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RESEARCH ARTICLE

Figures and figure supplements

Symbiont location, host fitness, and possible coadaptation in a symbiosis between social amoebae and bacteria

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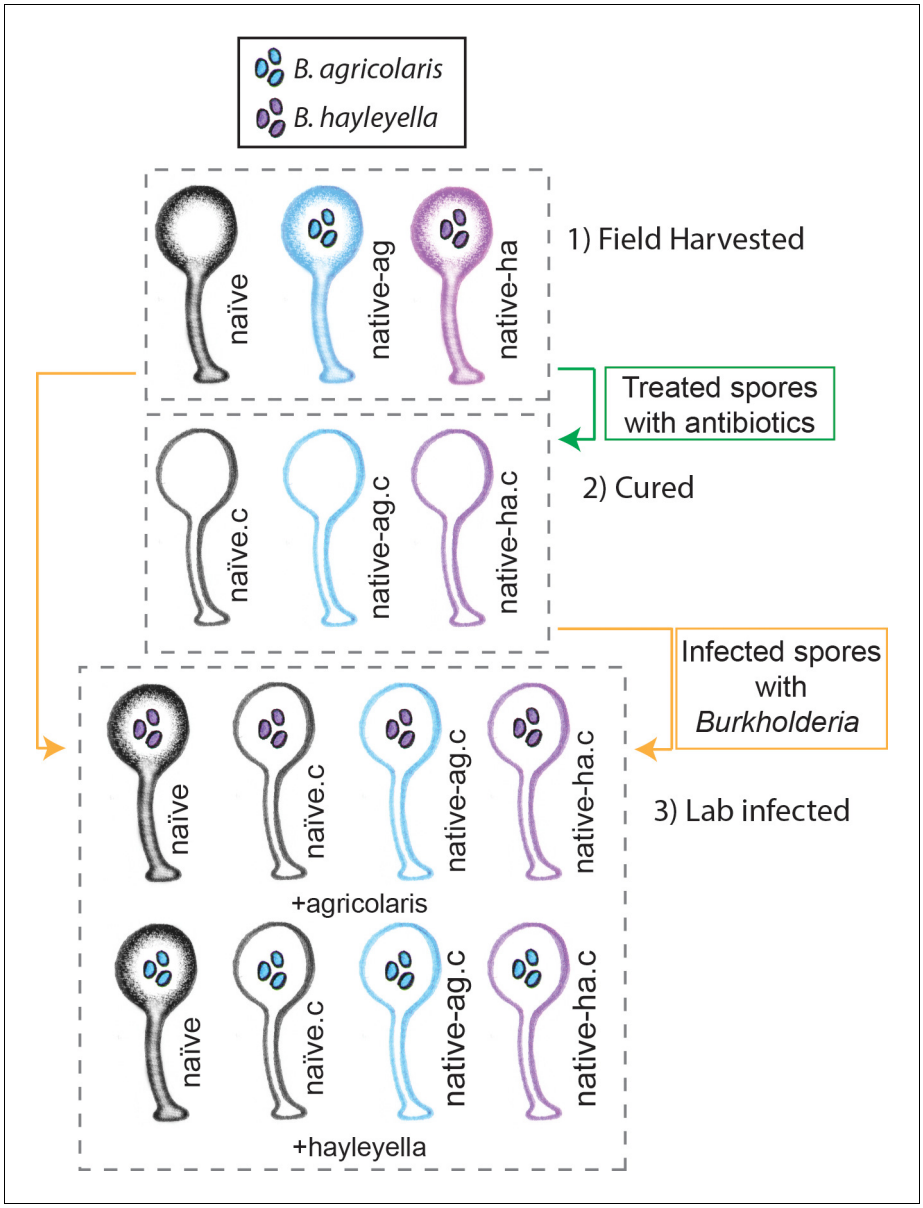

