Plant sexual and asexual contributions to the seed microbiome

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Summary

The seed microbiome plays a key role in the assembly of the plant microbiome, which has major impacts on plant functioning. Nonetheless, little is known about the origin of the seed microbiome. We investigated the relative contributions of two hypothesized transmission routes: sexual inheritance (via reproductive organs) and asexual inheritance (via the plant vascular system). To do that, we sampled shoot endophytes both before bloom and at seed maturity stages, apple flower ovaries and pollen sacs, and mature seeds from field-grown apple trees (*Malus domestica* BORKH. cv 'Gala Galaxy Selecta'). We showed that bacterial richness, diversity and composition differ among tissue types, with shoots before bloom harboring a higher diversity than ovary and pollen. Source tracking revealed that both sexual (30.3%) and asexual (23.8%) pathways contributed to seed microbiome assembly, with shoots at seed maturity being the dominant source. Notably, a large proportion (49.5%) of the seed microbiome originated from unknown sources. Lastly, the transmission pathways significantly differed among bacterial genera, with *Pseudomonas* primarily linked to shoots, *Rhizobacter* to pollen and *Burkholderia* to the ovary. Insights into seed microbiome origin offers new opportunities to enhance seed health and crop productivity through microbiome-assisted breeding.

Keywords: bacteria, community assembly, flower, microbial transmission, seed microbiome

Introduction

The concept of phylosymbiosis postulates that the similarity between host-associated microbial communities is mirrored by the evolutionary relationships among host (Brucker & Bordenstein, 2013). It suggests that closely related plant species harbor more similar microbiomes than distantly related species due to shared evolutionary history (Brooks *et al.*, 2016; Lim & Bordenstein, 2020). Although phylosymbiosis can arise through various mechanisms, including coevolution, shared environmental factors, or host selection effects (Kohl, 2020) one mechanism that is commonly overlooked is microbial inheritance. Microbial inheritance is the process in which plant-associated microorganisms pass from parents to seed, and persist seed dormancy and germination (Abdelfattah *et al.*, 2023). By transmission through the seeds, inherited microorganisms can be maintained across generations, preserving part of the plant microbiome over time (Abdelfattah *et al.*, 2023; Sulesky-Grieb *et al.*, 2024). For this mechanism to contribute to phylosymbiosis, however, it would require that introgression events not only include genetic material but also microbial communities from each parent plant, which in turn would lead to a novel admixture of both partners. Yet, seed microbial communities are not solely shaped by inherited microorganisms. For example, part of the seed microbes may originate from environmental sources such as air, water, and pollinators (Prado *et al.*, 2020; Simonin *et al.*, 2022). Understanding the role of microbial inheritance is essential for evaluating the evolutionary role of seed microbiomes - and by extension, for testing whether microbial inheritance can reinforce phylosymbiotic patterns.

The transmission of microbes from plant to seed can occur via two main pathways: sexual, involving reproductive organs such as pollen and ovaries (Mitter *et al.*, 2017; Bergmann *et al.*, 2025) and asexual, originating from vegetative tissues like shoots (Abdelfattah *et al.*, 2021, 2023). For example, if the seed microbiome is predominantly originated from the sexual transmission pathway, one can expect that the microbial communities in shoots before bloom would also be important sources of microbes (Olimi *et al.*, 2022). This is because floral organs develop directly from these shoots, and at the time of flowering, shoot endophytes are likely the primary microbial source for emerging reproductive tissues (Abdelfattah *et al.*, 2023; War *et al.*, 2023). In other words, any microbes transmitted through pollen or ovary most likely originate from or pass through vegetative tissues. Conversely, if the seed microbiome is primarily shaped by asexual transmission, then shoots collected during seed maturation would be more important sources of the seed microbiome, as they remain connected through vascular system to the maturing seed and can enable microbial transfer through maternal tissues during

seed filling. These two scenarios are not mutually exclusive, but each pathway implies distinct temporal and spatial patterns of microbial transmission toward the seed. Understanding the relative contributions of sexual and asexual transmission pathways will not only clarify the mechanisms underlying microbial inheritance but also provide insights into the ecological and evolutionary forces shaping seed microbiomes. This knowledge can enhance our understanding of host-microbe coevolution and phylosymbiotic patterns within plant lineages.

Microbial communities are not homogeneously distributed within plants. Instead, different tissues such as stems, leaves, flowers, and reproductive organs tend to harbor distinct microbiomes shaped by distinct physiological characteristics (Hardoim et al., 2015; Compant et al., 2011). Studies have shown that pollen grains can carry diverse microorganisms that could be transmitted during fertilization (Khalaf *et al.*, 2023; Cardinale & Schnell, 2024; Armstrong *et al.*, 2024). Paternal breeding lines contributed substantially to the seed microbiota of oilseed rape (Wassermann *et al.*, 2022). Although the presence of microorganisms in the plant ovules was reported (Mundt & Hinkle, 1976) to this date much less is known about female than male reproductive parts (Bergmann *et al.*, 2025). As for asexual tissues, shoots and stems often support more diverse microbial communities than reproductive tissues, likely due to their extended developmental windows and greater exposure to microbes in the environment. Interestingly, transmission pathways may differ among microbial taxa, with certain genera associated with specific tissues or transmission route (Chesneau *et al.*, 2020). Altogether it is important to consider both tissue-specific microbes and taxon-specific transmission strategies when studying plant-to-seed microbial inheritance.

This study aims to address the critical knowledge gap regarding the origins of the seed microbiome. Specifically, we aim to disentangle the contributions of sexual (pollen sacs and ovaries) and asexual (shoot endophytes) pathways to seed microbiome origin and assembly (Fig. 1). We hypothesize that:

1. Microbial diversity and composition differ between shoots, ovary and pollen, with:

    a. Shoots harboring a higher diversity of microorganisms than the ovary and pollen.
    b. Shoot endophytes before bloom and at seed maturity are compositionally more similar to each other than to the ovary and pollen tissues.

2. Both sexual (ovary and pollen) and asexual (shoots before bloom and at seed maturity) pathways are hypothesized to contribute to seed microbiome assembly, though their relative importance can vary. Thus, we expected that:

a. If sexual transmission dominates, shoots at bloom can serve as the main source for microbes colonizing emerging floral organs, making shoot at bloom, ovaries, and pollen sacs similar in their community composition to seed
b. If asexual transmission dominates, we expect shoots at seed maturity to contribute the largest proportion to the seed microbiome, due to microbial transmission through the vascular system to developing seeds

3. Some microbial taxa are predominantly associated with either asexual (e.g., *Allorhizobium* and *Pseudomonas* from shoots) or sexual (e.g., *Rhizobacter* from pollen and *Erwinia* from ovary or pollen) tissues.

4. A notable proportion of the seed microbiome originates from unknown sources, possibly reflecting unsampled environmental reservoirs such as pollinators or air.

