

Supplementary Figure S1 Relative distribution at family level of detected microbial groups per gut region of the passalid beetle. Four gut regions are represented, the foregut (FG), midgut (MG), anterior hindgut (AHG), posterior hindgut (PHG). Sequences were obtained by 454-pyrosequencing of the 16S rDNA V9 region.



Supplementary Figure S2 UPGMA dendrogram based on Weighted-Normalized Unifrac β -diversity showing the clustering of the microbial communities associated with the passalid beetle. Microbial communities were screened with the 16S rDNA pyrosequencing. Four gut regions are represented, the foregut (FG), midgut (MG), anterior hindgut (AHG), posterior hindgut (PHG). All branches showed a Jackknife support > 50%.



Supplementary Figure S3 Phylogenetic reconstruction based on maximum parsimony. Majority rule consensus tree of the most parsimonious tree among clones for each gut region and GenBank selected *nifH* sequences. Only bootstrap values greater than 50% are indicated (100 replicates). GenBank accession numbers of sequences of the most closely related bacteria are shown in parentheses. The scale bar represents the expected number of substitutions per nucleotide position. An *amoA* sequence was used as an out-group.



Supplementary Figure S4 Incorporation of ¹⁵N into total RNA from beetle gut extracts following incubation in a ¹⁵N₂ headspace. Four gut regions are represented, the foregut (FG), midgut (MG), anterior hindgut (AHG), posterior hindgut (PHG). For the FG and PHG regions sufficient mass of RNA was only obtained for a single replicate beetle; for MG and AHG, RNA from duplicate beetles was analyzed. Delta values are expressed relative to atmospheric N showing substantial ¹⁵N enrichment.