6 production fields were subsequently
90 planted in a single trial field near Veenklooster, the Netherlands, in the spring of 2019 (Fig. S1a). The
91 emerging plants from these seed tubers were cultivated for three months after which roots and
92 daughter tubers were harvested (Fig. 2a). The microbiome composition of these potato samples was
93 analyzed by sequencing both 16S rRNA gene and ITS amplicons. Using PCoA, we observed that the
94 bacterial community composition of both roots and tubers harvested in 2019 from the trial field
95 clearly separated ($P = 0.001$) from the seed tuber samples harvested from the production fields in
96 2018 (Fig. 2b; Table S4). In addition, the bacterial communities found on the roots are distinct from
97 those on daughter tubers, indicating that these two belowground potato organs harbor distinct
98 bacterial microbiomes within one field ($P = 0.001$, Fig. 2b; Table S4). A similar separation was
99 observed for the fungal communities on seed tubers, roots, and daughter tubers (Fig. S4a, Table S4).

100 We then focused on shared bacterial amplicon sequence variants (ASVs) between the microbiomes
101 of seed tuber, daughter tuber, and root samples (Fig. 2c). A total of 3986, 9205, and 11622 unique
102 bacterial ASVs were detected in seed tuber, daughter tuber, and root samples, respectively.
103 Whereas 86% ((6830+1050)/9205) of the bacterial ASVs on the daughter tubers were shared with
104 those on roots of the potato plants in the same trial field, only 13% ((1050+156)/9205) and 12%
105 ((1050+393)/11622) of the ASVs on the daughter tubers and roots, respectively, were also detected
106 on the seed tuber (Fig. 2c). Analysis of the fungal microbial communities showed similar results with
107 84% ((758+182)/1117) of the fungal ASVs from daughter tubers shared with those on roots, while
108 only 18% ((182+22)/1117) and 16% ((182+37)/1405) of the ASVs detected on the daughter tubers
109 and roots, respectively, were also detected on the seed tubers (Fig. S4b). This suggests that the
110 majority of microbes on potato daughter tubers and roots are not inherited from the seed tubers
111 but originate from the trial field.

112 The most abundant bacterial phyla in the microbiomes of all tuber and root samples were the
113 *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes*. Whereas *Bacteroidetes* were relatively
114 abundant in samples from plants in the trial field, *Firmicutes* had relatively low abundance in
115 samples from this field, especially on the daughter tubers. On those daughter tuber samples
116 *Bacteroidetes* were relatively more abundant, whereas *Actinobacteria*, *Firmicutes*, and
117 *Planctomycetes* had higher relative abundance on the roots of the potato plants in the same field
118 (Fig. 2d).

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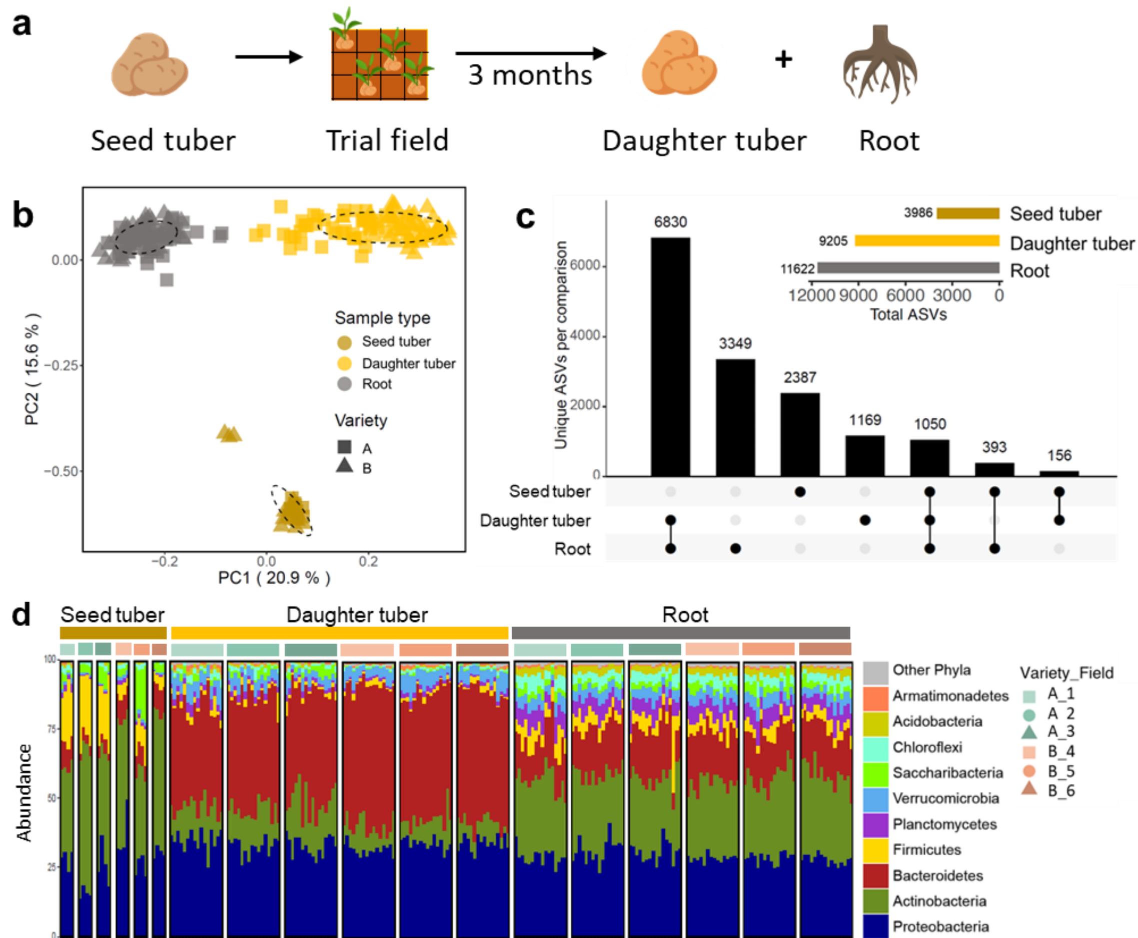

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Fig. 2 Analysis of bacterial communities on seed tubers from different production fields and their roots and daughter tubers the Veenklooster trial field. a Graphic representation of the experimental design. Seed tubers from 3 different production fields of each variety (*n* = 2) were planted together in a single trial field in Veenklooster (Fig. S1). For seed tubers from each production field, 4 replicate plots were randomly distributed across this trial field. Roots and daughter tubers from the emerging plants were harvested for microbiome analysis. **b** PCoA of potato-associated bacterial communities of seed tubers, daughter tubers, and roots. Square symbols represent Variety A and triangle symbols represent Variety B. Colors represent different sample types. Each ellipse represents a 68% confidence region and depicts the spread of data points within each group. **c** UpSet plot showing the number of bacterial ASVs that are shared between or are unique for seed tubers, daughter tubers and roots of both varieties combined. **d** Stacked bar chart of the taxonomic composition of bacterial communities of different sample types aggregated at the phylum level. Each stacked column represents an independent sample (n = 216). Different colors within a column represent different phyla. Only the top 10 most-abundant phyla were colored individually, all the rest are colored in gray and listed as "Other phyla". Samples are clustered by sample type and production field, which is shown by the colored bar on top of the stacked bar chart.

