

# Additional file 1

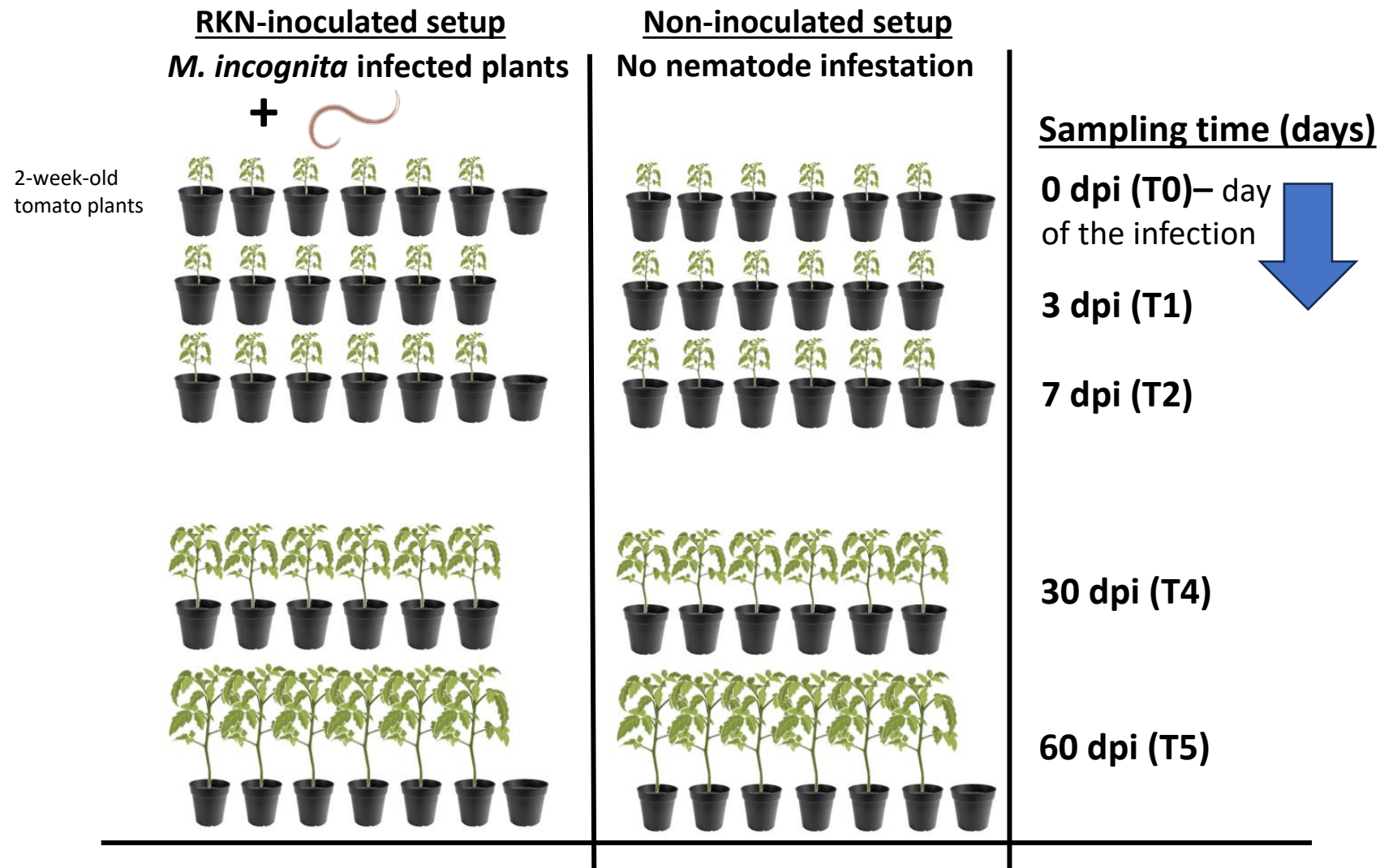
## **Distinct changes in tomato-associated multi-kingdom microbiomes during *Meloidogyne incognita* parasitism**