Materials and Methods

Study system and sample collection

To study the vertical transmission of endophytes in plants, we selected five apple trees (*Malus domestica*, cultivar 'Gala Galaxy Selecta') from the Botanical Garden of the University of Graz (47.0814° N, 15.4562° E). Sampling was conducted at two time points: before bloom (April 15, 2020) and at seed maturity (September 9, 2020) (Fig. 1b). All samples were collected using sterile forceps and gloves, transported on ice and processed under sterile conditions in a laminar flow cabinet.

To investigate the floral community, five flower buds from each of five trees were collected right before tree bloom in April (Fig. 1a). Alongside flower buds, shoots leading to the sampled flowers were collected at the same time. In September, mature apples were harvested, and seeds were extracted under sterile lab conditions (Fig. 1a). Alongside seeds, shoots leading to the sampled fruits were collected at the same time. The final set of samples included 25 pollen, 25 ovary and 25 shoot samples during bloom and 25 seed and 25 shoot samples during seed maturity (Fig. 1b).

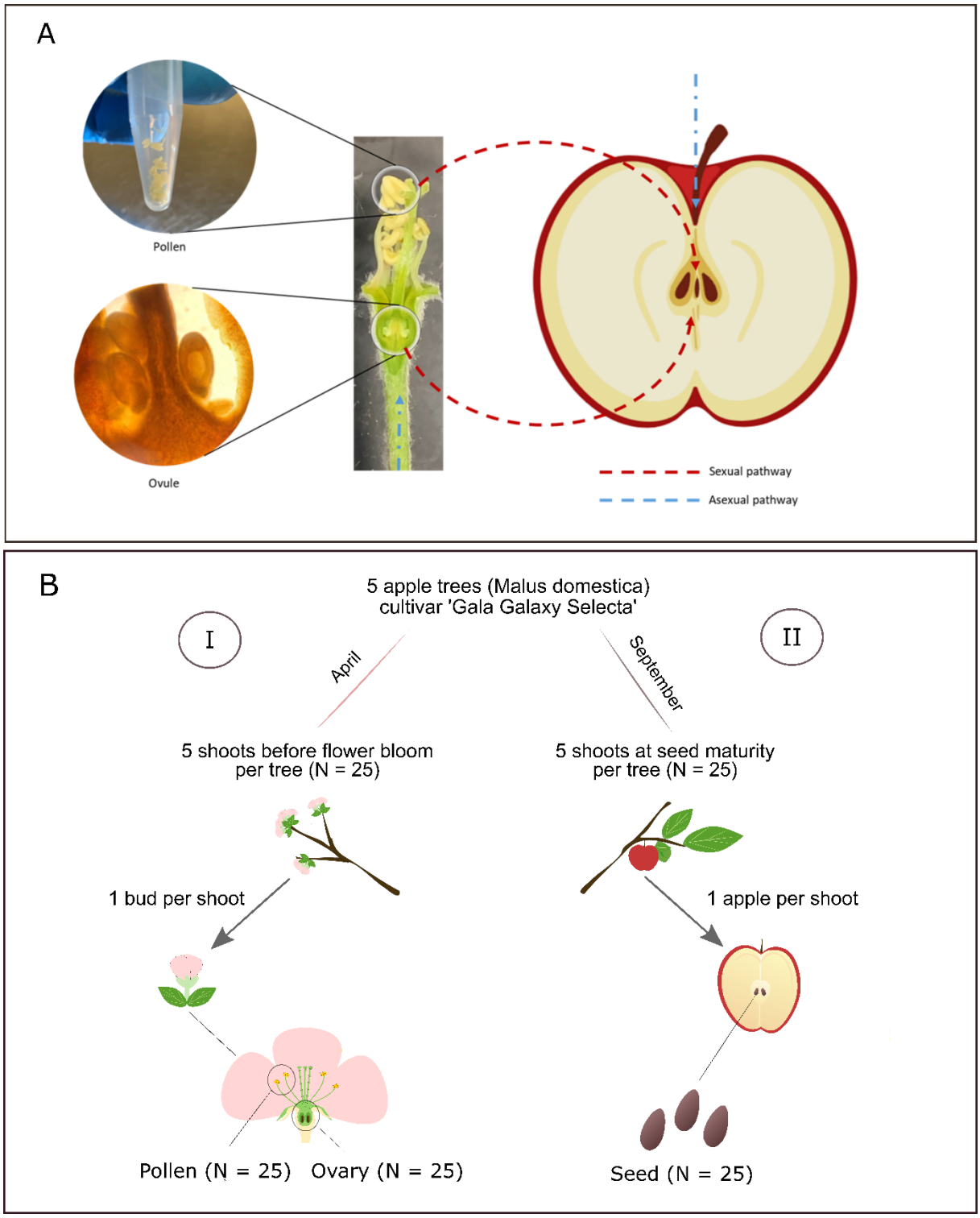


Fig. 1. Schematic representation of microbial transmission pathways to the seed in apple trees. (A) Dashed orange arrows indicate the sexual transmission pathway, where microbes are transferred via floral organs including pollen and ovary tissues. Dashed blue arrows indicate the asexual transmission pathway, through which microbes colonize seeds via shoot tissues both before flower bloom and during seed maturity. (B) Experimental design showing the sampling scheme. From each of five 'Gala Galaxy Selecta' apple trees, we collected samples

133 from five shoots (I) prior to flowering (April) and (II) at seed maturity (September). From the
134 five shoots prior to flowering, we collected shoot tissue and five floral buds. In the laboratory,
135 each flower bud was dissected to isolate ovary and pollen. From the five shoots at seed
136 maturation, we collected shoot tissues, five apples and seeds. A total of 25 replicate samples
137 were collected for each tissue type.

138 *Preparation of ovary, pollen, shoot and seed samples*

139 Under sterile laboratory conditions, each flower bud was dissected to isolate the female (ovary)
140 and male (pollen sac) reproductive organs (Fig. 1a). The outer ovary layer was removed, and
141 the entire inner portion was processed as a single ovary sample. Closed pollen sacs (hereafter
142 referred to as *pollen*) were isolated from the same buds, with all pollen sacs from a single bud
143 pooled into a single sample. Samples were stored at −20°C until DNA extraction.

144 Shoots collected before bloom and during seed maturity were surface sterilized in a 4% Sodium
145 Hypochlorite (NaOCl) solution then rinsed three times with autoclaved Ultraopure water, each
146 rinse lasting three minutes. After sterilization, the bark of the shoots was removed using a
147 scalpel, and the first and last centimeters of the shoots were discarded. The shoots were then
148 cut into 1 cm pieces, weighed, and transferred into sterile bags. To prepare the samples, 10 mL
149 of 0.85% sodium chloride (NaCl) solution was added, and the tissue was homogenized using a
150 sterilized mortar and pestle. The homogenate was transferred into two Eppendorf tubes and
151 centrifuged at 16,000 g for 20 minutes at 4°C. After discarding the supernatant, 2 mL of NaCl
152 solution was added to each tube for a second centrifugation under the same conditions. Seeds
153 were processed using the same protocol. For each seed sample, approximately 8 seeds were
154 ground in 10 mL of 0.85% NaCl solution, followed by centrifugation steps. All resulting pellets
155 (from seeds and shoots) were stored at −20°C until DNA extraction.