120

121 **Origin of seed tubers affects the root and tuber microbiomes of emerging plants**

122 Within the trial field, the bacterial and fungal microbial communities of both potato roots and
123 daughter tubers were significantly (P = 0.001) affected by potato genotype (Fig. 3a-b, Fig. S2g and j,
124 Table S1, Table S2). The effect size of potato genotype was larger for the tuber samples (R^2 = 0.08)
125 than for the root samples (R^2 = 0.03). Interestingly, also the field of production of the seed tubers
126 had a significant effect on microbiome composition of daughter tubers (P = 0.001, R^2 = 0.07) and

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127 roots (P = 0.001, R^2 = 0.08) of the plants emerging from these seed tubers (Fig. 3c-f, Fig S2g-l, Table
128 S1, Table S2). Thus, the impact of the production field stretches across a generation and influences
129 microbiome assembly on the roots and tubers of the daughter plants emerging from the seed tubers
130 in the subsequent growing season.

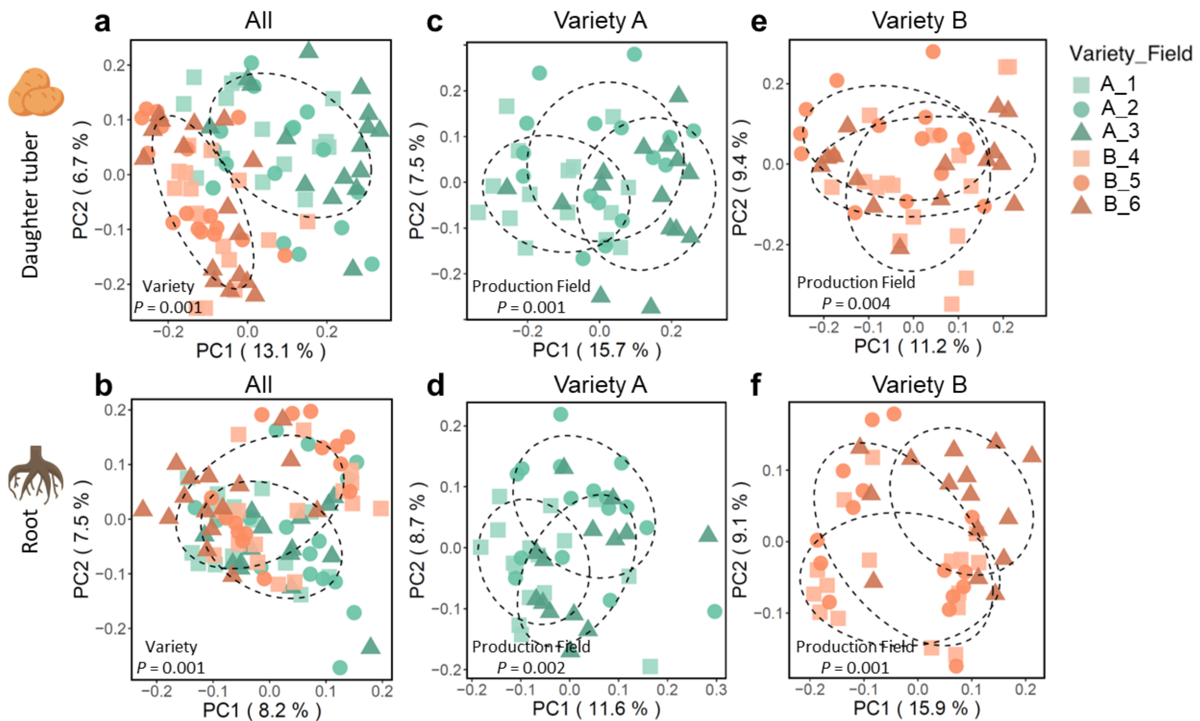

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Fig. 3 Bacterial community composition of daughter tubers and roots. PCoA of 16S rRNA amplicon sequencing data of **a)** daughter tubers of Variety A and B, **b)** roots of Variety A and B, **c)** daughter tubers of Variety A only, or **d)** roots of Variety A only, **e)** daughter tubers of Variety B only and **f)** roots of Variety B only. Each symbol represents the bacterial community of one replicate potato peel sample. Each daughter tuber sample consisted of a pool of potato peels collected from 6 daughter tubers of one plant. Each root sample is a subset of the whole root of the same plant from which the daughter tubers were sampled. For each variety, 4 replicate samples were collected from each of the 4 randomly distributed replicate plots. Green symbols represent Variety A and orange symbols represent Variety B. Different shapes within a same color represent different production fields. The P from PERMANOVA is shown in each PCoA plot. Each ellipse represents a 68% confidence region and depicts the spread of data points within each group.

132

133 **Inheritance of field-unique ASVs in daughter tubers and roots**

134 We hypothesized that the intergenerational influence of the seed tuber production field on the
135 microbiome of roots and daughter tubers is the result of vertical, seed tuber-mediated transmission
136 of field-unique microbes from one generation of potatoes to the next. To be able to track the
137 vertical inheritance of field-unique microbes from seed tubers to the emerging plants, we focused
138 on Variety A seed tubers from Field 1 and identified bacterial and fungal ASVs that were uniquely
139 detected in seed tuber samples from Field 1. We observed that 50.6% of the bacterial ASVs on seed
140 tubers from Field 1 were not detected on seed tubers from Field 2 and 3 and defined these 952 ASVs
141 as Field-1-unique on seed tubers (Fig. S5a). With the same definition, we identified 1451 bacterial
142 ASVs (29.7% of total daughter tuber ASVs) as Field-1-unique on daughter tubers that originate from
143 Field-1 seed tubers and 1244 bacterial ASVs (20.2% of total root ASVs) as Field-1-unique on roots
144 that originate from Field-1 seed tubers (Fig. S5c-d). An additional 54, 132 and 137 fungal ASVs were
145 defined as Field-1-unique on seed tubers, daughter tubers, and roots, respectively (Fig. S5e-h).

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146 We subsequently investigated whether the Field-1-unique ASVs were transmitted to the roots and
147 daughter tubers of the plants emerging from these Field-1 seed tubers. To our surprise, the results
148 did not support our original hypothesis, and instead, we found only a very small overlap between
149 Field-1-unique ASVs of seed tubers and daughter tubers and roots derived from these Field-1 seed
150 tubers (Fig. 4a-b and d-e, Fig. S6a-b and d-e). Namely, only 24 bacterial and 3 fungal Field-1-unique
151 ASVs were shared between seed tubers and the emerging daughter tubers. Similarly, only 26
152 bacterial and 1 fungal Field-1-unique ASVs were shared between seed tubers and the roots of
153 emerging plants (Fig. 4a-b, Fig. S6a-b). Moreover, these ASVs were lowly abundant in daughter tuber
154 (Bacteria: 0.1%, fungi: 0.3%) and root (Bacteria: 0.05%, fungi: 0.09%) microbial communities (Fig. 4g-
155 h, Fig. S6g-h). Thus, although we can distinguish ASVs unique to the field of production of the seed
156 tuber on the next season daughter tubers and roots, the large majority of the field-unique ASVs in
157 the daughter generation cannot be immediately traced back to the seed tuber.

158 When looking into the entire microbial community on seed tubers instead of only the field-unique
159 ones, we found that 83% (1556/1882, Fig. 4d) and 78% (1472/1882, Fig. 4e) of the seed tuber
160 bacterial ASVs were lost during vertical transmission to daughter tubers and roots, respectively.
161 Furthermore, 77.2% of the daughter tuber (Fig. 4g) and 74.5% (Fig. 4h) of the root bacterial
162 communities were acquired from the environment during the 3 months of growth in the trial field.
163 Around a quarter of daughter tuber (22.8%, Fig. 4g) and root (25.6%, Fig. 4h) microbial communities
164 were shared with those on the peel of the seed tuber. However, since these ASVs were not Field 1-
165 unique, it cannot be verified to what extent they are inherited from the seed tuber or simply
166 common in different fields. Similar results were observed for the fungal communities on the
167 daughter tubers and roots from Field 1 (Fig. S6). These results indicate that even though the field-
168 unique ASVs were rarely inherited cross generations, we did observe vertical inheritance for other
169 ASVs from seed tubers to daughter tubers and roots. However, the majority of the microbial
170 population in daughter tubers and roots were acquired from the environment where they were
171 formed.