Figure 1. Illustration of host-symbiont pairs used throughout the study. *D. discoideum* clones were originally harvested from the wild in three different states: uninfected (indicated as naïve), or naturally infected with *B. agricolaris* or *B. hayleyalla* (indicated as native-ag, and native-ha respectively). Clones were treated with antibiotics to eliminate symbionts and are indicated with a '.c'. Clones were subsequently exposed to *Burkholderia* to initiate new infections. Thus, experimental types include 1) Field harvested, 2) cured, and 3) lab infected hosts.
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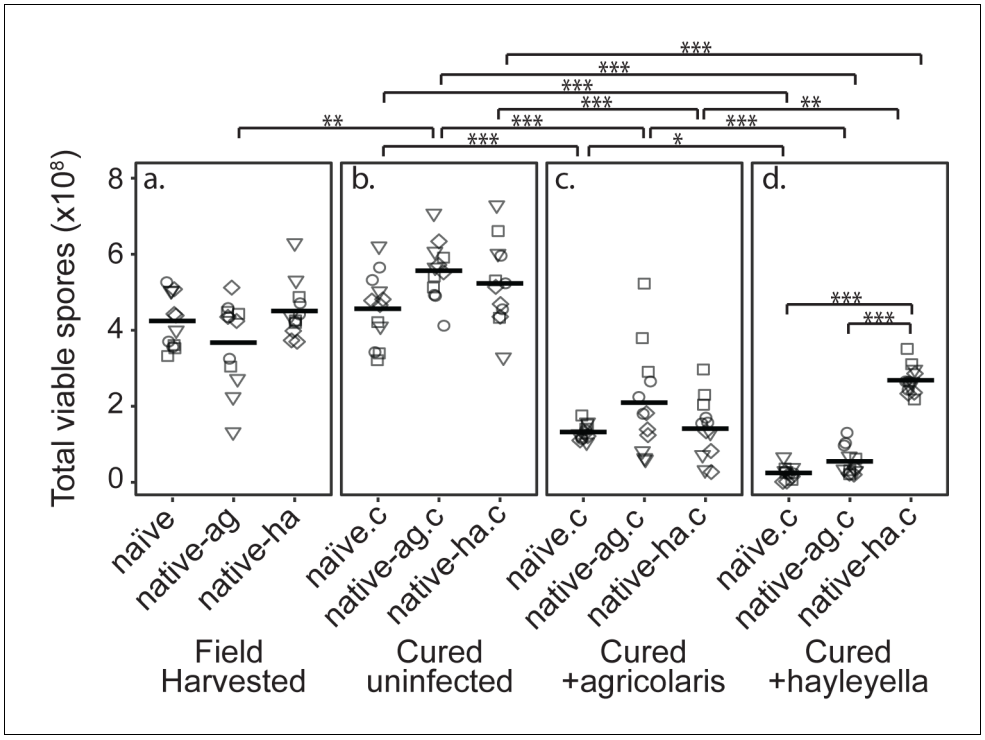

Figure 2. *Burkholderia* Infections Differentially Alter Spore Viability According to *Burkholderia* Species and Host Background. Total viable spores were determined for naïve and native hosts in their field harvested (**a**), cured (**b**), *B. agricolaris* lab-infected (**c**), and *B. hayleyella* lab-infected state (**d**). Four clones were measured for each type with three replicates for each (squares, triangles, circles, and diamonds represent set 1–4 clones respectively). Spore viability for wild harvested *B. agricolaris* and *B. hayleyella* host clones is higher than their cured-re-infected counterparts. Notably, spores from infected *B. agricolaris* and *B. hayleyella* native hosts (either naturally infected or cured and re-infected with their original *Burkholderia*) have a higher fitness than *Burkholderia* infected non-native counterparts. Bars represent significant differences ($p < 0.05$, and as indicated in supplemental tables).
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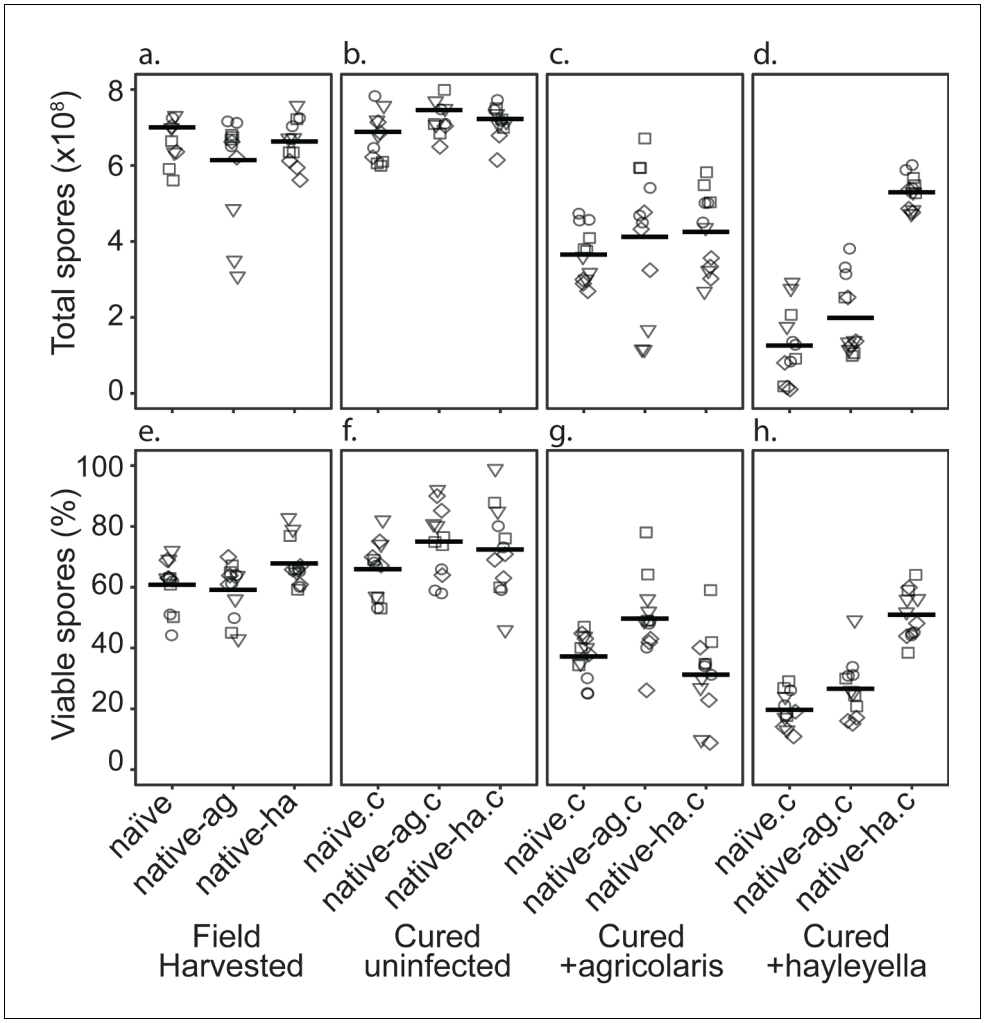