156 *Molecular methods and bioinformatics*

157 DNA extraction was performed using the FastDNA™ SPIN Kit for Soil (MP Biomedicals).
158 DNA extractions were carried out separately for each sample type (ovules, pollen, seeds, shoots
159 before bloom and at seed maturity) to prevent microbial cross-contamination, particularly
160 between pollen and ovule samples. The 16S rRNA gene was amplified using primers 515f and
161 806r (Walters *et al.*, 2015). To minimize host mitochondrial and plastid 16S rDNA
162 amplification, Peptide Nucleic Acid (PNA) clamps were added to the PCR mixture (von
163 Wintzingerode *et al.*, 2000; Lundberg *et al.*, 2013). PCR reactions were performed in a total
164 volume of 30 µL and were purified using the Wizard SV Gel and PCR Clean-Up System
165 (Promega, Madison, WI, USA; see Supporting Information for details). DNA concentration

was measured with a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), samples were pooled at equimolar concentrations and sequenced using 250 bp paired-end Illumina MiSeq sequencer.

Raw sequence data were processed using QIIME2 (version 2021.2) (Caporaso *et al.*, 2010; Bolyen *et al.*, 2019). Following paired-end read joining and quality filtering with Phred-score 33, error correction, denoising, chimera removal, and Amplicon Sequence Variant (ASV) table generation were performed using DADA2 (Callahan *et al.*, 2016). Taxonomic assignment of ASVs was conducted using the SILVA reference database (version 132, 99% identity, 06.05.21), with VSEARCH implemented for de novo clustering. Sequences of host mitochondrial and chloroplast origin were filtered out, and ASVs detected in negative controls were removed for each apple tissue type independently.

Statistical analysis

All analyses were conducted using R (version 4.4.3) (R Core Team, 2022). To investigate bacterial community composition, species richness, evenness, and diversity (Shannon index), the ASV table was rarefied to 200 reads per sample. Pairwise comparisons of microbial richness, diversity and evenness among tissues, such as ovaries, pollen, shoots and seeds, were assessed using Tukey's post hoc test. Cumulative Sum Scaling (CSS) from the *MetagenomeSeq* package was applied to account for differences in sequencing depth (Paulson *et al.*, 2013). Beta diversity was calculated using Bray-Curtis dissimilarity metrics (Anderson, 2001; McArdle & Anderson, 2001), and multivariate microbial community composition was visualized through Principal Coordinates Analysis (PCoA) using *plot_ordination* function in the *ggplot2* package (Wickham, 2009). Statistical testing of beta diversity was performed using PERMANOVA with the *adonis2* function in the *vegan* package (Oksanen *et al.*, 2022). Finally, to assess whether microbial communities in shoots before bloom were more similar to those at seed maturity than to those in ovary or pollen tissues, we calculated Bray–Curtis dissimilarities between sample pairs from shoots at bloom and each of the following: shoot at seed maturity, ovary, and pollen. Distances were extracted from the dissimilarity matrix and grouped by tissue pair. We then performed pairwise Wilcoxon rank-sum tests to compare the distributions of these between-group distances.

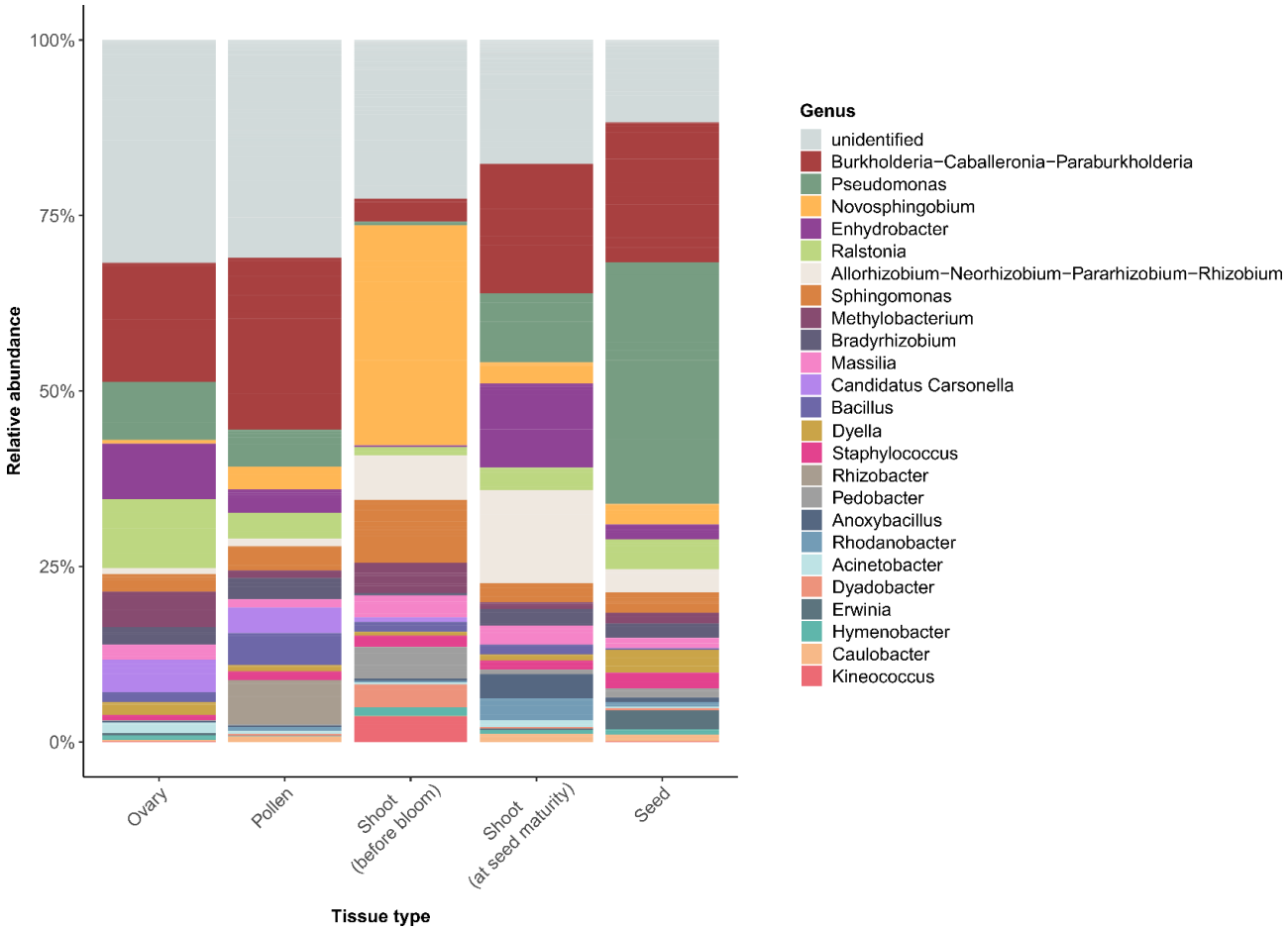
To infer the relative contributions of sexual (ovaries and pollen) and asexual (shoot) tissues to seed microbiome assembly, we applied two complementary source-tracking approaches. First, we used a Bayesian source-tracking method implemented in SourceTracker v2.0.1 (Knights *et al.*, 2011), which identifies taxa that differ among sources and sinks. Second, we applied