172 To investigate whether cold storage would already lead to the depletion of the above defined field-
173 unique seed tuber microbes pre-planting, we examined the occurrence of ASVs on the post-storage
174 seed tubers from Field 1. These post-storage seed tubers were stored under cold and dark condition
175 much longer than common practice, thus used as an extreme case to study the influence of storage
176 on field-unique seed tuber microbes. We found that 66% (1051/1593) of the total bacterial ASVs
177 detected on the post-storage seed tubers were also detected on the pre-storage seed tubers from
178 the same field (Fig. 4c and f) and that these ASVs represent 91.8% of the bacterial community (Fig.
179 4i). These results indicated that the large majority of the seed tuber bacterial community persists
180 during cold storage. Moreover, a large part of the field-unique ASVs were maintained over the
181 storage period (Fig. 4f and i, “Unique-Unique”). Similar results were observed for fungal
182 communities on the seed tuber and post-storage seed tubers from Field 1 (Fig. S6f and i).

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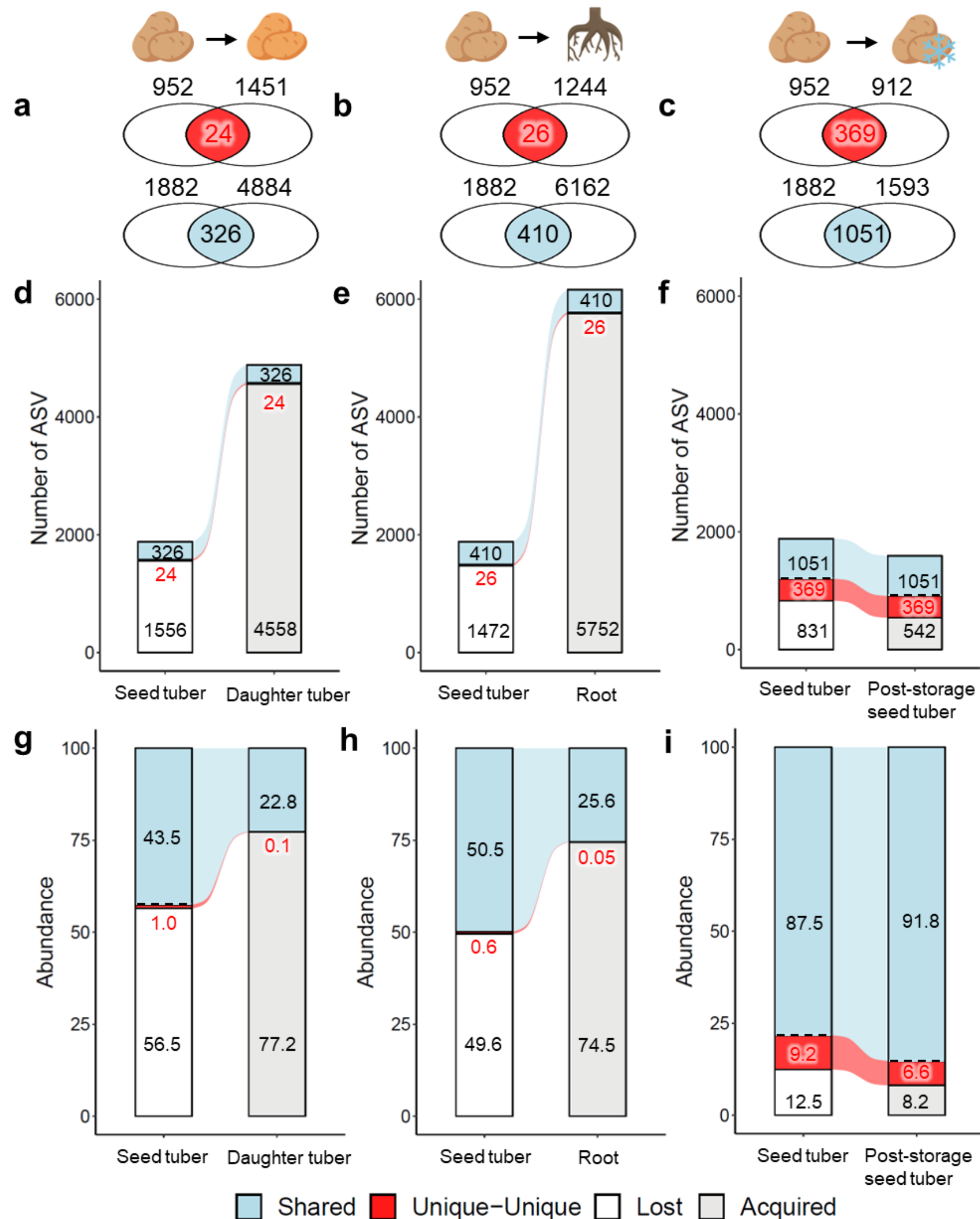

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Fig. 4 Comparison of bacterial ASVs on daughter tubers, roots, post-storage seed tubers and seed tubers of Variety A originating from Field 1. Venn diagrams showing the overlap between **a)** seed tubers and daughter tubers, **b)** seed tubers and roots, **c)** seed tubers and post-storage seed tubers of Field-1-unique bacterial ASVs (in red) or all bacterial ASVs (in blue). Sankey diagram of bacterial ASVs transferred from seed tubers to **d, g)** daughter tubers and **e, h)** roots that emerged from the seed tubers; and **f, i)** post-storage seed tubers. "Shared" in blue represents ASVs detected on both sample types. "Unique-Unique" in red represents the overlap of Field-1-unique ASVs on both sample types. The "Unique-Unique" in red is included in the "Shared" in blue. "Lost" in white represents ASVs lost from the seed tuber during vertical transmission. "Acquired" in light grey represents ASVs not transmitted from seed tubers but acquired from the environment. In **a-f),** numbers in the bars indicate the number of ASVs in each category mentioned above. In **g-i),** numbers in the bars indicate the accumulative relative abundance of ASVs in each category mentioned above.

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185 **Tracking the microbial transmission from different seed tuber compartments to sprouts**

186 Even though the microbiomes on daughter tubers and roots of next-season potato plants could be
187 distinguished based on the field of production of the seed tuber, we found little evidence for direct
188 vertical transmission of microbes from the peel of the seed tuber to the peel of the tubers or roots
189 on the daughter plants. This could mean that: 1) potato daughter plants do not inherit their
190 microbiome from the peel but other compartments of the seed tuber; or 2) vertical transmission is
191 apparent only during early stages of plant development after which transmitted microbes are
192 replaced by members from the trial field resident microbiome. To gain further insight into the
193 potential of vertical microbiome transmission from seed tubers to next-generation daughter plants,
194 we investigated the contribution of different seed tuber compartments (namely peel, eye, heel end,
195 flesh, and adhering soil, Fig. S1c) in shaping the potato sprout microbiome. We made use of material
196 from a parallel study in which we harvested tubers from 6 potato varieties produced in 25 distinct
197 fields of production (Variety A from 5, Variety B from 5, *Festien* (Variety C) from 3, *Challenger*
198 (Variety D) from 5, *Sagitta* (Variety E) from 5, and *Seresta* (Variety F) from 2 fields, respectively; Fig.
199 S1b). Samples from 50 seed tubers were pooled into a single sample per compartment per field and
200 thus a total of 1250 (50 x 25) tubers were sampled from these 25 fields. DNA was isolated and
201 bacterial and fungal microbiome composition was determined through 16S rRNA gene and ITS
202 amplicon sequencing.