Figure 2—figure supplement 1. Total Spore Number and Percent of Viable Spores for *Burkholderia* Infections in Diverse Host Backgrounds. Total spores (top panel) and percent viable spores (bottom panel) were determined for naïve and native hosts in their field harvested (**a**), cured (**b**), *B. agricolaris* lab-infected (**c**), and *B. hayleyella* lab-infected state (**d**). Four clones were measured for each type with three replicates for each (squares, triangles, circles, and diamonds represent set 1–4 clones respectively). These data were used to determine total viable spores represented in ***Figure 2***.

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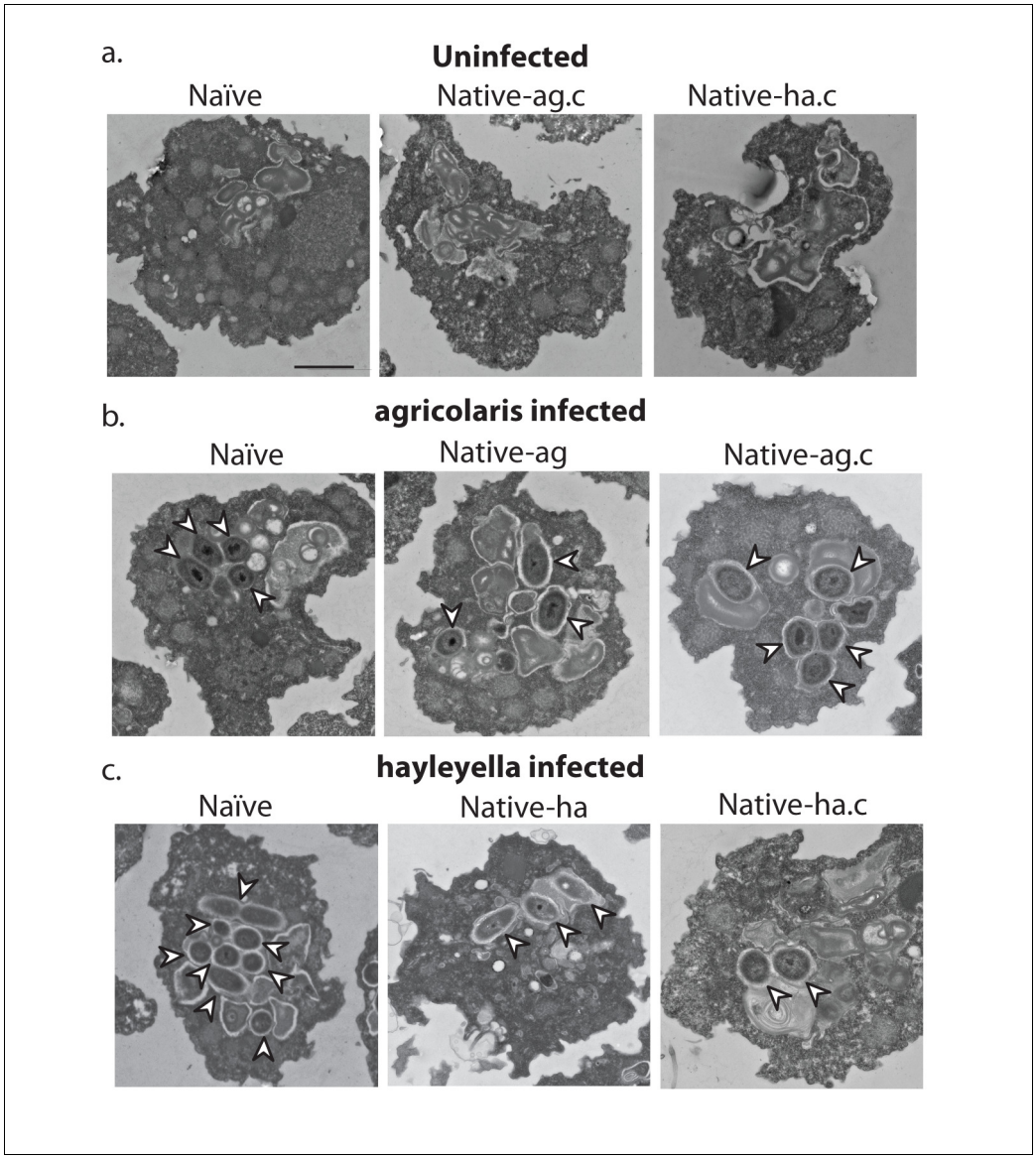