199 FEAST v0.1.0 (Shenhav et al., 2019) with a maximum of 5000 iterations. To enhance the
200 robustness of our findings, we applied both methods, as they offer complementary strengths.
201 SourceTracker provides taxon-specific insights by identifying differences among sources and
202 sinks, while FEAST is computationally efficient but lacks taxonomic resolution. In both source
203 tracking approaches, seeds were set as the "sink" samples, while ovary, pollen, shoot samples
204 before bloom and at seed maturity were defined as "source". The algorithm identified an
205 unexplained fraction, named 'unknown' source, which represented ASVs found in the sink, but
206 originating from other unsampled sources. Since both methods showed quantitatively and
207 qualitatively similar results, we present SourceTracker results in the main text, while FEAST
208 results are presented in the supplementary material (Fig. S1-S3).

209

210 **Results**

211 The bacterial taxa with the highest relative abundance varied across tissues. In ovaries, the
212 dominant genera were *Burkholderia*, *Enhydrobacter*, and *Ralstonia*, followed by *Pseudomonas*
213 (Fig. 2). In pollen, the dominant genera were *Burkholderia*, *Pseudomonas*, *Candidatus*, and
214 *Rhizobacter* (Fig. 2). For shoots, the composition shifted with developmental stage: before



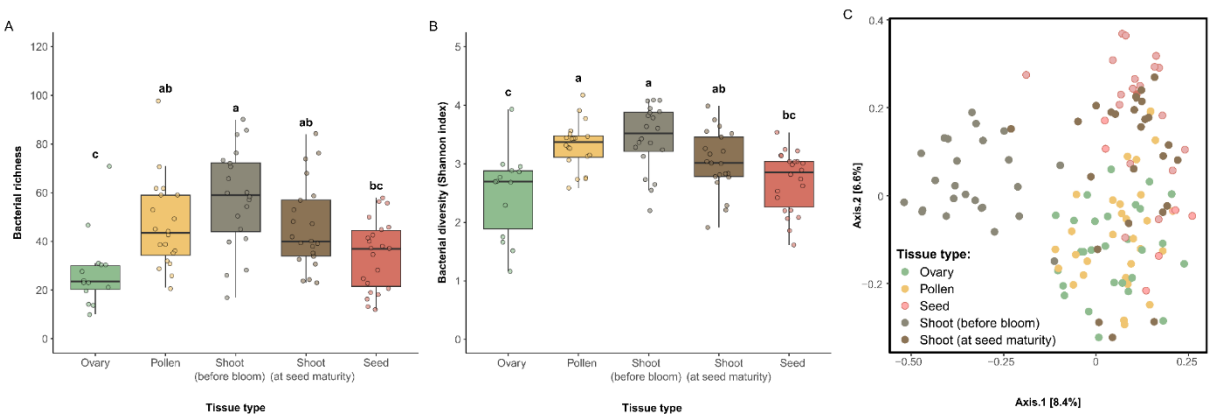
bloom, *Novosphingobium* and *Allorhizobium* were the most prevalent, whereas at seed maturity, *Burkholderia*, *Allorhizobium*, and *Enhydrobacter* dominated (Fig. 2). Finally, in seeds, *Burkholderia* and *Pseudomonas* were the most abundant bacterial genera (Fig. 2).

Fig. 2. Relative abundance of bacterial genera in ovary, pollen, shoots before bloom and shoots at seed maturity. Shown are the 25 genera with the highest relative abundance.

The richness, diversity and composition of the shoot, ovary, pollen and seed microbiomes

Bacterial richness varied significantly among tissue types, with the lowest richness in the ovary and seed, intermediate richness in pollen, and the highest richness in shoots ($F_{4, 97} = 9.97$, $p < 0.0001$). Post-hoc comparisons revealed that the ovary had significantly lower richness than pollen, shoots before bloom, and shoots at seed maturity, but was equal in richness to seeds (Fig. 3a; Table S1a). Moreover, bacterial richness in seeds was significantly lower than in shoots before bloom (Fig. 3a; Table S1a). A comparable pattern emerged for Shannon diversity, which also varied significantly across tissue types ($F_{4, 97} = 9.63$, $p < 0.0001$; Fig. 3b). As for richness, post-hoc tests showed that ovary samples had significantly lower diversity than pollen, shoots before bloom, and shoots at seed maturity, but was equal in richness to seeds (Fig. 3b; Table S1b). Moreover, pollen and shoots at bloom had significantly higher diversity than seeds (Fig. 3b; Table S1b).

Bacterial community composition varied significantly across tissue types ($R^2 = 0.14$, $F = 5.30$, $p = 0.001$; Fig. 3c), with all pairwise comparisons showing a significant differences (Table S2). The most pronounced compositional difference was observed between shoot endophytes before bloom and seeds ($R^2 = 0.17$) (Fig. 3c; Table S2). While shoot tissues also differed significantly from each other ($R^2 = 0.15$), their microbial community composition were more similar to each other than to those in ovaries (Wilcoxon rank-sum test, $p = 0.045$), but not significantly more similar than to those of pollen (Wilcoxon rank-sum test, $p = 0.128$).



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240 Fig. 3. **The differences in bacterial richness (a), diversity (b) and community composition**
241 **(c) among ovary, pollen, shoots before bloom, shoots at seed maturity and seed.**
242 (a-b) Differences in species richness and diversity among tissue types. The horizontal line
243 within each box represents the median, the box edges show the interquartile range (IQR), and
244 the whiskers demonstrate the data range. The small circles represent raw data points, which are
245 horizontally jittered to avoid overlap. (c) Difference in community composition among tissue
246 types visualized with two-dimensional principal coordinates analysis (PCoA) of bacterial
247 communities based on Bray–Curtis dissimilarity index.

248 *Sources of the seed microbiome*

249 Both sexual and asexual sources made substantial contributions to the seed microbiome,
250 accounting for 30.3% and 23.8%, respectively (Fig. 4a). From the sexual sources, the ovary
251 (22.1%) contributed more than pollen (8.2%; Fig. 4a). From the asexual sources, shoot
252 endophytes at seed maturity contributed a much large fraction of microorganisms to the seed
253 microbiome (21.6%) than the shoot endophytes at bloom (2.2%; Fig. 4a). From the sexual
254 sources, the ovary (22.1%) contributed more than pollen (8.2%; Fig. 4a). Unknown sources
255 accounted for 45.9% of the seed microbiome. Source tracking at the individual tree level
256 showed among-tree variation in source contribution to the seed microbiome (Fig. S1-S3).