203 Again we found that potato genotype significantly influenced the composition of bacterial and
204 fungal communities in the distinct seed tuber compartments (Fig. 5a, Fig. S7a, Table S1, Table S2).
205 Moreover, we found that each distinct tuber compartment harbored a bacterial community that is
206 significantly different ($P < 0.001$) from the communities in the other compartments (Fig. 5b-c, Table
207 S5), with the exception of the pairwise comparisons between eye and peel ($P = 0.143$) and between
208 eye and heel end ($P = 0.061$). The richness of the bacterial communities decreased from the outside
209 of the potato to the inside, with highest diversity in the potato-adhering soil and increasingly lower
210 diversity in respectively the potato peel, heel end, eye, and flesh compartments (Fig. S8a). At phylum
211 level, *Bacteroidetes* and *Proteobacteria* have a higher relative abundance in the heel end
212 compartments compared to the other 4 tuber compartments (Fig. 5c).

213 Similar to the bacterial communities, fungal communities found in distinct compartments were
214 significantly different from each other ($P < 0.001$, Fig. S7b, Table S6), except for the eye and peel
215 compartments which harbored nearly identical fungal communities ($P = 0.83$, Table S6). The highest
216 richness for fungal communities was observed in adhering soil samples; however, diversity did not
217 differ significantly between the other compartments (Fig. S8b). At family level, *Cladosporiaceae* was
218 most abundant in the adhering soil, whereas *Plectosphaerellaceae* was relatively more abundant in
219 the heel ends (Fig. S7c).

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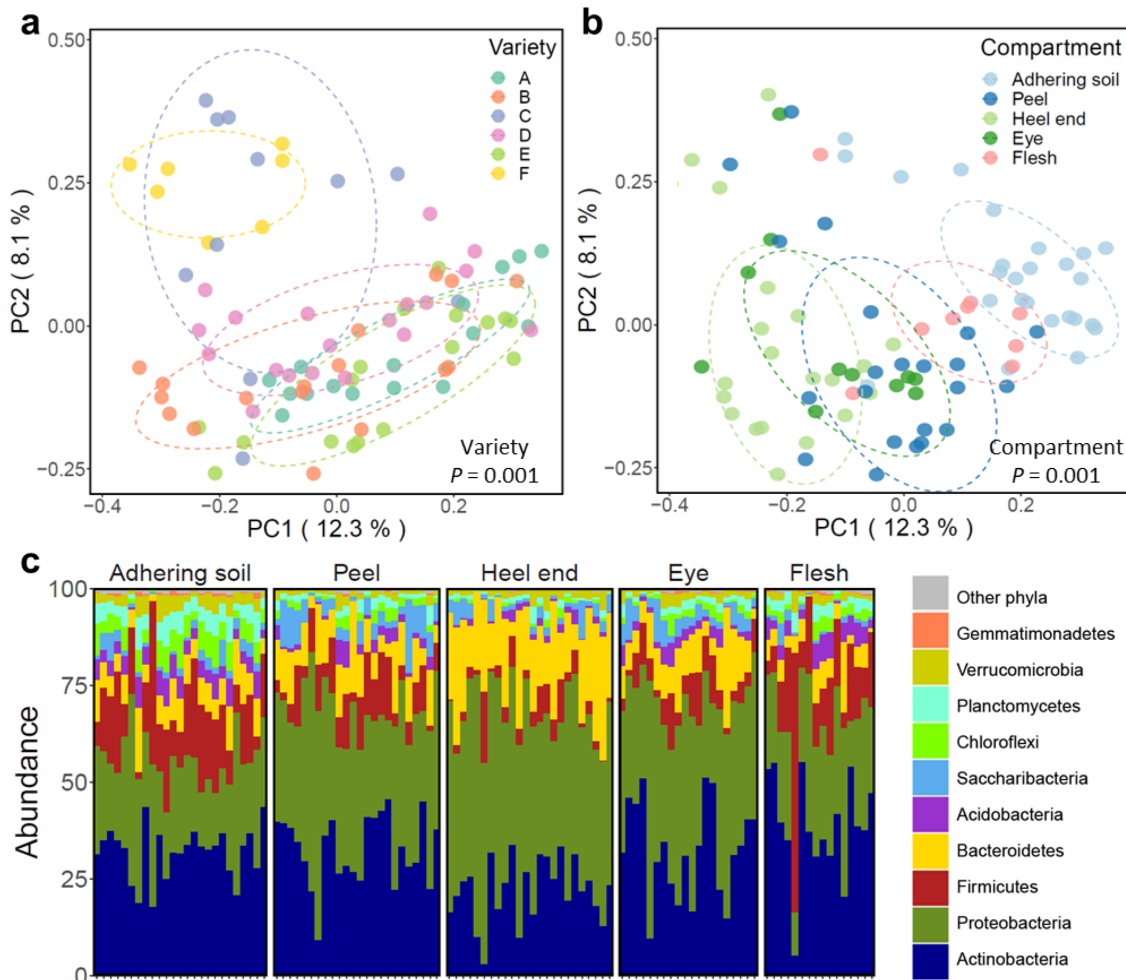

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Fig. 5 Distinct compartments on the potato tuber harbor distinct microbial communities. PCoA of the potato tuber-associated bacterial community based on 16S rRNA gene amplicon sequencing and colored by **a**) potato varieties (A, B, C, D, E and F, respectively) and **b**) potato tuber compartments (adhering soil, peel, heel end, eye and flesh, respectively). *P* as determined by PERMANOVA is shown in each PCoA plot. Each ellipse represents a 68% confidence region and depicts the spread of data points within each group. **c** Bar plot showing the phylogenetic composition of the bacterial community. Only the top 10 most abundant phyla are colored individually, the other phyla are shown together in grey. Each sample was isolated from the pooled compartments from 50 seed tubers per field.

221

222 The spout is the first daughter tissue to emerge from the seed potato, and thus the most likely tissue
223 for vertical transmission of microbiota. To investigate vertical transmission of microbes from the
224 seed tuber to the emerging plant, seed tubers of all 6 varieties and from 2 fields per variety sprouted
225 on Petri dishes for 7 days. Subsequently, we isolated microbial DNA of sprouts of 5 replicate tubers
226 per field and analyzed microbiome composition of the samples through 16S rRNA gene and ITS
227 amplicon sequencing. The bacterial community composition of sprouts was significantly ($P < 0.001$)
228 different from those of all five distinguished compartments of the seed tuber (Table S7). At phylum
229 level, the bacterial community of the sprout was dominated by *Actinobacteria*, which were detected
230 at a relative abundance of 72% of the total community, whereas *Firmicutes* (15%) and
231 *Proteobacteria* (11%; Fig. S9) were also abundantly detected on sprouts. Also on sprouts, our
232 analysis revealed a significant impact of plant genotype on microbial community composition (P =
233 0.001; Fig. 6a). Interestingly, 4 of the 6 varieties of sprouts emerging from seed tubers originating

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234 from different production fields had distinct microbiomes (Fig. 6b-g). These results indicate that the
235 sprout-associated microbiome is influenced by plant genotype, but also by the field of production of
236 the seed tuber.