Figure 3. Bacterial cells are found within *Burkholderia* exposed vegetative amoebae. Transmission electron micrographs of vegetative amoebae show naïve and cured native amoebae with intracellular morphologies suggestive of active bacterial digestion with no evidence of intact intracellular bacteria (**a**). In contrast, bacterial cells can be found within *B. agricolaris* (**b**) and *B. hayleyella* (**c**) infected hosts. Arrows point to bacterial cells. More bacteria are observed in the *B. hayleyella* infected naïve host than in field harvested native-*hayleyella* and cured and re-infected native-*hayleyella* hosts (**c**). Bacterial cells appear to be within vacuole-like compartments. Scale bar (applicable to all): 2 um.

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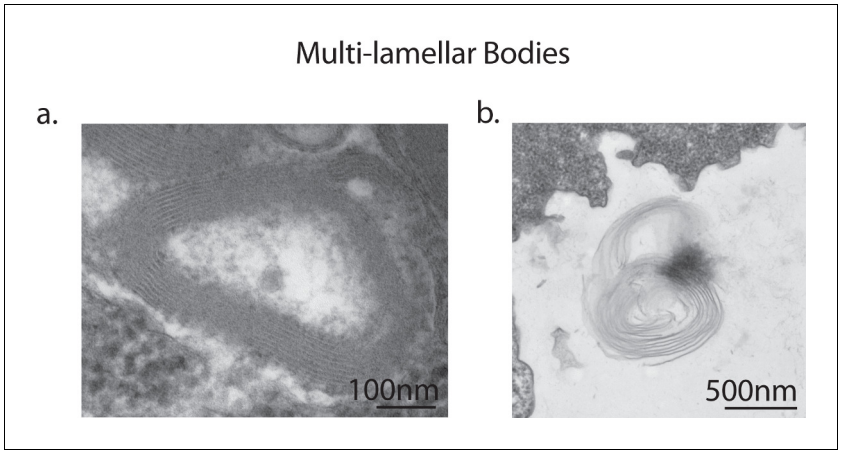

Figure 3—figure supplement 1. Multi-lamellar bodies excreted by vegetative amoebae. Transmission electron micrographs of vegetative amoebae identified multi-lamellar bodies inside uninfected amoebae, indicating successful digestion of bacterial food (**a**). Multi-lamellar bodies are eventually secreted into the surrounding medium (**b**).

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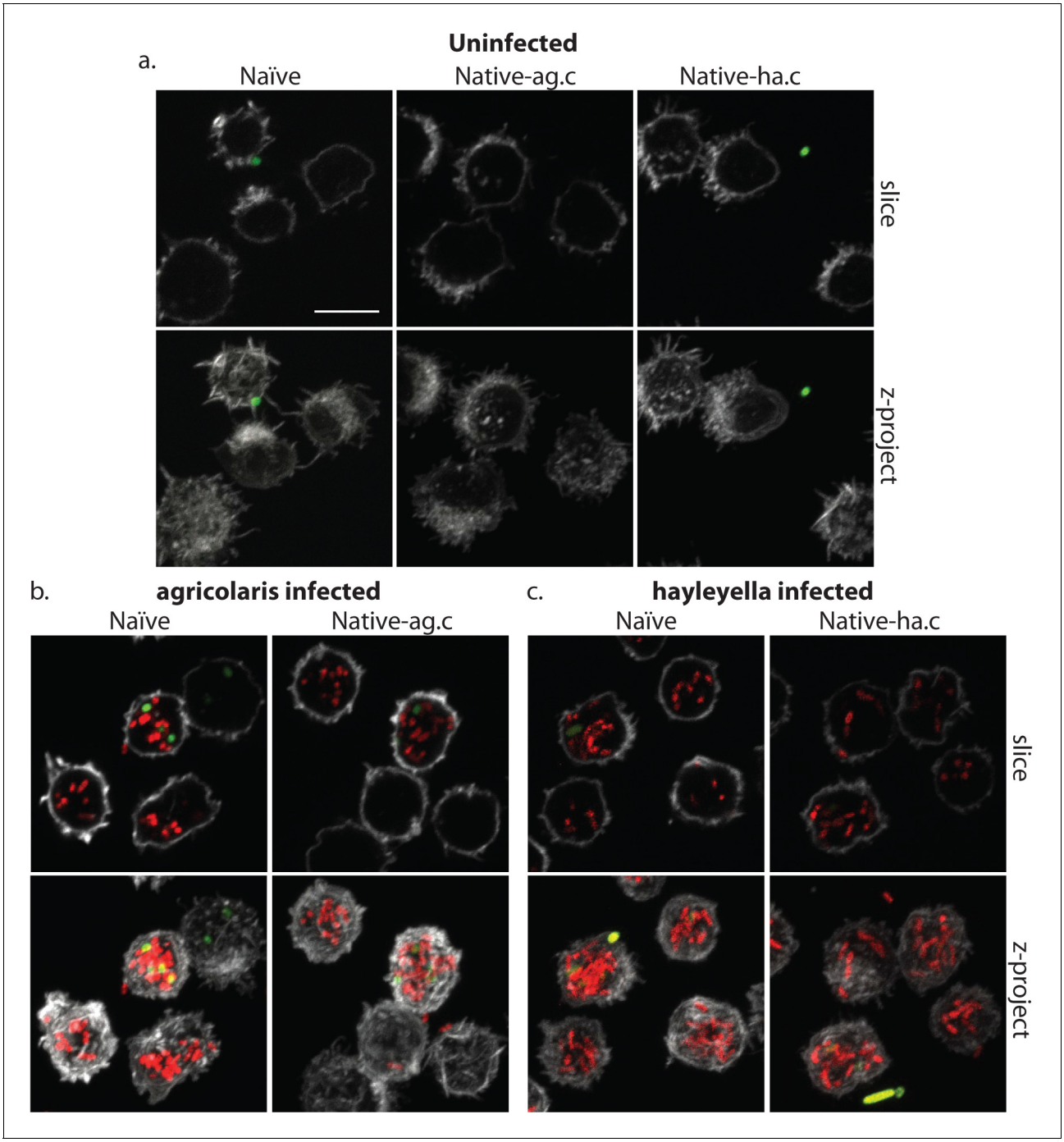