257 SourceTracker analysis also revealed taxon-specific transmission patterns to seeds (Fig. 4b).
258 *Pseudomonas* and *Burkholderia* colonised the seed microbiome from shoot endophytes
259 collected at seed maturity. In contrast, *Erwinia* and *Dyella* colonised seed from both shoots and
260 unknown sources. Several other taxa, such as *Staphylococcus* and *Rhizobacter*, were primarily
261 associated with pollen or ovaries. Notably, a substantial number of seed-associated taxa,
262 including *Pedobacter* and *Allorhizobium*, colonised seed from unknown sources.

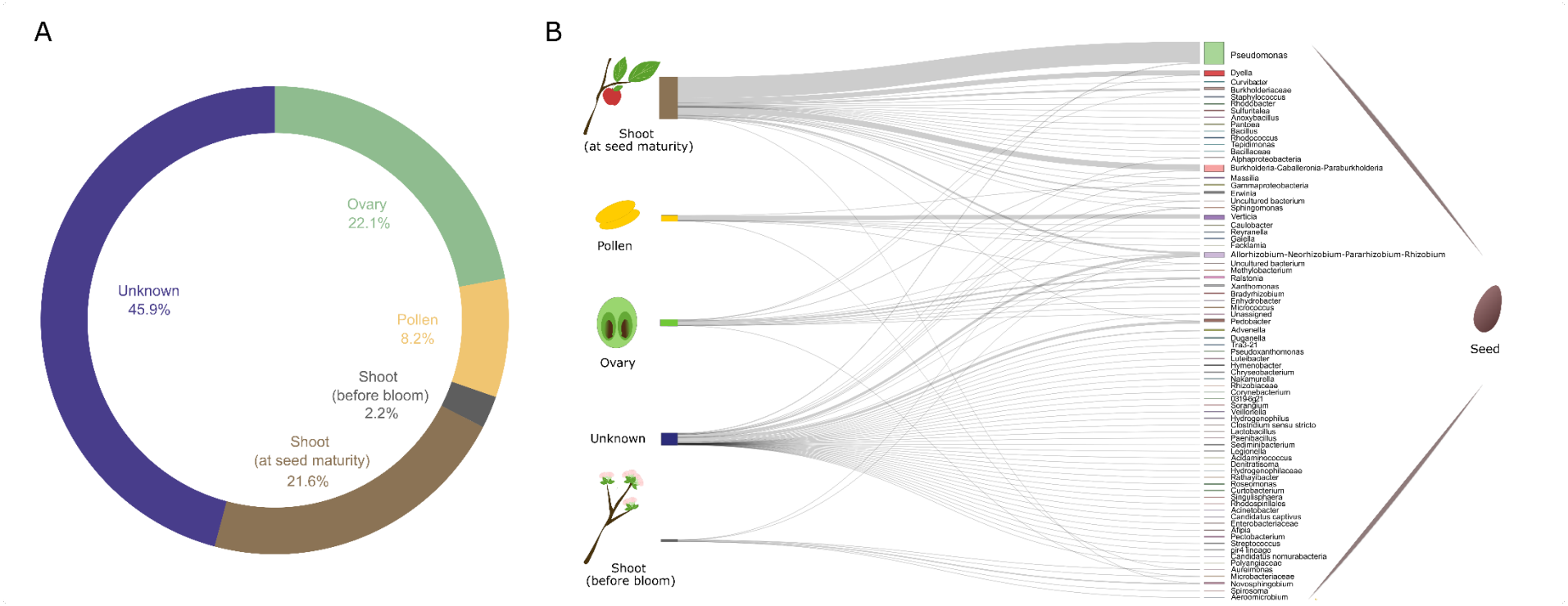

Fig. 4. Identification of potential asexual and sexual pathways of microbiome transmission to apple seeds. (A) The donut chart shows the relative contribution of ovary, pollen and shoot endophytes to the seed microbiome, as estimated using fast expectation-maximization for microbial source tracking (SourceTracker). For corresponding standard deviations (SD) and variation between individual trees see Fig. S1. (B) Relative contribution of different sources to the individual genera of the seed microbiome, as estimated using SourceTracker. Each line represents a taxon, with the thickness of the line corresponding to the relative contribution of a given source to the abundance in the seed microbiome. The thickness of each bar on the right shows the overall relative abundance of that genus in the seed microbiome. Taxa are shown at the genus level.