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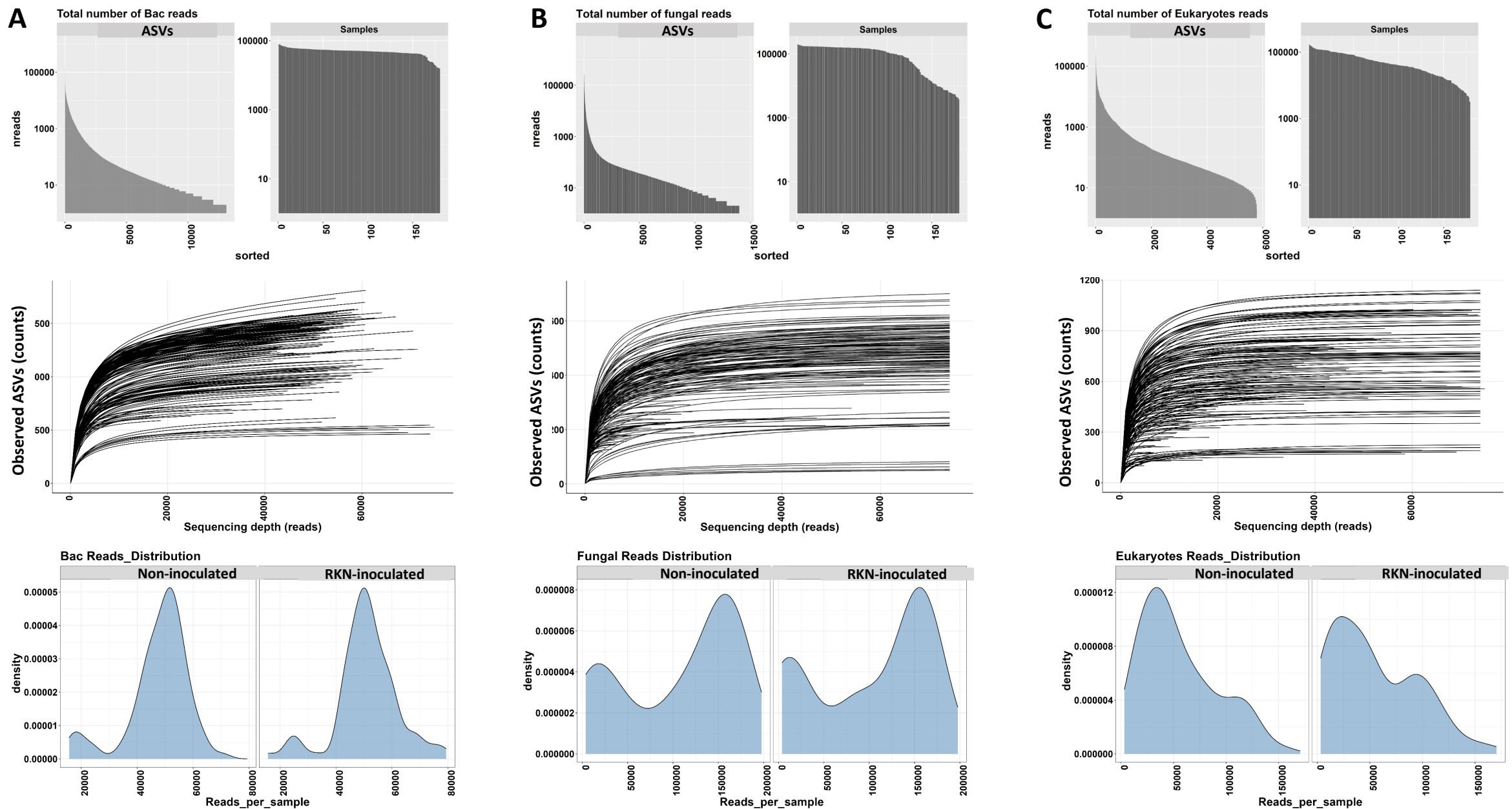
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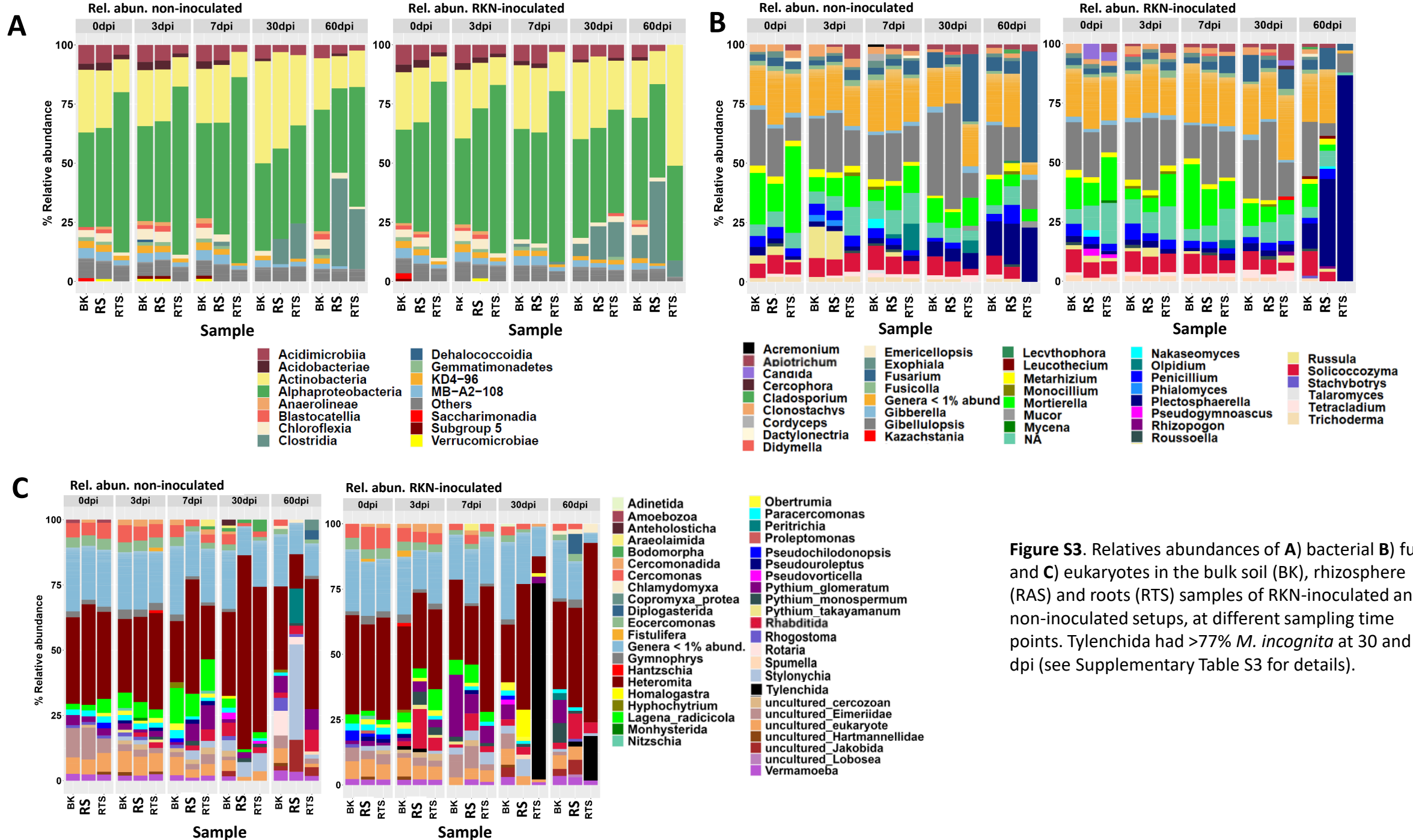
Correspondence: Mette Vestergård (mvestergard@agro.au.dk)