Figure 4. *Burkholderia* is found abundantly in colonized vegetative amoebae. Confocal imaging of fixed and stained vegetative amoebas show little to no intracellular bacteria in uninfected clones (**a**). However, abundant *Burkholderia* (*Burkholderia-RFP* shown in red) is found in *B. agricolaris* (**b**) and *B. hayleyella* (**c**) infected hosts. Occasional intracellular food bacteria (*Klebsiella*-GFP shown in green) is seen in *B. agricolaris* hosts (**c**). Spore coats are stained with phalloidin shown in grey. Scale bar 10 um.

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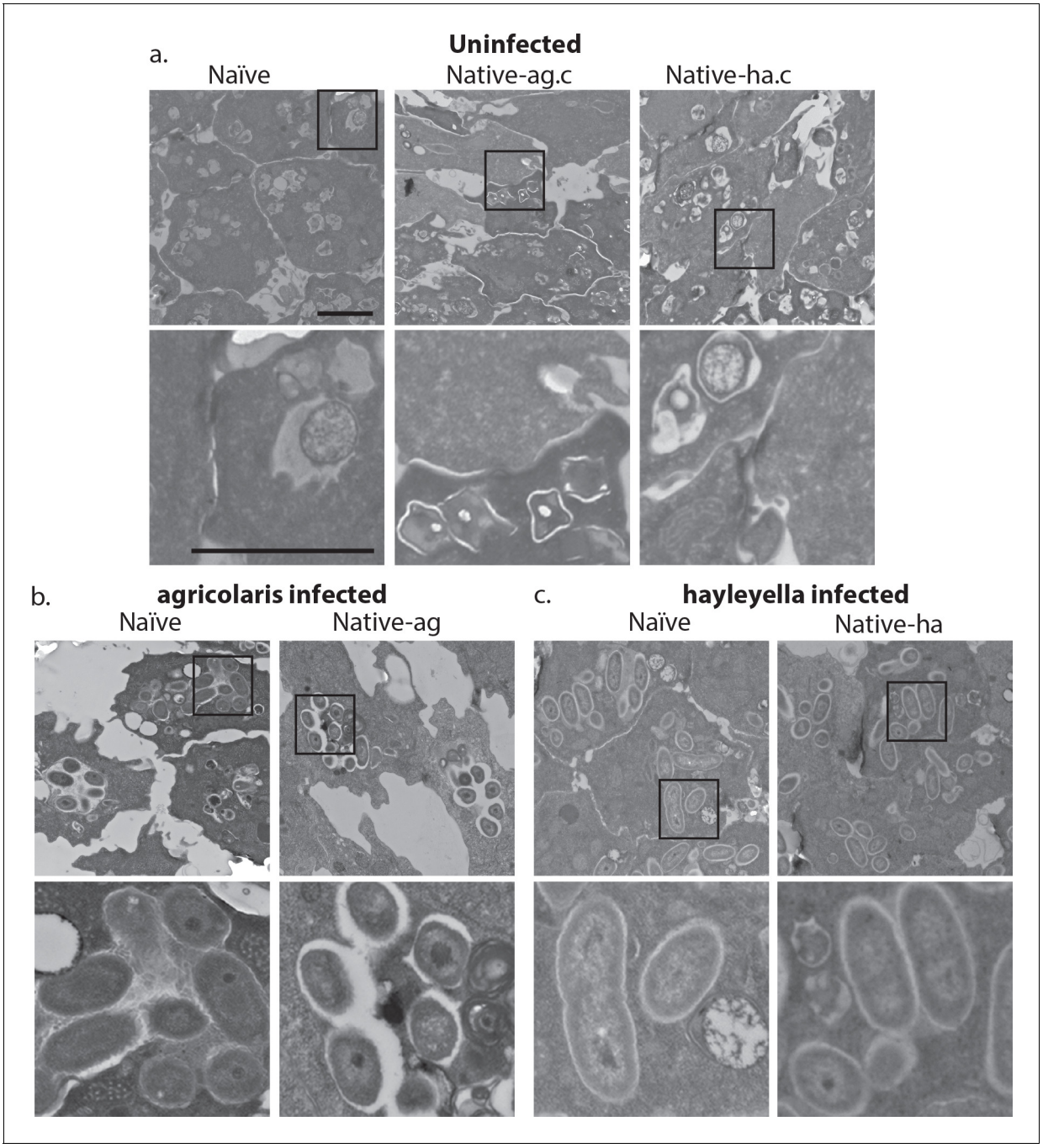