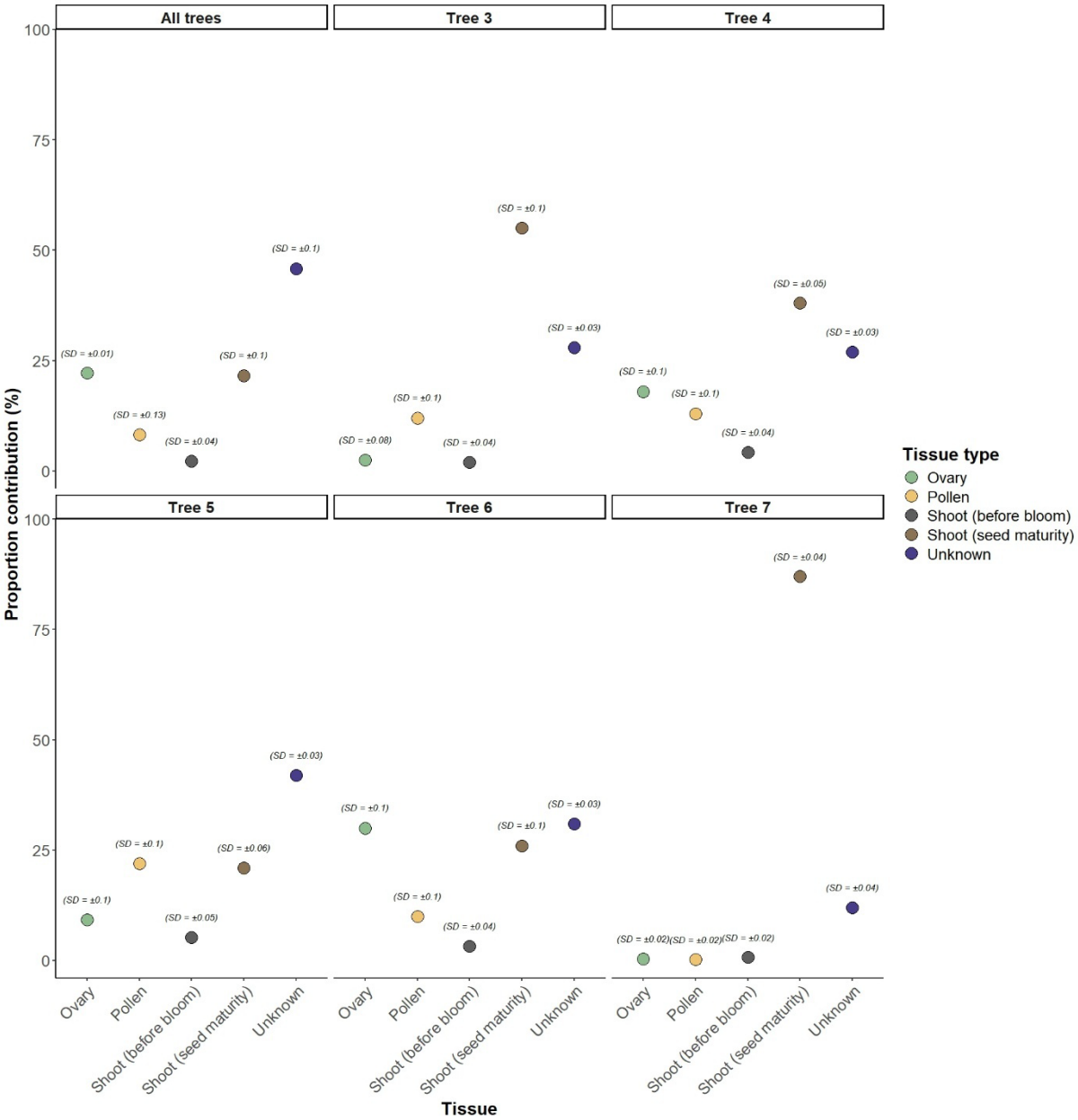

Fig. S1. **Identification of potential asexual and sexual pathways of microbiome transmission to apple seeds using fast expectation-maximization for microbial source tracking (SourceTracker).** Circles represent mean proportion contributions from each tissue type, and the text above denotes the corresponding standard deviation (SD). "Pooled Trees" refers to the aggregated results from all trees, while individual trees show the predicted contribution of each of tissue types to the seed microbiome for each of five trees.

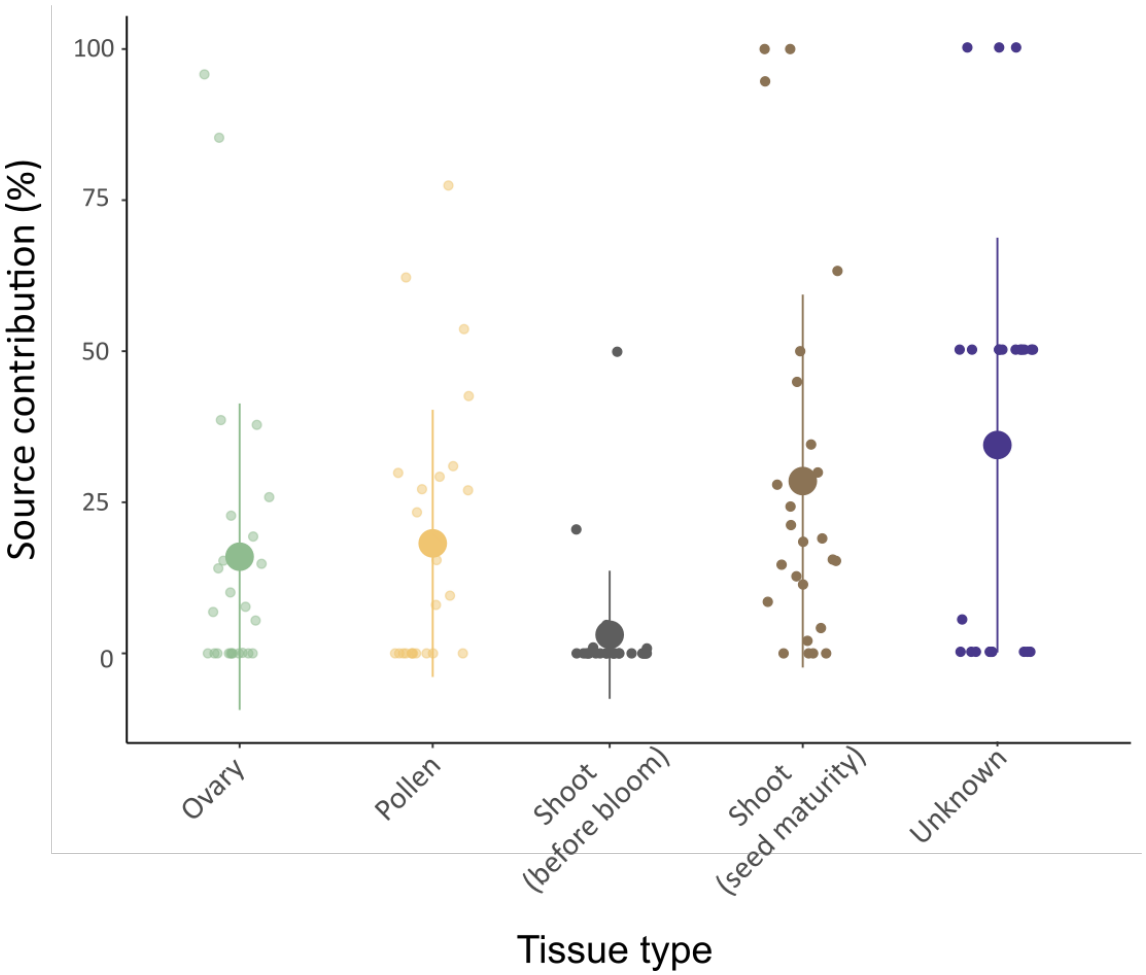

Fig. S2. Identification of potential asexual and sexual pathways of microbiome transmission to apple seeds using fast expectation-maximization for microbial source tracking (FEAST). The circle represents the mean values and the error bars represent standard deviations. The small circles represent raw data points, which are horizontally jittered to avoid overlap.