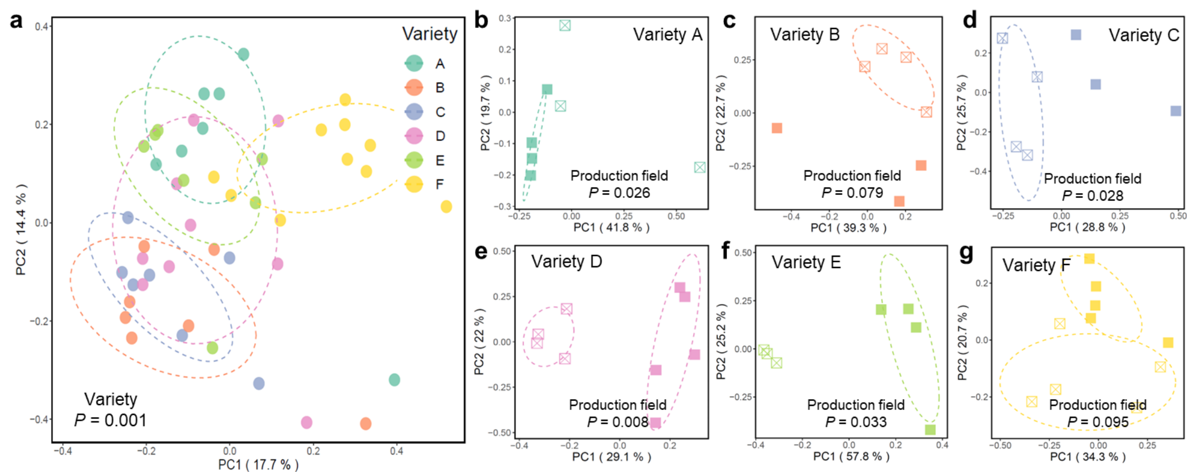

Fig. 6 Field of production of the seed tuber affects the sprout microbiome. PCoA of bacterial sprout microbiomes of **a)** all varieties together and **b-e)** each variety separately. Each color represents one variety. Open and closed symbols represent distinct seed tuber production fields. The *P* from PERMANOVA is shown in each PCoA plot. Each sprout sample is a pool of 3-4 sprouts from one single tuber. Each ellipse represents a 68% confidence region and depicts the spread of data points within each group.

237 We next compared the microbiomes of the sprouts to the distinct compartments on the seed tubers
238 that were analyzed above to identify the sources for the sprout microbiome. For bacteria, the
239 analysis revealed that 79% (177 of 223) of the ASVs detected in the sprout microbiome were also
240 detected in the microbiomes of at least one of the five seed tuber compartments (Fig. 7a). Thirty-
241 one percent of these ASVs (70 of 223) were present in all compartments, but these 70 ASVs
242 represented on average 60% of the total abundance of the sprout microbiome. Concomitantly, the
243 46 sprout-unique ASVs only made up 1.2% of the total bacterial abundance on the sprout (Fig. 7a).
244 Thus, with 98.8% of the total bacterial abundance on the sprout, the seed tuber was the main source
245 of the sprout microbiome in this soil-free system. Nonetheless, the taxonomic composition of the
246 sprout microbiome was distinct from the compartments on the seed tuber (Fig. S9), indicating that
247 the sprout compartment favors proliferation of a distinct subset of microbes that originate from the
248 seed tubers.

249 We further analyzed whether specific compartments on the seed tuber contribute differentially to
250 the sprout microbiome. Of the 223 bacterial ASVs detected on sprouts, 148 ASVs (66%) were also
251 detected in adhering soil, 124 in heel end (56%), 128 in peel (57%), 109 in eye (49%) and 103 in flesh
252 compartments (46%; Fig. 7b). We subsequently identified the top 18 most-abundant bacterial ASVs
253 (ASVs with relative abundances over 1%) in the sprouts that made up 80% of the total bacterial
254 sprout community and were able to trace them back in at least 2 of the 5 tuber compartments, but
255 with significantly lower abundances comparing within the sprouts (Fig. 7c). For fungi, 8 ASVs out of
256 the 74 ASVs that were detected in sprout samples were not found in any of the tuber compartments,
257 and the 8 ASVs represented only 2% of the sprout fungal community (Fig. S10a). On the other hand,
258 46% (34 of 74) of the sprout ASVs were present in all compartments and represent on average up to
259 65% of the total abundance of the sprout fungal community (Fig. S10b). Furthermore, the top 16
260 most-abundant fungal ASVs (ASVs with relative abundances over 1%) in the sprout totaled 95% of
261 the fungal sprout community (Fig. S10c). The relative abundance of these 16 fungal ASVs in the

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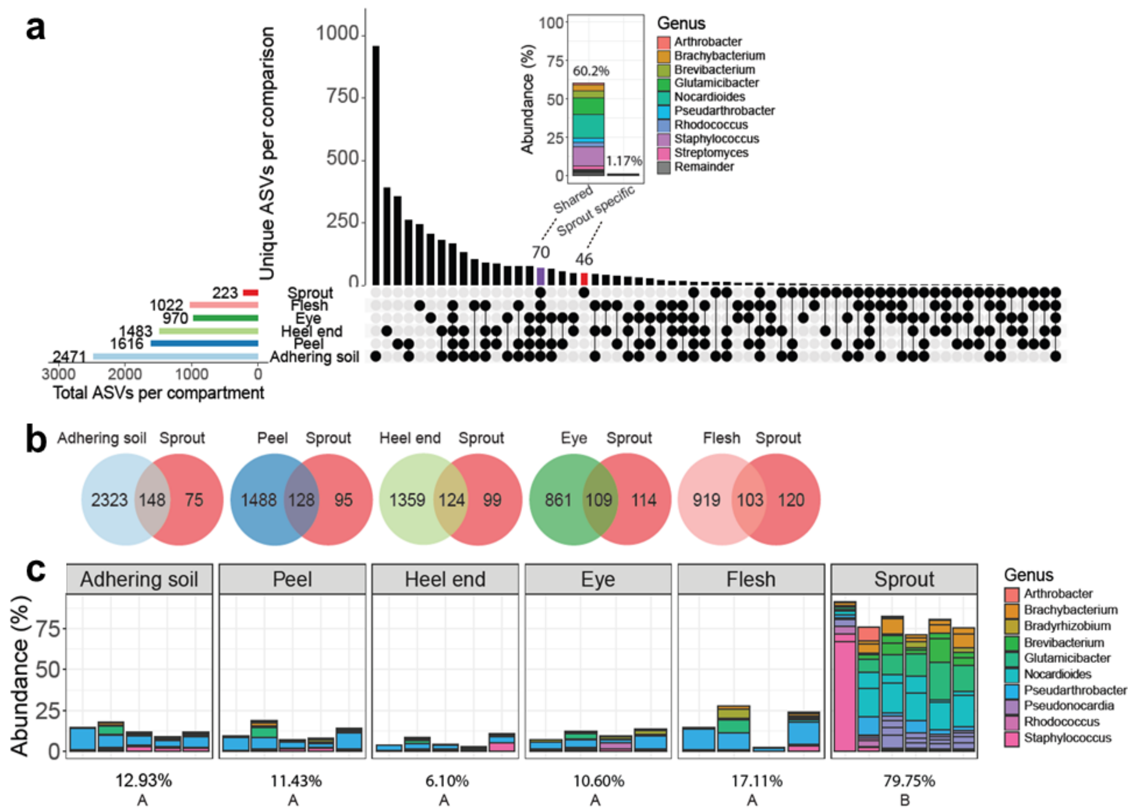
262 sprout did not differ significantly (ANOVA, Turkey, $P > 0.05$) between the distinct tuber
263 compartments (Fig. S10c). Together these data show that both bacteria and fungi on seed tubers
264 have the potential of being vertically transmitted to the sprouts, and that the sprout compartment
265 subsequently promotes proliferation of a select number of microbes that are relatively lowly
266 abundant in all compartments of the seed tubers.