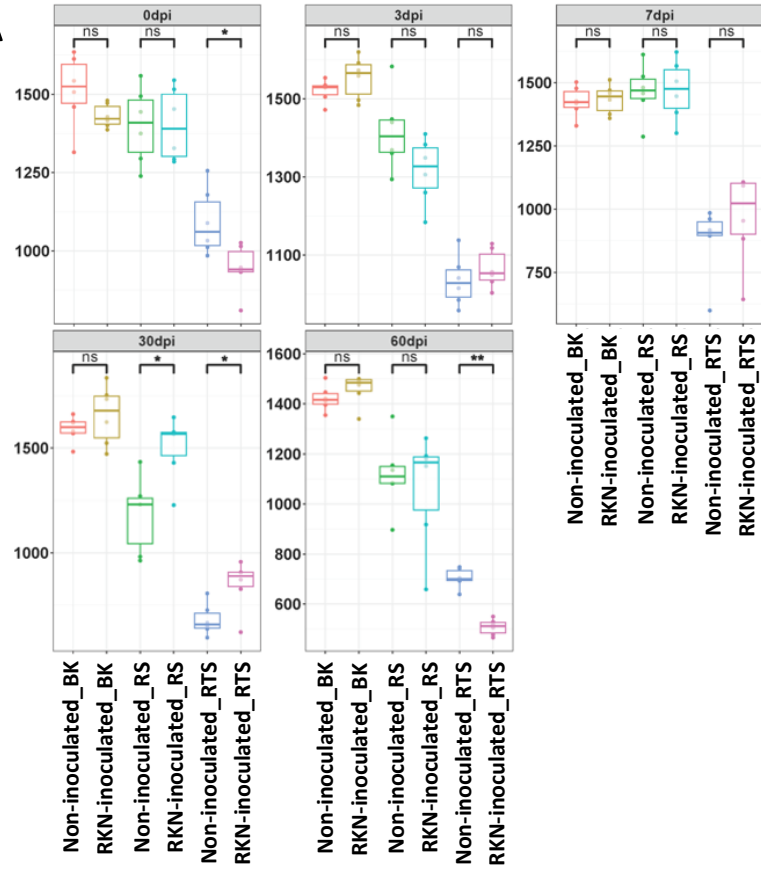
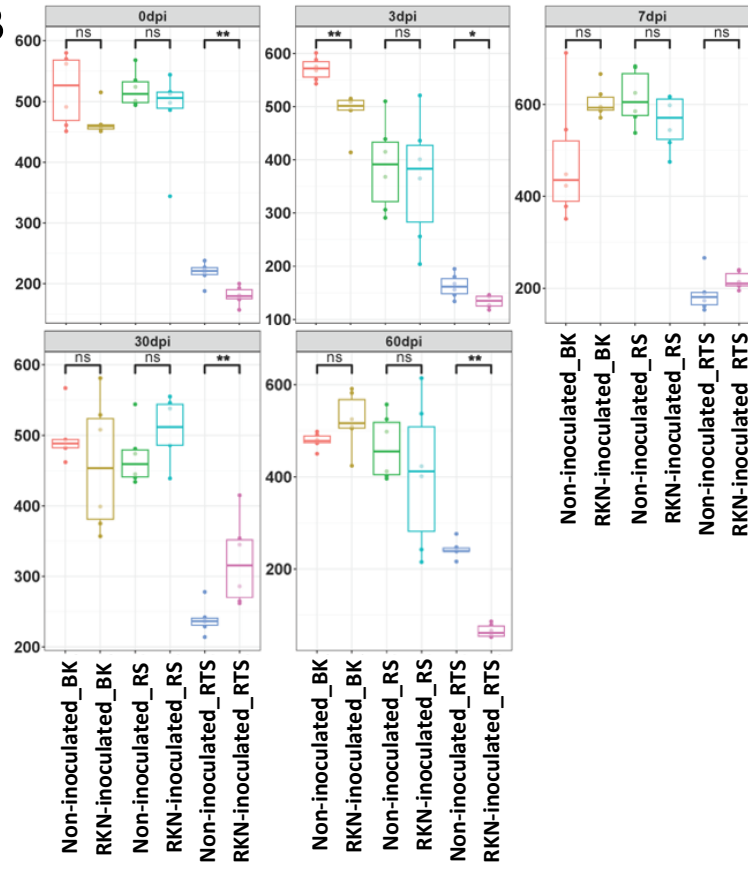
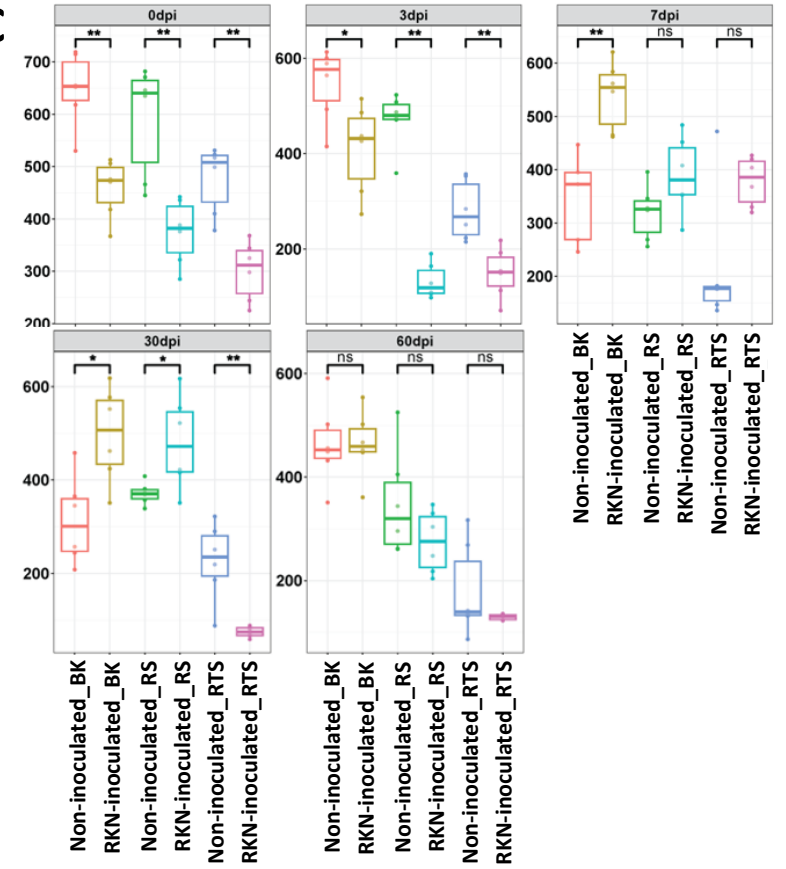


**Figure S1:** Experimental scheme used in this study. Two-weeks old tomato plants were inoculated with root-knot nematode (RKN) *M. incognita* (RKN-inoculated), while control setup had no RKN (Non-inoculated). Samples were taken at time 0 (day of inoculation) and subsequently at 3, 7, 30 and 60 days post RKN inoculation (dpi).