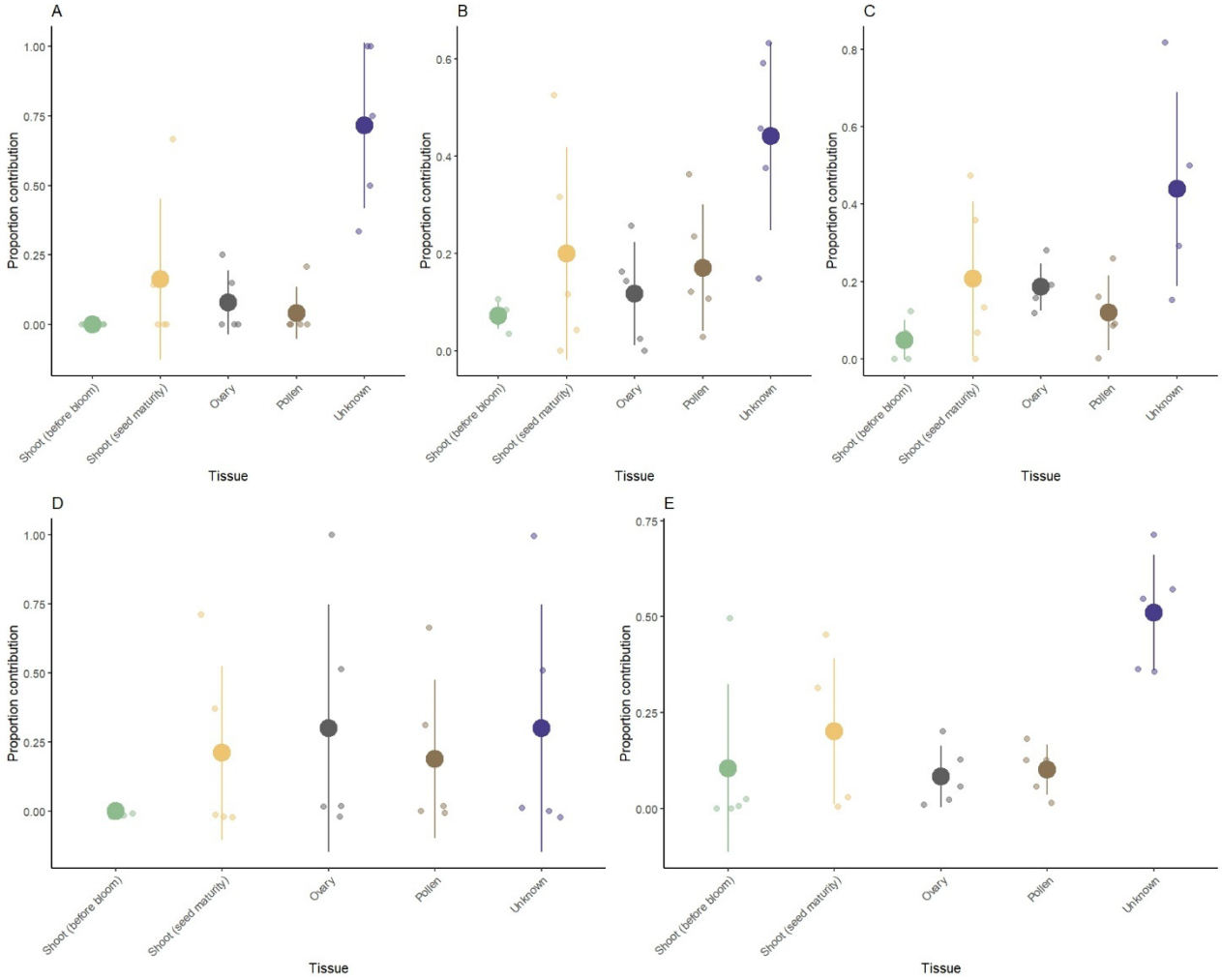

Fig. S3. Identification of potential asexual and sexual pathways of microbiome transmission to apple seeds using fast expectation-maximization for microbial source tracking (FEAST). (A-E) Show the predicted contribution of each of tissues types to seed microbiome for each of five sampled trees.

295 **Discussion**

296 In this study we disentangled the role of sexual (pollen sacs and ovaries) and asexual (shoot
297 endophytes) pathways to seed microbiome. We found that microbial richness, diversity and
298 community composition varied significantly among ovaries, pollen, shoots at bloom, shoots at
299 seed maturity and seed. Sexual and asexual pathways contributed similar proportions to the
300 seed microbiome, with shoots at seed maturity contributing the largest proportion, as followed
301 by the ovary and pollen. We also observed pathway-specific associations for several bacterial
302 taxa: *Pseudomonas* and *Allorhizobium* were primarily linked to shoots, *Rhizobacter* and
303 *Verticia* to pollen, and *Ralstonia* and *Burkholderia* to the ovary. Notably, nearly half of the
304 seed-associated bacterial community could not be traced to the sampled tissues, suggesting that
305 environmental reservoirs such as air or pollinators likely play a substantial role in the origin of
306 the seed microbiome. Overall, these findings indicate the importance of both sexual and asexual
307 pathways in shaping seed microbiomes. Such dual transmission pathways may have
308 evolutionary implications, for example by reinforcing phylosymbiosis.

309 As expected, we found that shoots at bloom and at seed maturity had higher richness and
310 diversity than the ovary. However, their richness and diversity was not significantly higher than
311 that of pollen. In addition, our hypothesis that shoot endophytes before bloom and at seed
312 maturity are compositionally more like each other than to the ovary and pollen was only
313 partially supported. While microbial communities in shoots were significantly more similar to
314 each other than to ovary, it was not true for pollen. Pollen may serve as microhabitat that support
315 a diverse microbial community due to their high nutrient content, such as sugars, lipids, and
316 proteins (Ambika Manirajan *et al.*, 2016; Vannette, 2020). In contrast, the lower diversity
317 observed in the ovary may reflect selective filtering mechanisms, possibly driven by structural
318 barriers or antimicrobial compounds in the ovary (Chesneau *et al.*, 2022; Abdelfattah *et al.*,
319 2023). Interestingly, even though ovary had lower bacterial richness than shoots and pollen,
320 they contributed a similar proportion of the seed microbiome. One interesting avenue for future
321 research is to examine when and how microbes disperse between tissues during plant
322 development.

323 We expected that if sexual pathway played a greater role in microbial transmission, microbes
324 from shoots at bloom might colonize floral organs (ovary and pollen) during their formation,
325 which would later serve as sources for seed microbial communities. This is plausible given that
326 floral tissues originate from vegetative shoots and can share endophytic communities.
327 Alternatively, if asexual transmission dominated, shoots at seed maturity would contribute more

328 to the seed microbiome via vascular connections that facilitate microbial dispersal to the
329 developing seeds. Our findings showed sexual and asexual pathways are contributing nearly
330 equally to microbial transmission to the seed. First, we have demonstrated that sexual tissues
331 together contributed ~30%, with the ovary contributing more (22%) than pollen (8%), though
332 both were consistent sources across tree individuals. The contribution of both male and female
333 reproductive tissues to the seed microbiome may increase microbial diversity and functional
334 redundancy in seeds, particularly under cross-fertilization scenarios (Abdelfattah *et al.*, 2023).
335 In parallel, shoot endophytes at seed maturity were the most dominant contributors (~21.6%),
336 indicating that transmission may occur during later stages of seed development. This is
337 biologically plausible and supported by previous studies (Nelson, 2018; War *et al.*, 2023), that
338 suggest that as seeds mature alongside the fruit, vascular connections between maternal tissues
339 and the developing seed are likely strongest. Interestingly, while endophytes from shoots
340 collected before bloom had the highest richness and diversity among plant tissues, their
341 contribution to the seed microbiome was minimal compared to the ovaries, pollen and shoots
342 at seed maturity. To better understand the pathways of microbial inheritance, future studies can
343 track the spatial and temporal movement of microbes across tissues, and examine how plant
344 development, immune responses, and tissue connectivity shape transmission pathways.