267

Fig. 7 The sprout microbiome is derived from diverse seed tuber tissues. a UpSet plot shows shared and unique ASVs of each compartment of Variety A. Each row represents a sample type, and each column represents a set of ASVs, where filled-in black dots with an edge between the dots indicates that these ASVs are present in multiple sample types. The sets are ordered by the number of ASVs as indicated by the bar plot above each category. The total ASVs in each sample type is indicated by the rotated bar plot on the left. The inlay shows the abundance of ASVs (46) that are unique to sprouts and of sprout ASVs (70) that are shared with all tuber compartments. **b** Venn diagrams of ASVs shared between each tuber compartment and the sprout of Variety A. Color represents different compartment. **c** The distribution of the top 18 most-abundant sprout ASVs in all compartments of Variety A. Color represents the genus of the ASVs. The percentage under each figure shows the relative abundance of these top sprout ASVs in each compartment. Capital letters indicate significant difference ($P < 0.05$) in agglomerated abundance of the top sprout ASVs as determined by ANOVA with Tukey's post-hoc test.

268

269 ## Discussion

270 It has been well established that both soil type and plant genotype are important drivers in the
271 assembly of plant-root associated microbial communities [25]. However, seeds are also a source of
272 microbiota that can be transmitted to the plants that develop from them [26, 27]. Potatoes are
273 vegetatively propagated by transplantation of relatively large seed tubers that contain a complex
274 microbiome. Here we studied how the microbiome of seed potatoes is affected by the field of

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275 production and whether the seed tuber microbiome associated with production fields is transmitted
276 to the emerging potato plant in the next season.

277 First, we analyzed two important factors that likely determine potato tuber microbiome
278 composition. Soil has been reported to be the main source of microbes that colonize potato roots
279 and tubers [20, 28, 29]. In addition plant genotype is a factor that shapes plant-associated microbiomes
280 [30]. Plant roots actively and dynamically secrete root exudates that can selectively promote or deter
281 specific microbes [31, 32]. Although up to 85% of the total dry matter produced by the potato plant can
282 accumulate in the tubers [33], it is unclear whether the tuber actively exudes metabolites to interact
283 with the microbiome. In this light, it has been reported that the tuber surface is low in nutrients and
284 that the limited nutrients that are available to the microbiome are a result of cell decay or lesions
285 only [34]. Tubers might therefore control soil microbiota to a much smaller extent compared to roots.
286 In line with this, Buchholz, Antonielli [20] and Nahar, Floc'h [35] reported that the microbiome found on
287 potato tubers is largely independent from the potato genotype. Also, Weinert et al. show that tuber-
288 associated bacteria were not strongly affected by the plant genotype although a few cultivar-
289 dependent taxa were identified [36, 37].
290
291 In our study, however, when growing different genotypes in the same field we observed that not
292 only root, but also the tuber-associated bacterial and fungal communities were significantly affected
293 by the potato genotype (Fig. 3). Moreover, we found that the influence of potato genotype is larger
294 on the tuber microbiome than on the potato root microbiome (Fig. 3, Table S1). This suggests that
295 potato plants do exert control on the tuber microbiota, just like they selectively shape their root
296 microbiomes. Nonetheless, up to half of the bacterial ASVs found on seed tubers harvested from one
297 field were not found on seed tubers from the same variety that originated from other production
298 fields (Fig. 1, Fig. S5). Field of production determined more than half of the bacterial variation of the
299 seed tubers (Fig. 1, Table S1, S2). These results indicated that field of production is dominating over
300 genotype and is playing an even more vital role in tuber-associated microbiome assembly than
301 potato genotype, confirming previous findings [20, 29, 35].
302
303 Interestingly, we observed that both roots and daughter tubers in our trial field harbored
304 microbiomes that were distinguishable by the production field of their seed tuber. This implies that
305 there is intergenerational or vertical transmission of microbes from the seed tuber to the emerging
306 plant and subsequently to the newly emerging tubers, the latter most likely via the stolon. In this
307 light, Vannier et al. [38] reported that both bacteria and fungi of the clonal plant *Glechoma hederacea*
308 can be transmitted to daughter plants through the stolon. In potato, some bacteria may migrate via
309 the xylem or intracellular spaces to the above ground tissues of the potato plants as well as the
310 stolon [39] and subsequently into the emerging tubers [20]. These studies suggest that vertical
311 transmission of microbes from one potato generation to the next is possible. In our study, we
312 observe around a quarter of bacterial and up to half of fungal communities in the daughter tubers
313 and roots overlapped with the seed tuber microbiomes (Fig. 4, Fig. S6). However, when we looked at
314 ASVs that were uniquely found on roots and daughter tubers that originate from seed tubers from a
315 specific production field, we see that a very small part of these ASVs (< 0.5%) is also detected
316 uniquely on the seed tubers from that production field (Fig. 4, Fig. S6). We conclude that, based on
317 the tractable vertical transmission of field-unique microbes, intergenerational transmission of
318 microbiota is minimal and cannot explain the effects of field of production on microbiomes in the
319 subsequent crop.

320 To better understand the early events in transmission of specific microbiome members from the
321 seed tuber to plants emerging from these tubers, we analyzed the microbial composition of sprouts

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322 geminated in a soil-free system and compared it to the microbial communities of different
323 compartments of the seed tubers. Firstly, we observed that the tuber's adhering soil, peel, heel end,
324 eye and flesh constitute distinct compartments that have significantly different microbiomes (Fig. 5,
325 Fig. S7). Apparently the physical and chemical characteristics and activities in these distinct
326 microhabitats [40, 41] select for different microbes. Moreover, the bacterial richness decreased from
327 the surface of the tuber inwards (Fig. S8). Arguably this is a result of physical exclusion of microbes
328 by the barrier function of the distinct tuber tissues and increased selective pressure inside the tuber
329 by a combination of e.g., plant immunity and oxygen limitation [42].

330 In order to focus on the transmission from seed tuber to its sprouts without the interference of the
331 soil, we subsequently analyzed the microbiomes of sprouts emerging from the seed tubers in a soil-
332 free system. Our results showed that the early stage of microbial community assembly in the sprouts
333 are genotype related. Moreover, sprouts emerging from tubers of the same genotype but originating
334 from different production fields still show to some extent distinct microbial patterns (Fig. 6). These
335 results indicate that the influence of tuber genotype and the field of seed tuber production can
336 largely determine the early-stage microbial assembly on the potato sprouts. Moreover, the top 18
337 most abundant bacterial ASVs, comprising almost 80% of the total bacterial communities on the
338 sprouts, could be traced back to the seed tuber compartments that we analyzed (Fig. 7, Fig. S10).
339 However, these sprout-abundant ASVs microbiome comprised a significantly smaller part of the total
340 bacterial microbiome in the different seed tuber compartments. This suggests that the most
341 abundant ASVs on the sprouts originate from diverse compartments of the seed tuber, and their
342 proliferation was specifically stimulated by the sprout.