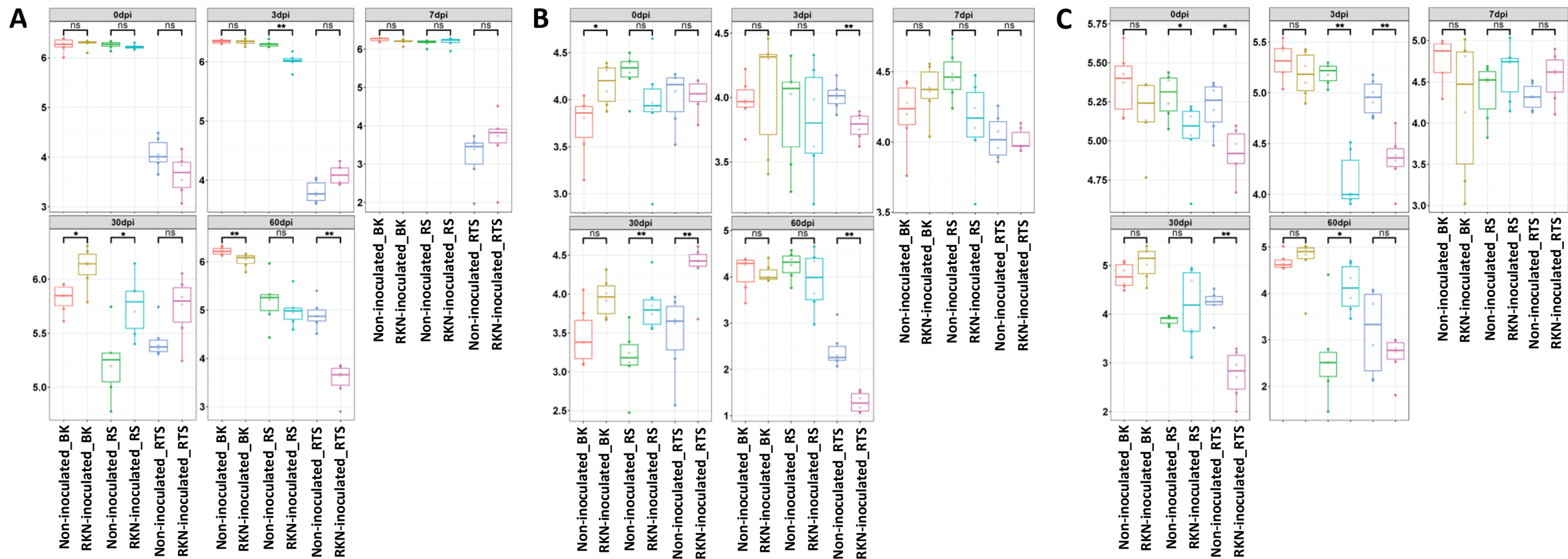


**Figure S2:** Distribution of (A) bacterial and (B) fungal and (C) eukaryotes sequence reads and ASVs, rarefaction curves showing the coverage of ASV richness (species richness in number of ASVs) as a function of sequencing depth (sample size in number of reads) and density plots.