Figure 5. Intracellular bacteria are retained in naïve migrating slugs exposed to *Burkholderia* and in native *Burkholderia* hosts. Transmission electron micrographs of uninfected (**a**) show closely packed amoebae with internal structures reminiscent of previous bacterial digestion but without evidence of intact internal bacteria. In contrast, *B. agricolaris* (**b**) and *B. hayleyella* (**c**) infected slugs retain intracellular bacteria. Bottom panels represent magnified versions (see box) of upper panels. Scale bar (applicable to all panels in row) 2 um.

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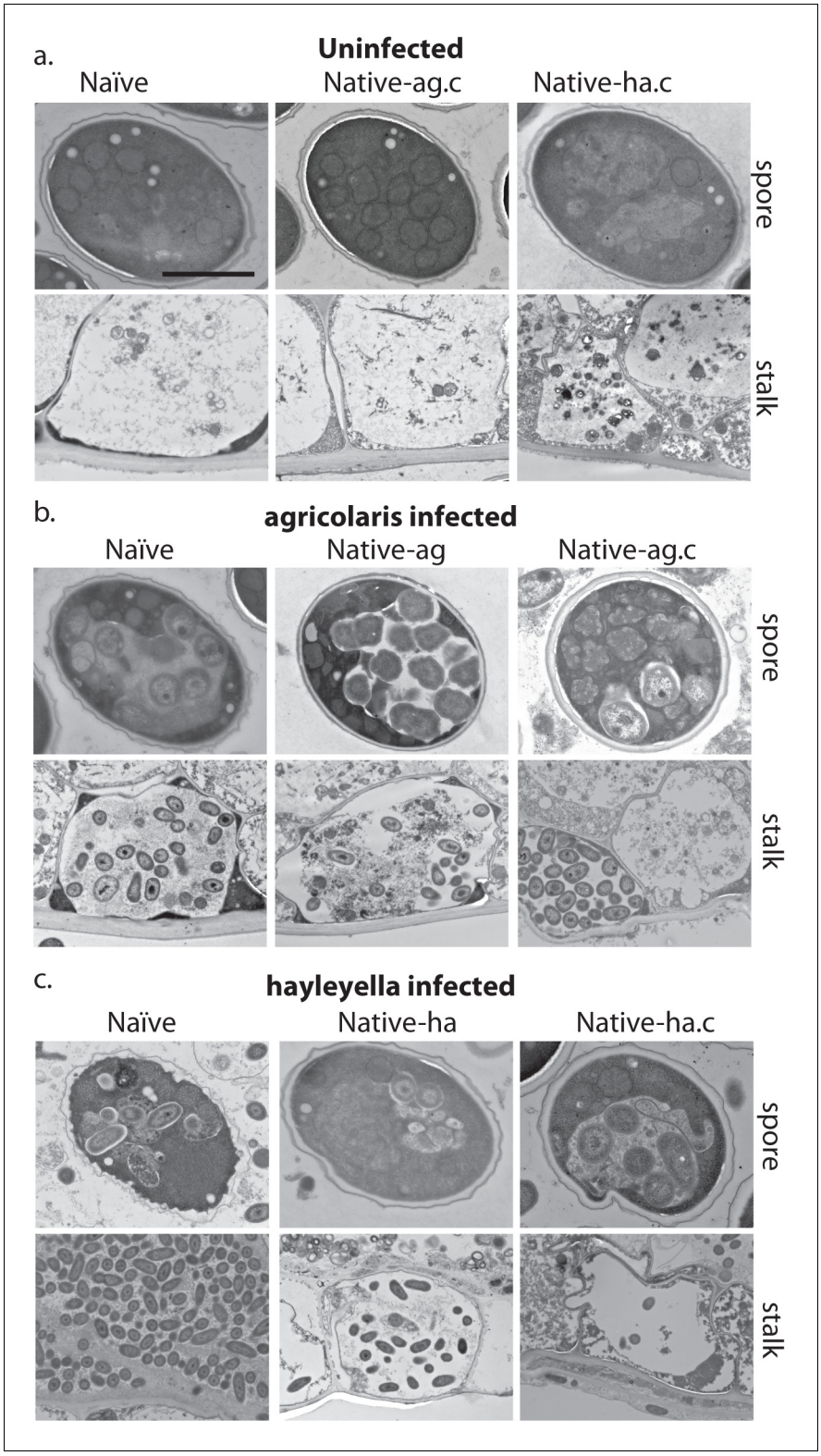

