

Fig. S1 Metabolites compound of aerial root mucilage of *Heterotis rotundifolia*. **a**, Analysis of different compound (740 metabolites) in aerial root mucilage (ARM) and underground root exudates (URE). **b**, Analysis of Carbohydrate content in URE and ARM.

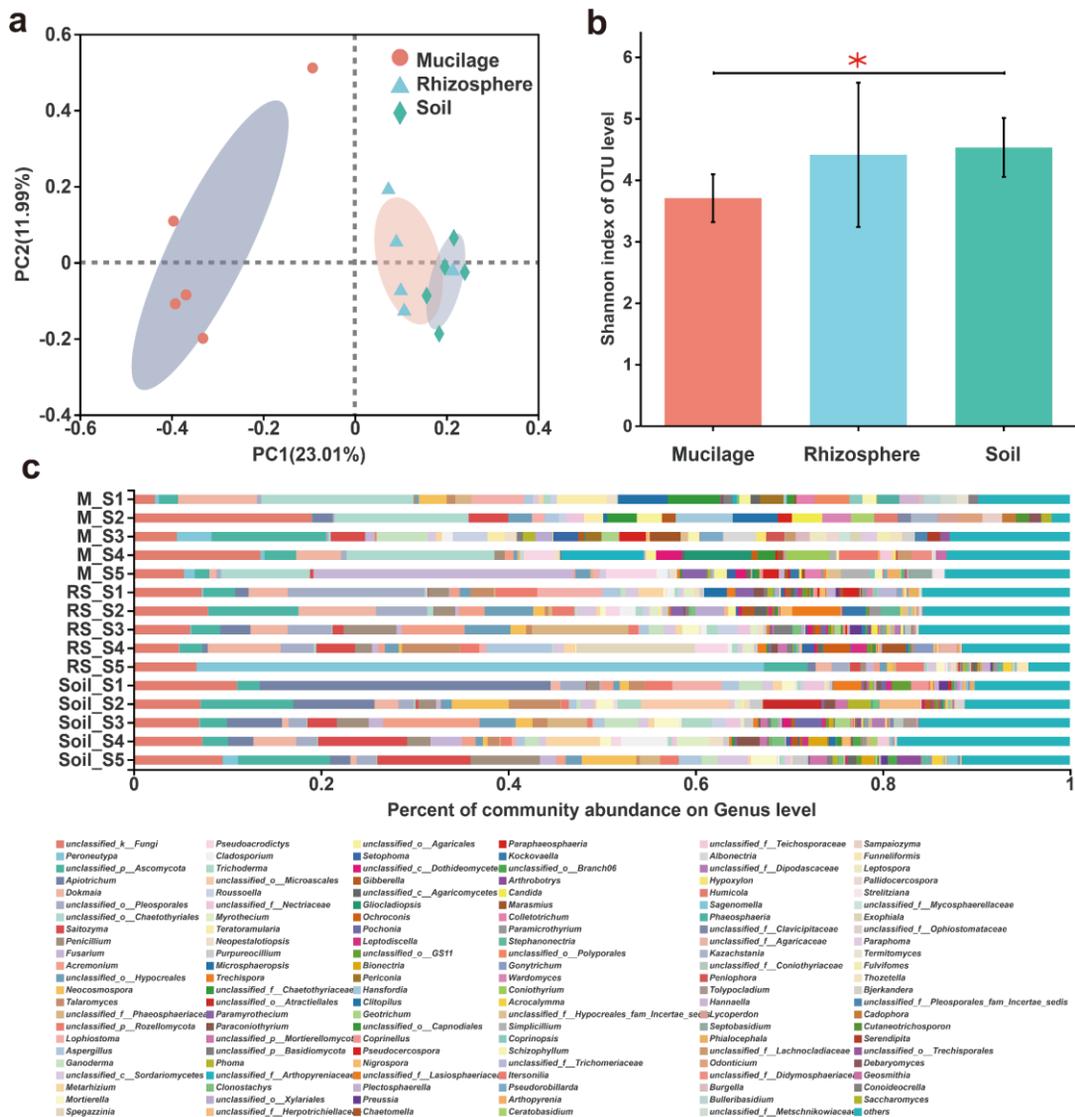


Fig. S2 Fungal diversity of aerial mucilage and rhizosphere. **a**, Unconstrained PCoA with Bray–Curtis distance showing that the fungi of mucilage separate from those of rhizosphere and soil in the first axis ($P < 0.001$, permutational multivariate analysis of variance (PERMANOVA) by Adonis). **b**, Shannon index of the fungal of aerial root mucilage, underground rhizosphere soil and the corresponding bulk soils. **c**, Genus-level distribution of fungal communities in mucilage and rhizosphere soil. The numbers of replicated samples in this figure are as follows: aerial mucilage ($n = 5$), rhizosphere soil ($n = 5$), soil ($n = 3$).