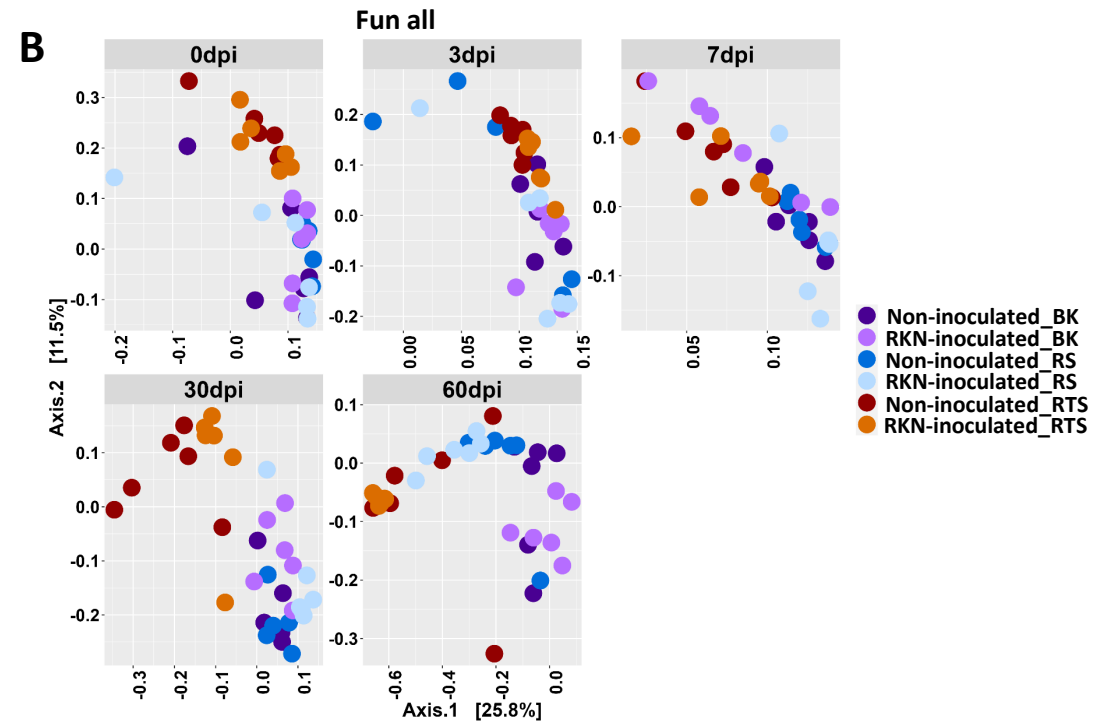
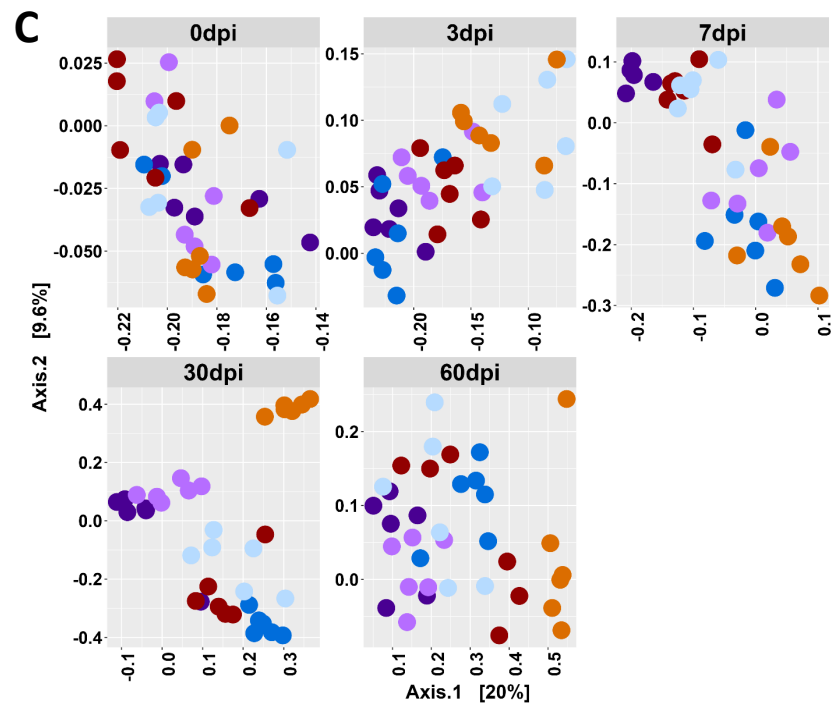
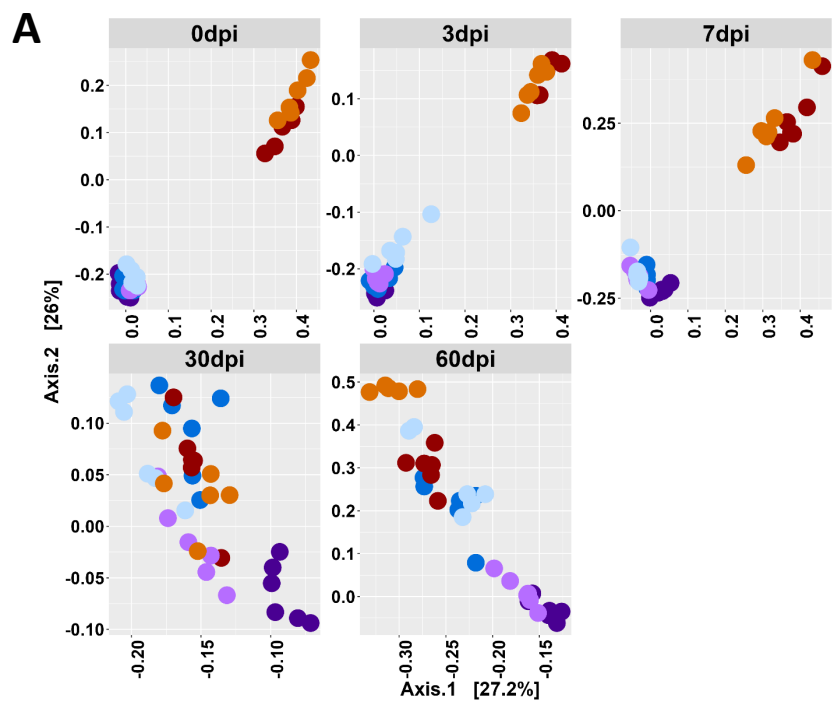


**A****B****C**

**Figure S4.** Observed richness of **A)** bacterial **B)** fungal and **C)** eukaryotes in RKN-inoculated and non-inoculated setups across different sampling time points

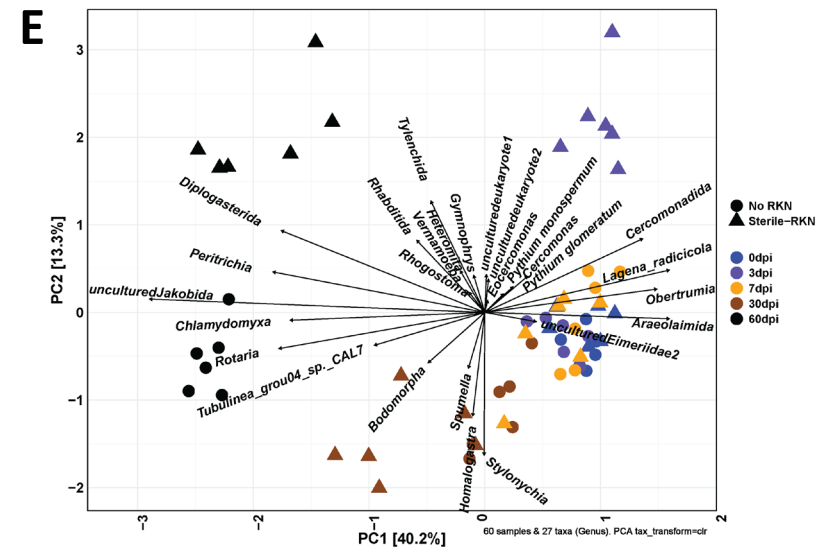
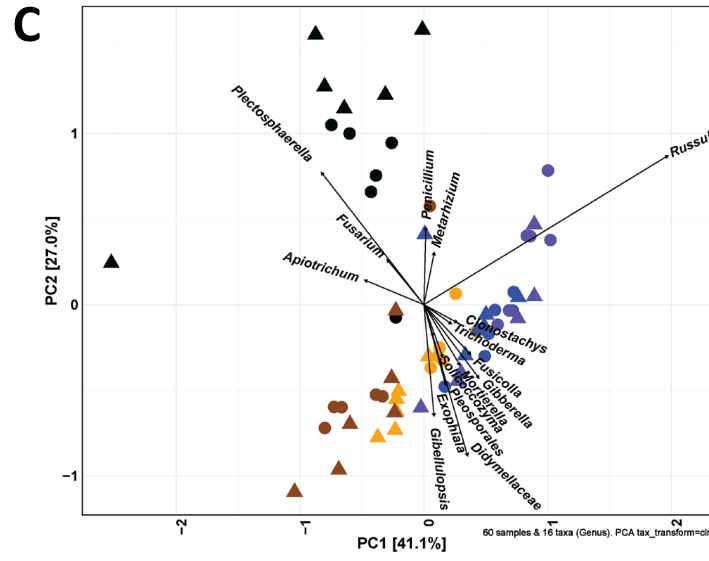
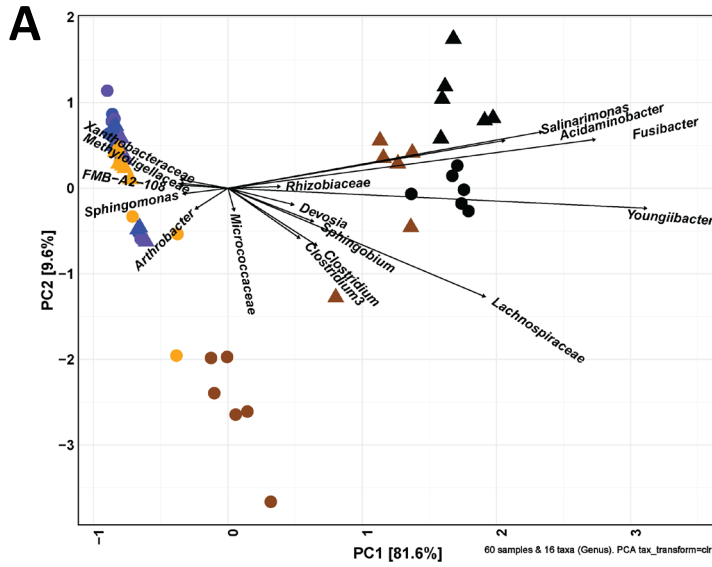