343 Together our data show that microbiome composition is intergenerationally affected by the field of
344 production of the seed tuber. The potato tuber and root microbiomes on the daughter plants were
345 comprised mostly of microbes derived from the soil environment in which the next-season potato
346 plants were cultivated. The composition of a potato tuber microbiome is typically influenced by a
347 combination of factors: the resident soil microbiome, potato genotype, and the specific physical,
348 chemical, and (micro)biological conditions under which the tubers develop. In this study we
349 demonstrate that the potato tuber microbiome is also affected by the field in which the seed tuber
350 was produced. However, although we show that vertical transmission of microbes can occur from
351 seed tuber to the emerging sprouts in a soil free system, most microbes that occur on the roots and
352 daughter tubers of field-grown potato cannot be traced back to the population of seed tubers from
353 which they emerged. We speculate that the abiotic and biotic environmental conditions in the fields
354 of production differentially imprinted the seed tubers, leading to so far unknown epigenetic and/or
355 metabolic changes in the seed tubers that in turn differentially altered interactions of the emerging
356 plant with the soil microbiome, resulting in distinguishable microbiome signatures on daughter
357 tubers and roots, depending on the field of production of the mother seed tuber.

358 In conclusion, we show that seed tuber imprinting by the field of production shapes the microbiome
359 of the emerging potato plant. As it is accepted that plant microbiomes contribute to plant nutrition
360 and health, the initial microbiome is a much-undervalued trait of seed tubers specifically, or planting
361 materials in general. Elucidating the relative importance of the initial microbiome and the
362 mechanisms by which the origin of planting materials affect microbiome assembly will pave the way
363 for the development of microbiome-based predictive models that may predict the quality of seed
364 tuber lots, ultimately facilitating microbiome-improved potato cultivation.

365

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366 # Materials and Methods

367 **Potato varieties**

368 In total, 5 potato varieties form the Royal HZPC Group and Averis Seeds B.V. were used in this study,
369 namely variety *Colomba* (Variety A), *Innovator* (Variety B), *Festien* (Variety C), *Challenger* (Variety D),
370 *Sagitta* (Variety E) and *Seresta* (Variety F).

371 **Sampling of seed tubers and post-storage seed tubers**

372 In the autumn of 2018, seed tubers of two potato varieties (labelled A and B in this study to protect
373 the commercial interests of the potato breeding companies that produced them) were harvested
374 from 3 fields of production for Variety A and 3 other fields for Variety B (Fig. S1a-b). These tubers
375 were shipped to a central location where they were subsequently stored in the dark at 4 °C. Seed
376 tubers were taken from cold storage and sampled in December 2018 as "seed tuber" and July 2nd,
377 2019, as "post-storage seed tuber". For seed tuber samples, peels were sampled from 24 seed
378 tubers per production field and the peels of 6 tubers were pooled into a composite replicate sample,
379 resulting in 4 replicated samples per variety per field. For post-storage seed tuber samples, peels
380 were sampled from 36 seed tubers per field and the peels of 6 tubers were pooled into a composite
381 replicate sample, resulting in 6 replicated samples per variety per field. In total, 144 seed tubers and
382 216 post-storage seed tubers were sampled and resulted in 24 seed tuber samples and 36 post-
383 storage seed tuber samples. These samples were frozen in liquid $N_{2,}$ freeze-dried and stored in 50-
384 mL falcon tubes at -20 °C prior to analysis.

385 **Sampling of daughter tubers and roots emerging from seed tubers**

386 Seed tubers of Variety A and B of the above-mentioned 6 production fields were subsequently
387 planted in a single trial field near Veenklooster (Fig. S1a; GPS location: 53.30353, 6.02670), the
388 Netherlands. The chemical composition of this sandy field was analyzed by Normec Groen Agro
389 Control B.V. and found to contain 1630 mg N/kg, 34 mg P_2O_5/l, 108 mg K/kg, 216 mg MgO/kg, 9 mg
390 Na/kg, 3.4% organic matter and a sulfur supply capacity 7.2kg S/ha per year. The field pH was 5.1
391 and the cation exchange capacity was 57 mmol/kg. On April 16th, 2019, 24 seed tubers were planted
392 in each of the 4 replicate plots which were randomly distributed across the field. On July 2nd, 2019, 4
393 potato plants were collected from the centre of each plot, from which the root material of each
394 plant was sampled as a root sample, resulting in 4 root samples per plot. In detail, for each plant, the
395 loosely attached soil was shaken off the roots, then the roots were cut into 5 cm fragments by sterile
396 scissors and a random subset of the root fragments were stored in a 50-mL falcon tube. In the
397 meantime, the peel of 6 newly formed tubers of each plant were samples and pooled as a composite
398 daughter tuber sample, resulting in 4 daughter tuber samples per plot. For both tuber and root
399 samples, the soil tightly attached to the peel and root was retained. In total, 96 potato plants and
400 576 daughter tubers were sampled resulting in 96 root and 96 daughter tuber samples. These
401 samples were freeze-dried and stored in 50-mL falcon tubes at -20 °C prior to analysis.

402 **Sampling of seed tuber compartments**

403 To dissect the contribution of microbiomes of different seed tuber compartments, namely peel,
404 eyes, heel ends, flesh, and adhering soil (Fig. S1c), in shaping the sprout microbiome, we made use
405 of material from a parallel study in which we harvested tubers from 6 potato varieties produced in
406 25 distinct production fields (Variety A from 5, Variety B from 5, Variety C from 3, Variety D from 5,
407 Variety E from 5, and Variety F from 2 fields, respectively; Fig. S1). In detail, the adhering soil was
408 gently rubbed from the tuber surface and collected in 50-mL falcon tubes. Subsequently, 1 cm thick
409 cores were sampled from potato heel ends and eyes using a sterilized Ø 0.6-cm metal corer. Then,
410 peel was sampled from around the minor axes of a tuber using a sterilized peeler. Flesh was sampled
411 by halving a tuber using a sterile scalpel and sampling 1-cm core using a sterile Ø 0.6-cm metal corer

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412 from the centre of the tuber. Samples from 50 seed tubers were pooled into a single sample per
413 compartment per field. In total, 1250 tubers were sampled to access the microbial composition of
414 different tuber compartments, resulting in 125 compartment samples. These samples were freeze-
415 dried and stored in 50-mL falcon tubes at -20 °C prior to analysis.

416 **Sampling of sprouts**

417 To study early events in transmission of specific microbiome members from seed tubers to plants
418 emerging from these tubers, the sprout microbiome was characterized. Seed tubers of all 6 varieties
419 (Variety A-F) from 12 of the above mentioned 25 fields were employed to study the sprout
420 microbiome (Fig. S1a-b). Five replicate tubers collected from each production field were germinated
421 on sterile Petri dishes in dark conditions (20 °C and RH 68%). These 60 seed tubers were randomized
422 in 6 trays and the position of the trays were rotated every day. After 7 days, 3–4 sprouts were
423 removed from each tuber using a sterile scalpel and pooled as a composite sample. These 60 sprout
424 samples were freeze-dried and stored in 2-mL Eppendorf tubes at -20 °C prior to analysis.

425 **Sample grinding**

426 To grind the samples in high-throughput, four 5-mm sterile metal beads were added to freeze-dried
427 samples in 50-mL falcon tubes and placed in a custom-made box. The samples were ground for 9
428 min on maximum intensity in a SK550 1.1 heavy-duty paint shaker (Fast & Fluid, Sassenheim, the
429 Netherlands). Freeze-dried sprout samples were ground in 2-mL Eppendorf tubes with one 5-mm
430 sterile metal bead per tube with a Tissuelyzer at 30 Hz for 1 min.