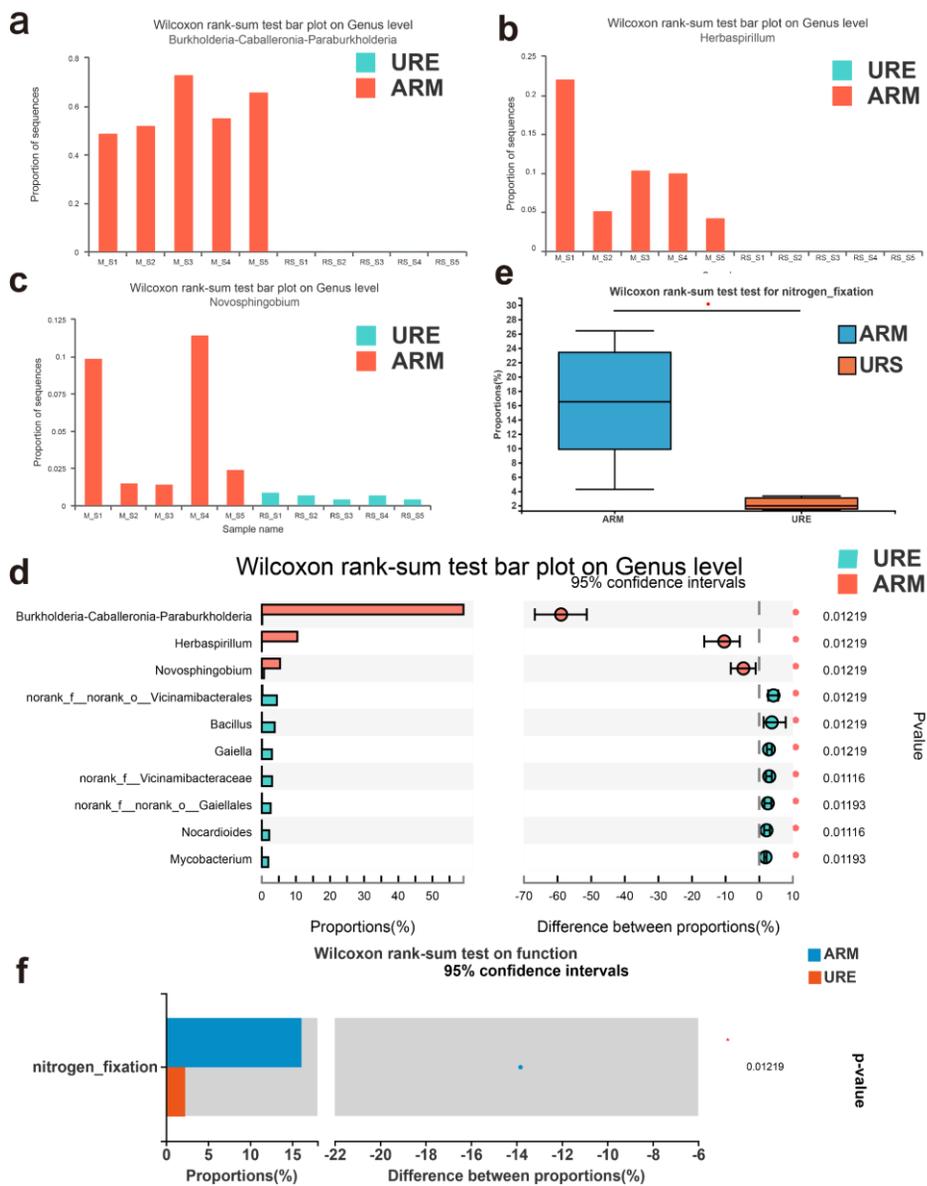


Fig. S3 Mucilage is the main site for nitrogen fixation in *H. rotundifolia* aerial root. a-d, Aerial root mucilage contained higher load of genera Burkholderia (a), Herbaspirillum (b) and Novosphingobium (c). **e-f**, Aerial mucilage has a higher nitrogen fixation capacity than rhizosphere soil (Wilcoxon rank-sum test, $P < 0.05$).

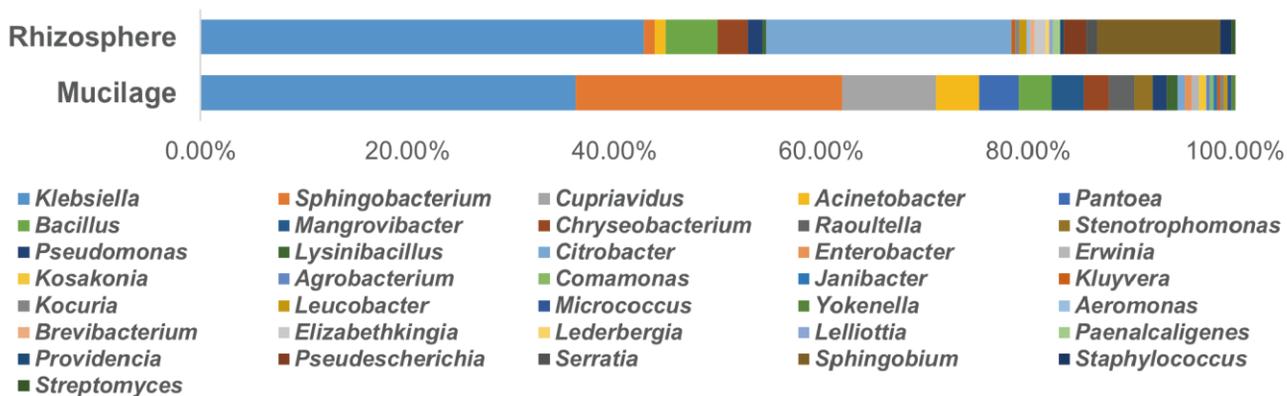
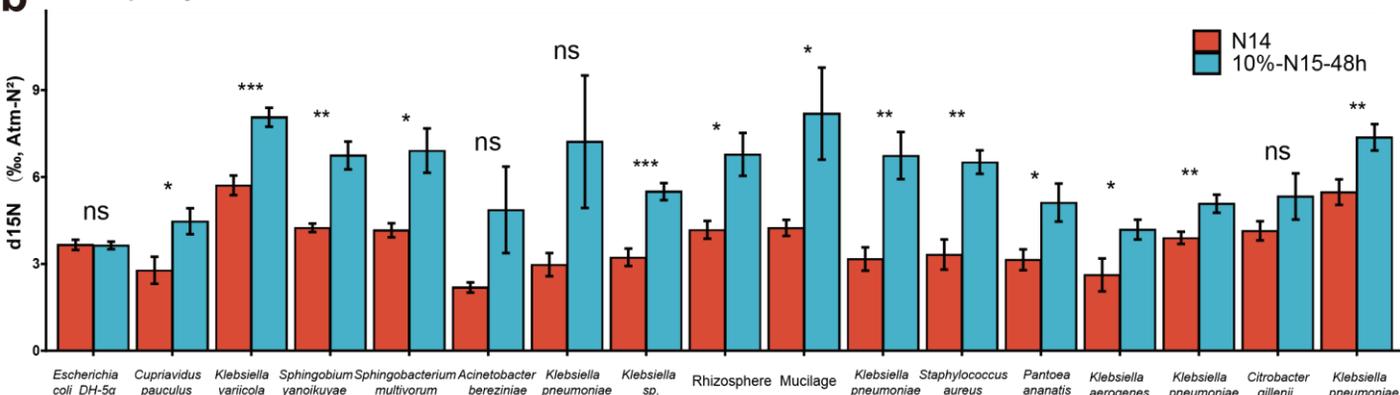
a**Cultured bacterial community****b**