Figure 6. Bacterial cells are retained in spore and stalk cells from *Burkholderia*-exposed hosts. As visualized through transmission electron microscopy, (**a**) uninfected hosts form sturdy spores and stalk cells with no detectable bacteria. Spores and stalk cells retain intracellular bacteria in B. *agricolaris* (**b**) and B. *hayleyella* (**c**) hosts. Naïve *B. agricolaris* hosts appear structurally similar to uninfected cells while naïve *B. hayleyella* hosts have compromised spore coats and collapsed stalk structures filled with bacteria. Scale bar: 2 um.

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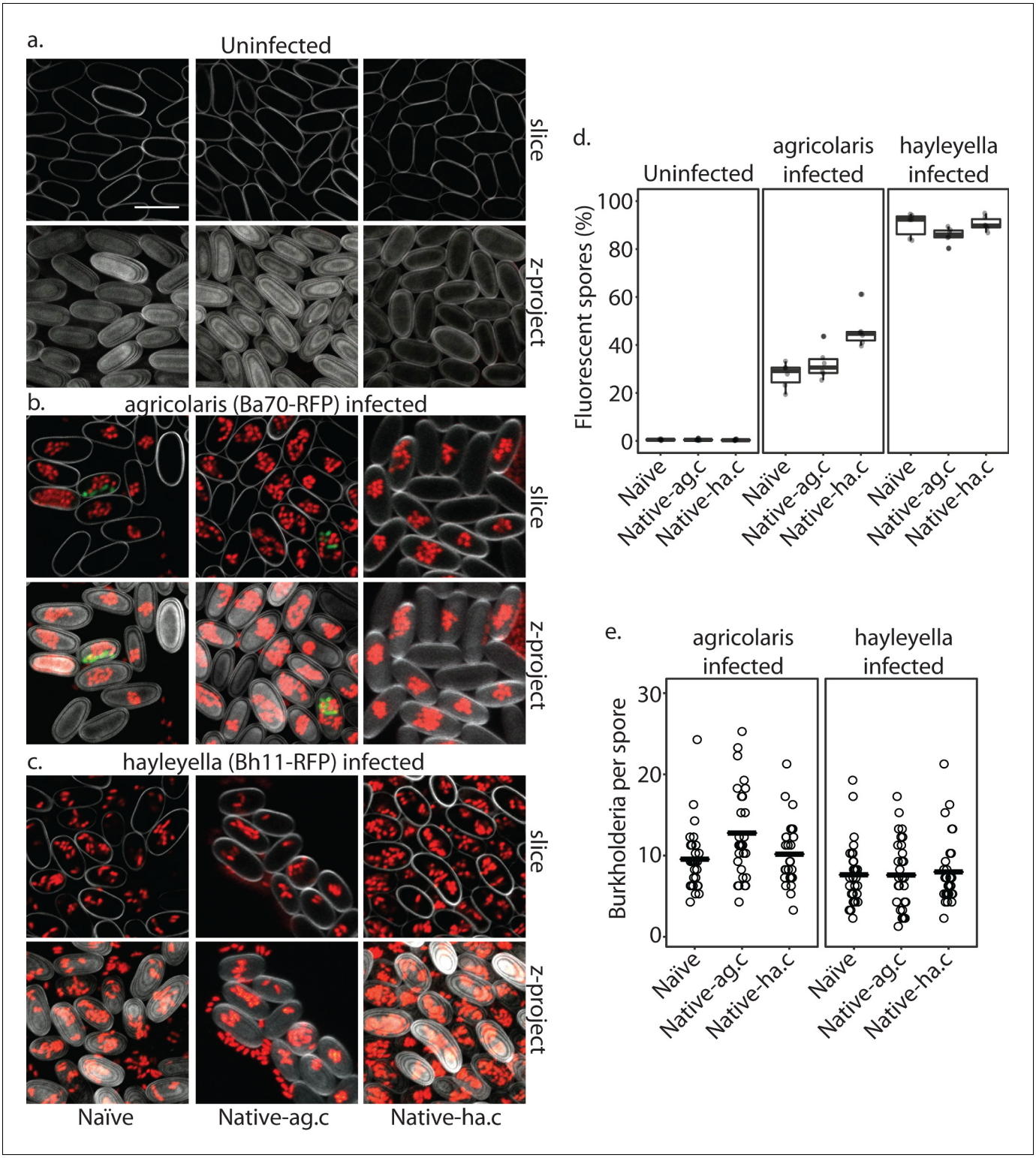

Figure 7. *Burkholderia* is retained in the sori of developed *D. discoideum* hosts and the percent of *Burkholderia* positive spores differs according to *Burkholderia* species. Confocal images show no intra- or extracellular bacteria in uninfected spores (**a**) Abundant *Burkholderia* is seen in *B. agricolaris* (**b**) and *B. hayleyella* (**c**) hosts, with more infected spores seen for *B. hayleyella* (**d**) but more *Burkholderia*-RFP cells detected per infected spore for *B. agricolaris* hosts (**e**). Co-infection by food bacteria is occasionally observed in *B. agricolaris* infected spores (**b**). For a-c: *Klebsiella*-GFP shown in green, *Burkholderia*-RFP shown in red, and calcofluor stain shown in grey. Top panels are image slices; bottom panels are max intensity projections of z stacks. Scale bar: 10 um.