431 **DNA isolation, library preparation and sequencing**

432 Genomic DNA was isolated from ±75 mg potato powder per sample using a Qiagen Powersoil KF kit.
433 The KingFisher™ Flex Purification System machine was used for high throughput DNA isolation. DNA
434 was quantified using a Qubit® Flex Fluorometer with the Qubit dsDNA BR Assay Kit (Invitrogen,
435 Waltham, MA, USA) and normalized to a concentration of 5 ng/µl. The resulting DNA samples were
436 then stored at -20 °C.

437 Bacterial 16S ribosomal RNA (rRNA) genes within the V3–V4 hypervariable regions were amplified
438 using 2.5 µL DNA template, 12.5 µL KAPA HiFi HotStart ReadyMix (Roche Sequencing Solutions,
439 Pleasanton, USA), 2 µM primers B341F (5'-
440 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') and B806R (5'-
441 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') [43] with Illumina
442 adapter sequences in combination with 2.5 µM blocking primers mPNA (5'-GGCAAGTGTTCTTCGGA-
443 3') and pPNA (5'-GGCTCAACCCTGGACAG-3') in 25 µL reactions. Blocking primers were used to avoid
444 the amplification of mitochondrial (mPNA) or plastidial (pPNA) RNA from the plant host [44]. Cycling
445 conditions for 16S rRNA were (1) 95 °C for 3 min; (2) 95 °C × 30 s, 75 °C × 10 s, 55 °C × 30 s, 72 °C × 30
446 s, repeated 24 times; (3) 72 °C × 5 min; (4) hold at 10 °C.

447 Fungal internal transcribed spacer 2 (ITS2) DNA was amplified using 2.5 µL DNA template, 12.5 µL
448 KAPA HiFi HotStart ReadyMix, 2 µM primers fITS7(5'-
449 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGARTCATCGAATCTTTG-3') and ITS4-Rev (5'-
450 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC-3') with Illumina adapter
451 sequences in combination with 2 µM blocking primers cl1ITS2-F (5'-
452 CGTCTGCCTGGGTGTCACAAATCGTCGTCC-3') and clITS2-R (5'-
453 CCTGGTGTCGCTATATGGACTTTGGGTCAT-3') in 25 µL reactions [43]. Cycling conditions for ITS2 were (1)
454 95 °C for 3 min; (2) 95 °C × 30 s, 55 °C × 30 s, 72 °C × 30 s, repeated 9 times; (3) 72 °C × 5 min; (4)
455 hold at 10 °C.

456 For both PCR reactions, DNA was cleaned using the KingFisher™ Flex Purification System. Twenty µL
457 of vortexed AMPure XP Beads (Beckman Coulter, Brea, USA) were added to 25 µL of PCR product in a

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458 KingFisher™ 96 deep-well plate. Beads with adjoined DNA were washed by subsequent transfer to 3
459 KingFisher™ 96 deep-well plates with 80% ethanol and DNA was then eluted in 30 µL C6 elution
460 buffer from the Qiagen Powersoil KF kit.

461 Index PCR reactions were performed using standard Illumina i7 (N701-N712) index primers for
462 columns and Illumina i5 (N501-N508) index primers for rows of each plate. Five µL DNA sample was
463 added to a mix of 2.5 µL 2 µM index primer, 12.5 µL KAPA HiFi HotStart ReadyMix and 5 µL Milli-Q
464 H2O. Cycling conditions for index PCRs were (1) 95 °C for 3 min; (2) 95 °C × 30 s, 55 °C × 30 s, 72 °C ×
465 30 s, repeated 9 times for 16S or 24 times for ITS2; (3) 72 °C × 5 min; (4) hold at 10 °C. After the
466 index PCR, DNA was cleaned using the abovementioned cleaning protocol. DNA concentrations of all
467 PCR products were measured using a Qubit® Flex Fluorometer with the Qubit dsDNA BR Assay Kit
468 (Invitrogen, Waltham, MA, USA) and normalised to 2 ng/µL, after which the samples were pooled
469 and sent for Illumina V3 2x300 bp MiSeq sequencing at USEQ (Utrecht, the Netherlands).

470 **Microbial community analysis and statistics**

471 Both 16S and ITS2 rDNA raw sequencing reads were denoised, joined, delineated into amplicon
472 sequence variants (ASVs), and assigned taxonomy in the Qiime2 (v.2019.7) environment [45]. Datasets
473 were demultiplexed and then filtered using the DADA2 pipeline [46]. ASVs with less than 30 reads or
474 present in less than 3 samples across all samples within a dataset were removed to minimize
475 potential errors in sequencing. The representative sequences were subsequently taxonomically
476 classified using a classifier trained with the 99% OTU threshold SILVA database [47] for bacteria and
477 UNITE reference database (v.8.0)[48] for fungi. For bacteria, we removed remaining 16S reads
478 annotated as mitochondria or chloroplasts and kept only reads assigned to Bacteria. On average, the
479 mitochondrial and chloroplast reads together accounted for 46%, 21%, 11% and 3% of 16S reads in
480 the seed tuber, seed tuber after storage, daughter tuber and root samples, respectively, and 0.08%,
481 22%, 26%, 46%, 67% and 90% in the adhering soil, heel end, peel, eye, flesh and sprout, respectively.
482 For fungi, we removed remaining ITS reads assigned as *Viridiplantae* and *Protista* and kept only
483 reads assigned to Fungi. On average, plant-originated reads accounted for 53%, 16%, 78% and 30%
484 of ITS reads in the seed tuber, seed tuber after storage, daughter tuber and root samples,
485 respectively; and 2%, 11%, 27%, 35 %, 55% and 46% in the adhering soil, heel end, peel, eye, flesh
486 and sprout, respectively.

487 The datasets with samples from seed tubers, post-storage seed tubers, daughter tubers, and root
488 samples were rarefied to 10000 bacterial and 4000 fungal reads per sample, respectively. The
489 datasets with samples from five compartments of the seed tuber were rarefied to 8000 reads per
490 sample, for both bacterial and fungal reads.

491 Bray-Curtis dissimilarity matrices were created in QIIME2 and visualized in R using the Qiime2R and
492 *ggplot2* package. Permutational multivariate analysis of variance (PERMANOVA, 999 permutations)
493 tests were performed using QIIME2 to test the effect of different factors on the microbiome
494 composition. Kruskal-Wallis tests were performed to test for differences in community diversity and
495 evenness. Distance matrices were created separately for each generation and variety to compare
496 the seedlots within the varieties using PERMANOVA tests. Venn diagrams were conducted by R
497 package VennDiagram (v1.7.1, https://CRAN.R-project.org/package=VennDiagram). UpSet plots
498 were generated by R package UpSetR [49]. Sankey diagrams were produced by R package ggalluvial
499 (http://corybrunson.github.io/ggalluvial/).
500

501 **Data availability**

502 The datasets generated during and/or analyzed during the current study are available from the
503 corresponding author on reasonable request.

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504 **Code availability**

505 Custom code for the analyses in the current study are available from the corresponding author on
506 reasonable request.

507 **Acknowledgements**

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516 farm, potato and root icons used in this manuscript were designed using images from Flaticon.com.
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519

520 **Author contributions**

521 R.L.B., P.A.H.M.B., R.J and C.M.J.P. conceived and designed the study. Y.S., C.D.J. and J.H.H.M.
522 conducted the experiments and collected the data. Y.S. and J.S. performed the statistical analysis.
523 Y.S. drafted the manuscript, and R.L.B., P.A.H.M.B., and C.M.J.P. provided critical revisions. All
524 authors approved the final version of the manuscript.
525

526 **Competing interests**

527 The authors declare that they have no competing interests. None of the authors have financial or
528 non-financial conflicts of interest that could be perceived as influencing the research presented in
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