**Figure S5.** Shannon diversity of **A)** bacterial **B)** fungal and **C)** eukaryotes in RKN-inoculated and non-inoculated setups across different sampling time points.

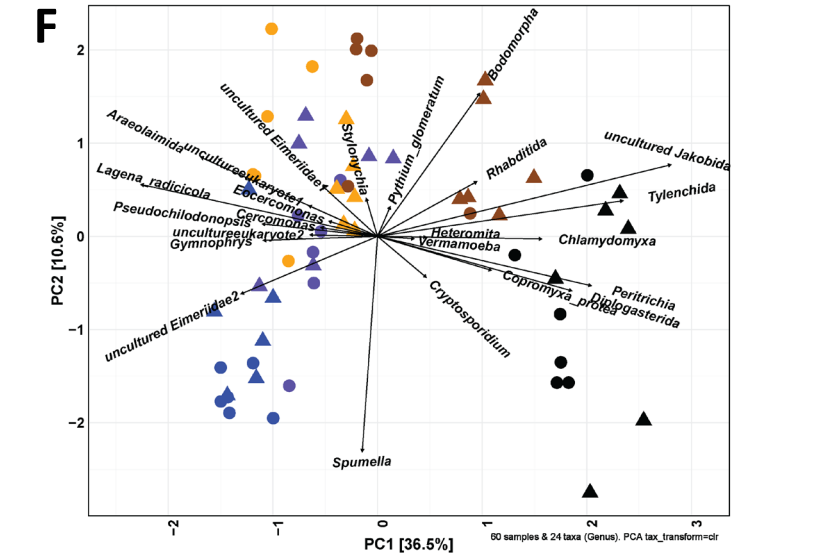
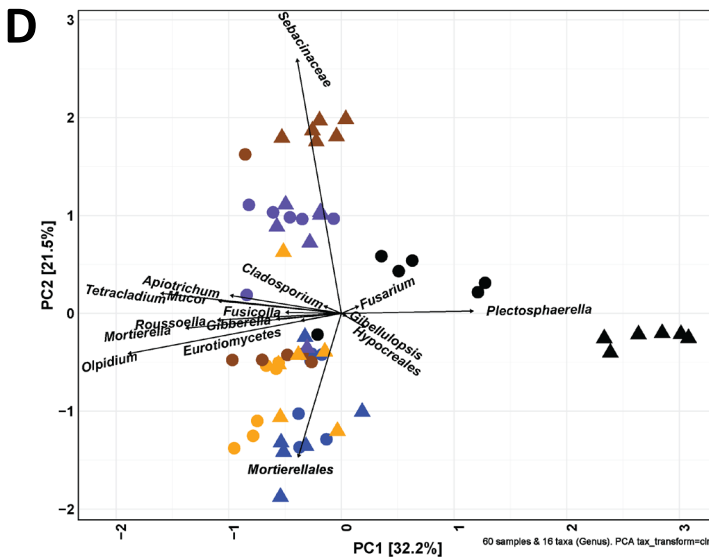
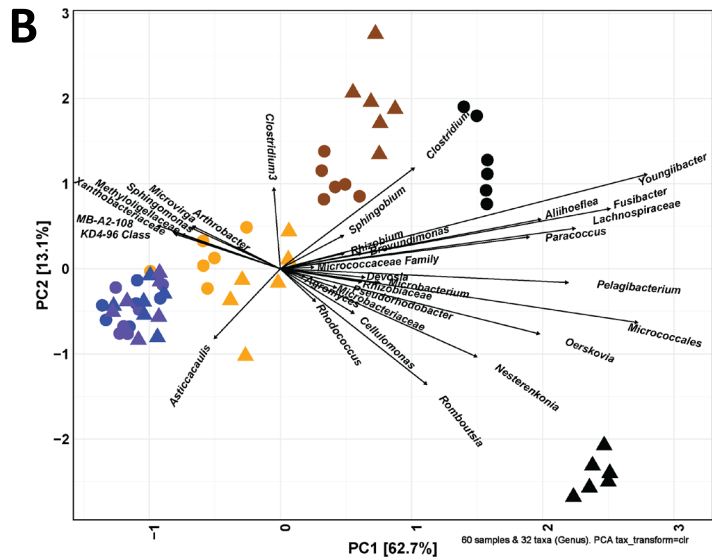


**Figure S6.** Principal coordinates analysis (PCoA) plots of **A)** bacterial **B)** fungal and **C)** protists/nematode communities in RKN-inoculated and non-inoculated setups at different dpi's using all the dataset.