Fig. S4 Bacterial isolates in mucilage and rhizosphere soil and their nitrogen fixation potential. a, Genus-level distribution of bacterial isolates in mucilage and rhizosphere soil. **b, d.** ¹⁵N₂-labeled experiments demonstrated that candidate bacterial strains have nitrogen fixation activity.

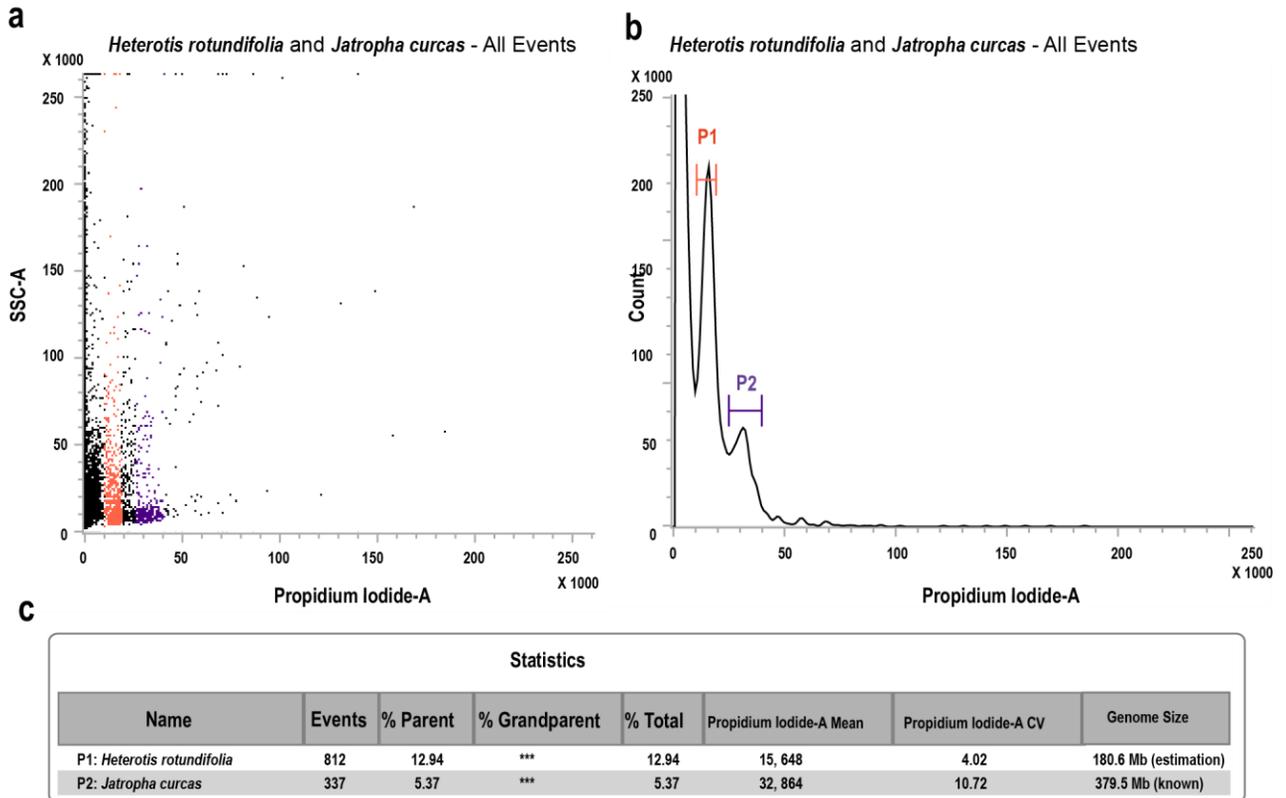


Fig. S5 Flow cytometry of. **a**, Sample preparation mass scatter plot. **b**, Peak of the genome of *Heterotis rotundifolia* and *Jatropha curcas*. **c**, The genome size of *Heterotis rotundifolia* was estimated based on the known genomic features of *Jatropha curcas*.