345 To date, very few published studies have explored the transmission of microbial communities
346 between plant organs (e.g., ovary, pollen and shoots) and seeds, so the range of bacterial taxa
347 that may colonize seeds via sexual and asexual transmissions is largely unknown. However, a
348 few studies that explored microbial inheritance using watermelons and oaks (Bergmann *et al.*,
349 2025) reported similar results for some of the taxa. For example, showing that, e.g. *Rastonia*
350 and *Bradirhizobium* are likely to be transmitted from the flower to the seed of the plants. As for
351 other taxa, *Pseudomonas*—a dominant seed-associated genus—was primarily traced back to
352 shoot endophytes at seed maturity, with a smaller contribution from the ovary. This aligns with
353 a meta-analysis across 50 plant species, which identified *Pseudomonas* as one of the most
354 prevalent and abundant seed-associated bacteria (Simonin *et al.*, 2022). Interestingly,
355 *Pseudomonas* has also been shown to transmit from seeds to the leaves and roots of oak
356 seedlings (Abdelfattah *et al.*, 2021), suggesting its central role in both vertical inheritance and
357 subsequent colonization of vegetative tissues. Another abundant taxon, *Burkholderia*, was
358 linked to shoot endophytes at seed maturity. This indicates partial specialization in microbial
359 inheritance, with some taxa primarily using sexual routes, others asexual, and some transmitting
360 by both. A few taxa were shown to be transmitted from pollen. That includes bacteria from
361 *Verticia* genus. Currently, there is no documented knowledge of this bacteria being transmitted

362 from pollen to seeds or being part of the apple seed bacterial community. Thus, its ecological
363 role and implications for seed microbiome establishment represent interesting directions for
364 future research. Among common pathogens of apple trees, *Erwinia* — a causal agent of fire
365 blight disease, was shown: 1) to be present in seeds; and 2) to be mainly transmitted to seeds
366 from unknown sources and to lesser extent from shoots at seed maturity. Several studies have
367 demonstrated the presence of *Erwinia* on stigmas of apple flowers with the possibility that
368 pathogen overwinters on shoots (Piqué *et al.*, 2015; Slack *et al.*, 2022). Interestingly, diversity
369 of *Enterobacteriaceae* within seeds correlated with higher resistance against pathogenic
370 *Erwinia* species of pumpkin plants in the field (Adam *et al.*, 2018), which had implications for
371 microbiome-assisted breeding. Several other abundant taxa, such as a Gram-negative genus
372 *Pedobcater* and *Allorhizobium*, were also shown to be transmitted from unknown sources.
373 *Pedobcater* has been mainly described from the soils (Viana *et al.*, 2018), while *Allorhizobium*
374 is primarily known as a nitrogen-fixing symbiont commonly associated with plant root nodules
375 (Kuykendall & Dazzo, 2015). Their presence in apple seeds suggests potential, yet unidentified,
376 transmission pathways or reservoirs within apple trees or surrounding environments,
377 highlighting knowledge gaps in our understanding of microbial inheritance in plant
378 microbiomes.

379 One interesting result is that nearly half of seed microbiota originated from parent tissues and
380 half from unknown sources (e.g., Abdelfattah et al., 2023). This large proportion of unknown
381 taxa could be explained by unsampled environmental sources of the microbes such as air or
382 mostly likely pollinators. In fact, studies have shown that pollinators can act as vectors of plant-
383 associated microbes, facilitating microbial exchange during flower visits and potentially
384 contributing to seed microbiome assembly (Vannette, 2020; Álvarez-Pérez *et al.*, 2024;
385 Cardinale & Schnell, 2024). During foraging, pollinators come into contact with floral organs
386 and nectar, carrying microorganisms on their body surfaces, mouthparts, or digestive tracts.
387 Moreover, the floral microbiome itself is shaped by pollinator identity and behavior, which may
388 influence the microbial communities on reproductive tissues (Prado *et al.*, 2020; de Vega *et al.*,
389 2021; Hietaranta *et al.*, 2023). As such, insect pollinators may play an important role in
390 transferring microbes from pollen to seeds and offer promising avenue for future research on
391 microbial transmission pathways and pollinator ecosystem services.

392 Our results also raise the question of whether the assembly follows stochastic or deterministic
393 principles. If you follow the transmission pathways, it looks like a deterministic process, as all
394 organs have very specific microbiomes. The pollen microbiome in particular is unique and

395 ensures the microbial male heritage. In addition, the maternal microbiome changes during fruit
396 ripening, which is also reflected in the seed microbiome, and even shows interesting parallels
397 to the human microbiome (Blaser & Dominguez-Bello, 2016). Overall, our findings highlight
398 the mechanisms behind microbial inheritance in plants, demonstrating that multiple pathways,
399 including sexual (ovary and pollen) and asexual (shoot endophytes), significantly shape the
400 seed microbiome. Understanding these transmission routes has important applied implications
401 for agriculture, as manipulating microbial inheritance could help enhance seedling health, for
402 example disease resistance, through microbiome-assisted breeding.

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406 **Competing interests**

407 None declared.

408 **Author contribution**

409 AA designed the experiment. AA conducted the sample collection, processing, and AA and DO
410 conducted the molecular work. MF analyzed the data with support from AA. MF wrote the first
411 draft with contributions from AA, AJMT and GB. All authors reviewed the final manuscript.

412 **Data availability statement**

413 The data that supports the findings of this study, including the ASV table, metadata, R code,
414 are archived in Zenodo repository. These resources can be accessed here via doi:
415 10.5281/zenodo.15639819 (https://zenodo.org/records/15639819). All amplicon sequencing
416 data generated in this study is deposited on the National Center for Biotechnology Information's
417 (NCBI) Sequence Read Archive under BioProject accession number PRJNA1308301. They can
418 be accessed through the following link: https://www.ncbi.nlm.nih.gov/bioproject/1308301.

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422

423 **Supporting Information**

424 **Methods S1** Detailed description of molecular methods and bioinformatics

425 **Table S1.** Pairwise comparisons of observed bacterial richness and Shannon diversity among
426 different plant tissues: seed, ovary, pollen and shoots before bloom and at seed maturity.

427 **Table S2.** Pairwise comparisons of bacterial community composition among different plant
428 tissues: seed, ovary, pollen and shoots before bloom and at seed maturity.

429 **Fig. S1**. Identification of potential asexual and sexual pathways of microbiome transmission to
430 apple seeds using fast expectation-maximization for microbial source tracking (SourceTracker).

431 **Fig. S2**. Identification of potential asexual and sexual pathways of microbiome transmission to
432 apple seeds using fast expectation-maximization for microbial source tracking (FEAST).

433 **Fig. S3**. Identification of potential asexual and sexual pathways of microbiome transmission to
434 apple seeds using fast expectation-maximization for microbial source tracking (FEAST).

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