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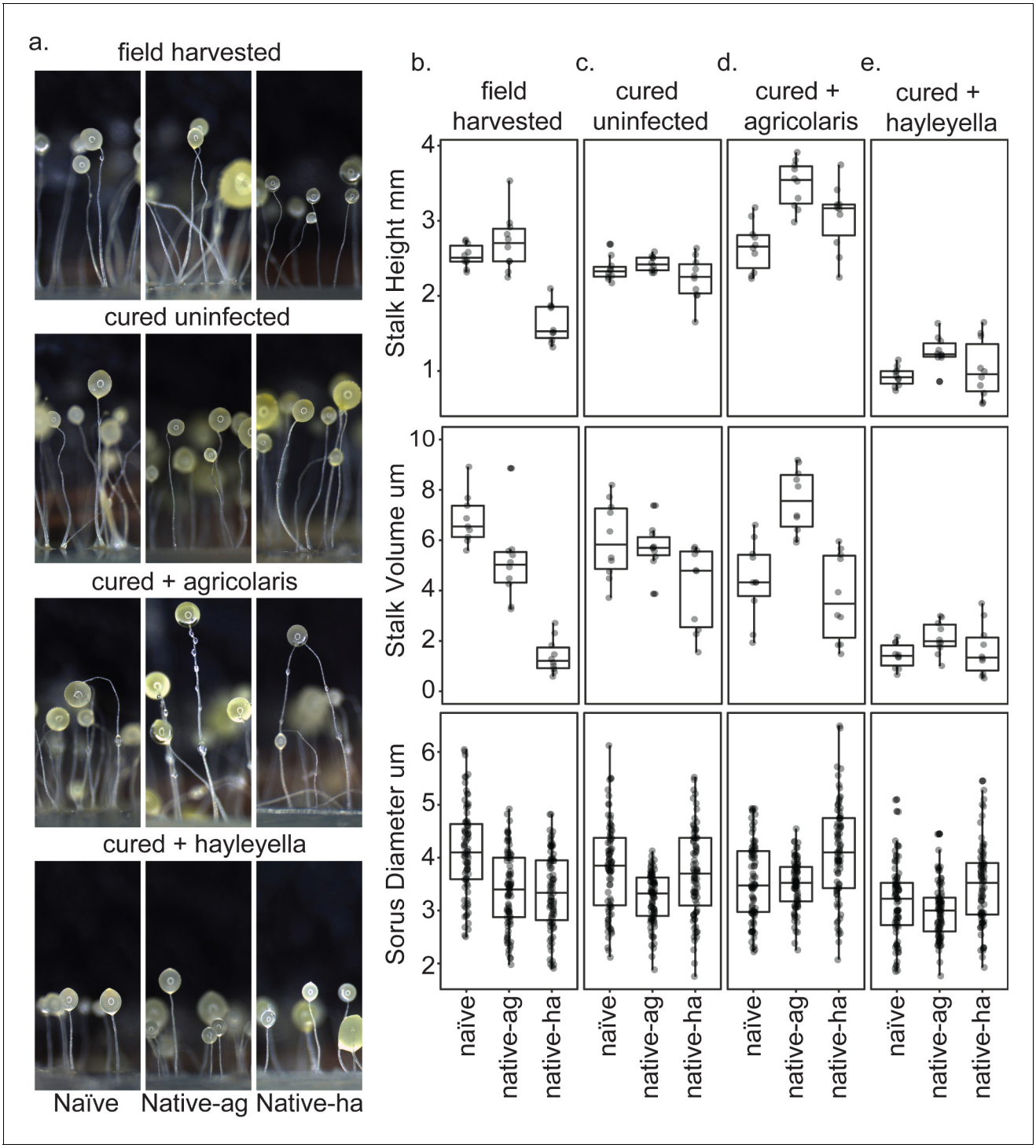

Figure 8. Fruiting body morphology is differentially altered by *Burkholderia* colonization. Macro photographs of fruiting bodies (**a**) show slightly different morphologies according to *Burkholderia* infection status. Sori measurements demonstrate that field collected native-*hayleyella* hosts produce shorter stalks and less voluminous sori (**b**). Cured hosts produce similar fruiting body measurements across host background (**c**). Cured hosts subsequently infected with *B. agricolaris* produce slightly taller stalks, which is most noticeable in cured and re-infected native-*agricolaris* hosts (**d**). Cured hosts subsequently infected with *B. hayleyella* all produce significantly shorter stalks with overall smaller fruiting body dimensions (**e**).
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