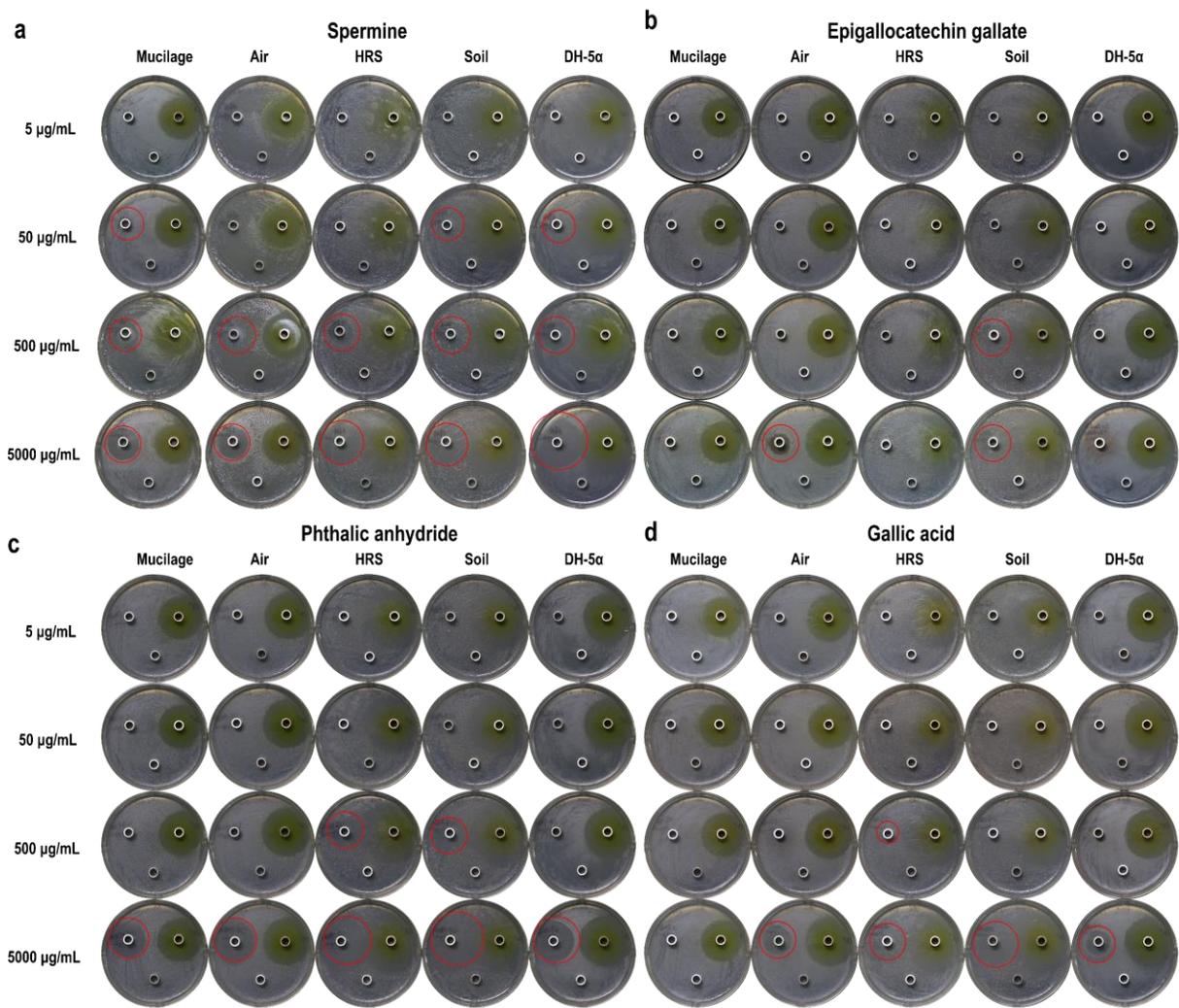


Fig. S6 In vitro activity assay of different compounds against various microbes. a, Spermine; b, Epigallocatechin gallate; c, Phthalic anhydride; d, Gallic acid. Each medium has three Oxford cups, with candidate metabolites on the left, CK⁺(antibiotic) on the right, and CK⁻ (blank solvent) on the bottom.

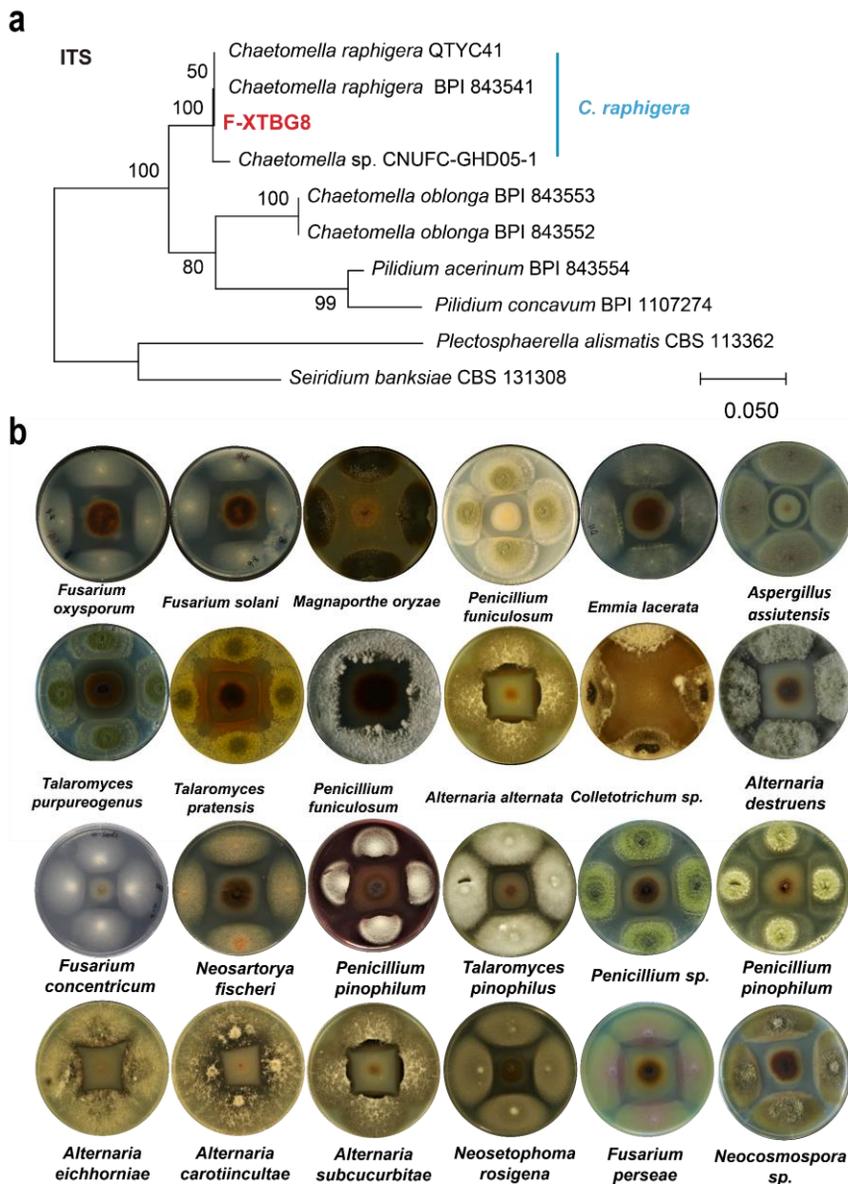


Fig. S7 Partners of mucilage and nitrogen fixing bacteria: a broad-spectrum anti-microbe fungi (F-XTBG8). **a**, Phylogram derived from maximum likelihood analysis of ITS regions Interaction between different microbes and environmental fungi. Only F-XTBG8 can resist the plant pathogenic fungi and various fungi in the environment. **b**, F-XTBG8 resistant to various pathogenic and environmental fungi.