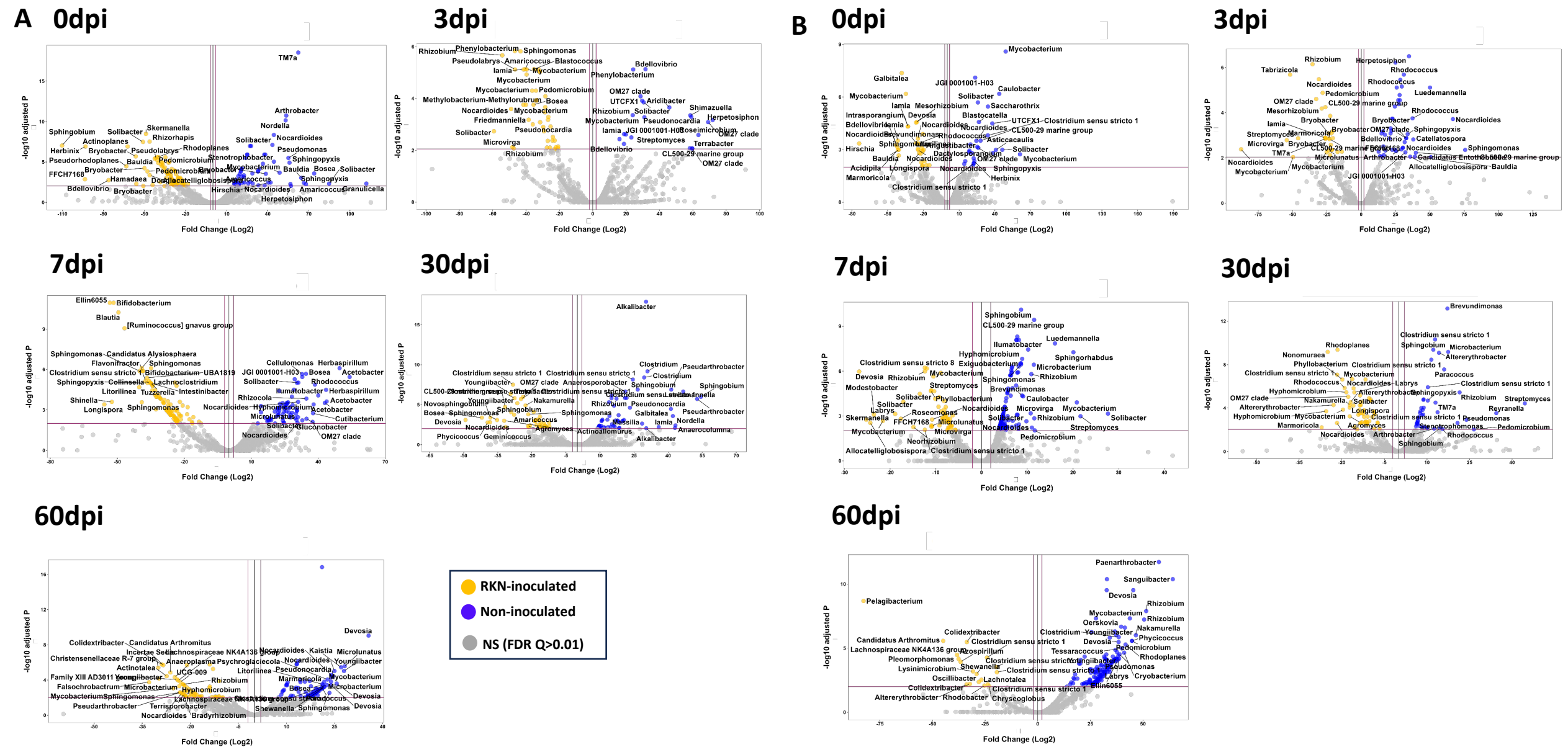
## Rhizosphere



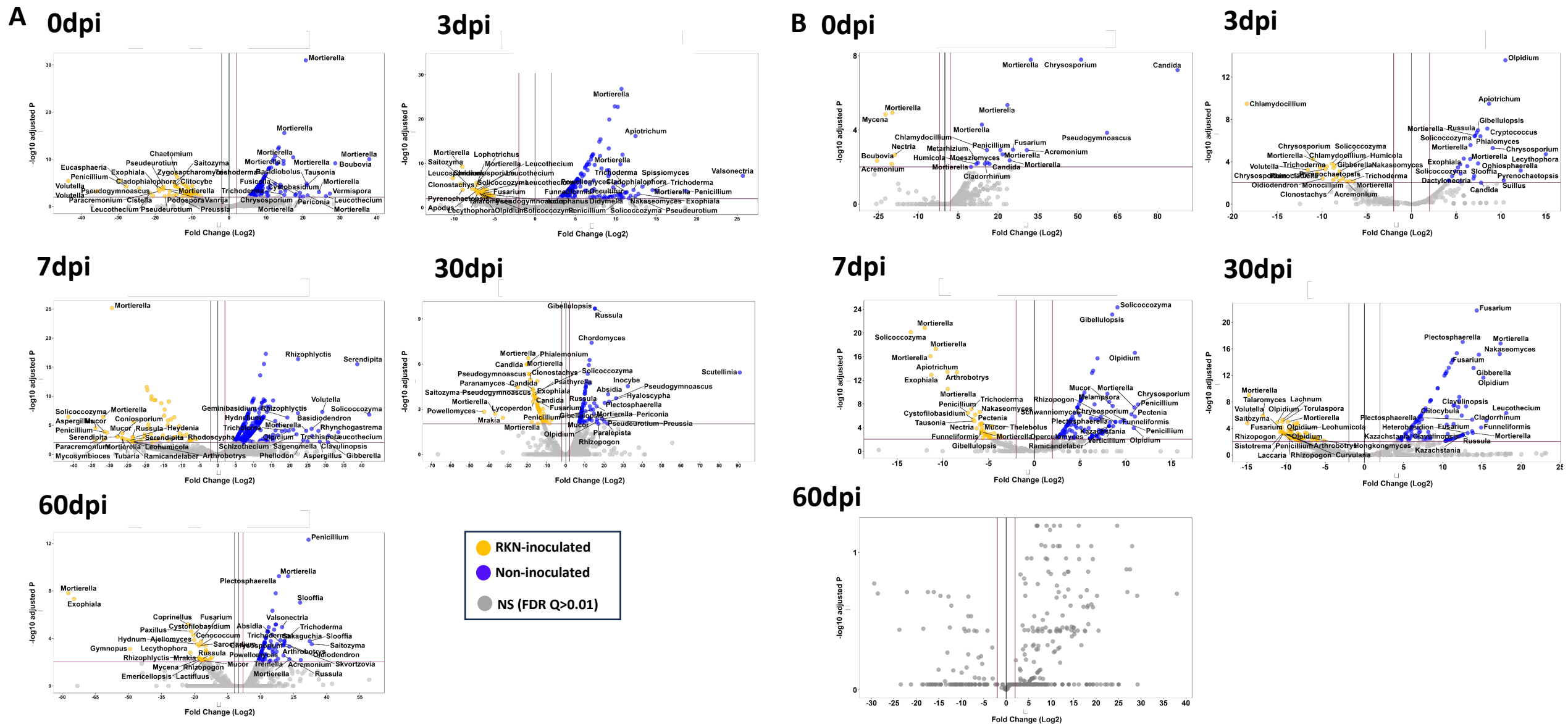
## Roots



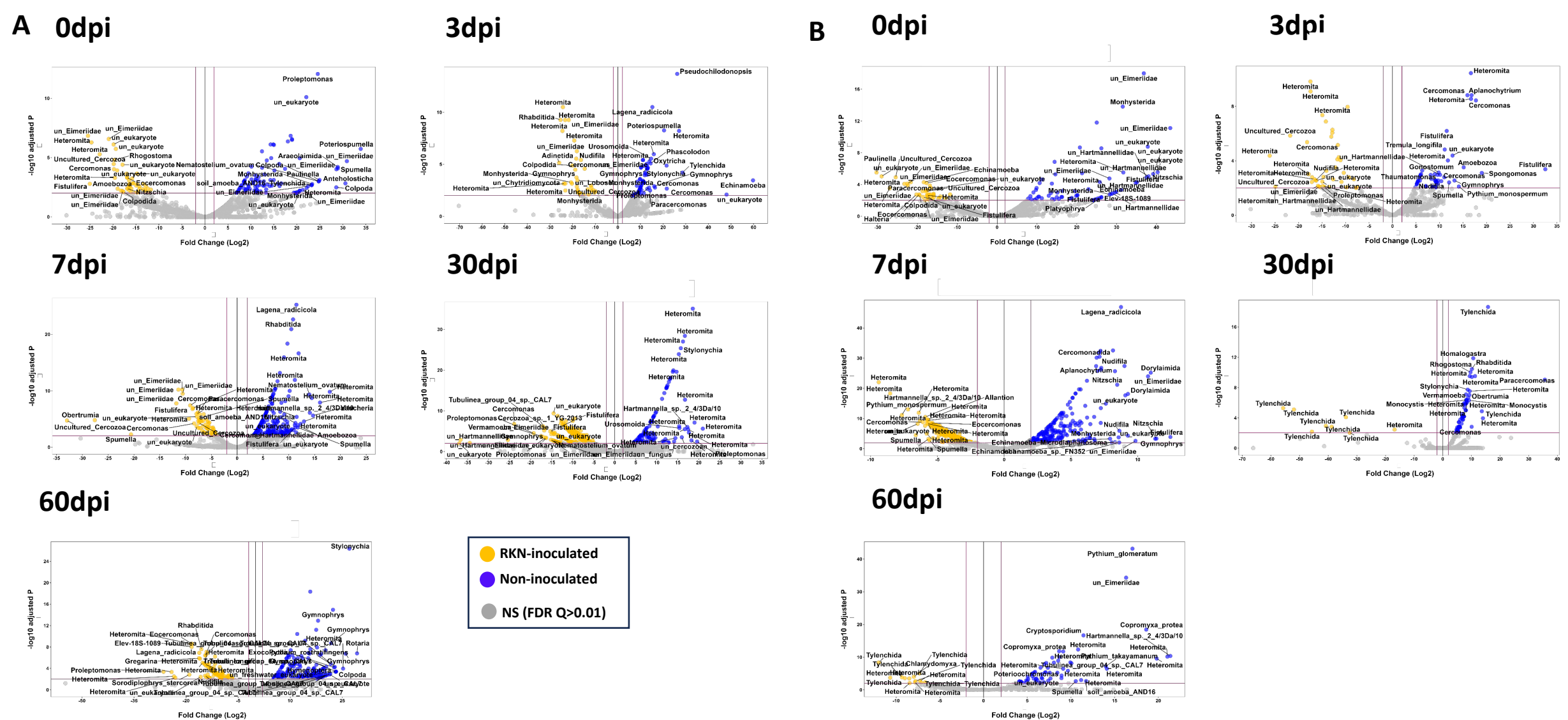
**Figure S7.** Unconstrained Principal Components Analysis (PCA) plots based on the Aitchison distance, showing rhizosphere and roots of bacterial (**A and B**), fungal (**C and D**) and protists/nematode (**E and F**) community compositions in RKN-inoculated and non-inoculated (controls) at different sampling time points.



**Figure S8.** Volcano plot visualization of differentially abundant bacterial taxa (at ASV levels) in **A**) rhizosphere and **B**) roots of RKN-inoculated and non-inoculated samples at different dpi's. Each point represented an individual ASV assigned to corresponding taxa names. The position along the x-axis represents the direction of fold change. The red line showed the threshold of significant differential ASVs ( $|\log_2(FC)| \geq 2$ ). Taxa names of 20 most differentially abundant taxa were shown in each compared set. Differentially enriched ASVs in non-inoculated and RKN-inoculated samples are shown in blue and yellow dots, respectively, while gray dots represent non-significant ASVs.



**Figure S9.** Volcano plot visualization of differentially abundant fungal taxa (at ASV levels) in **A**) rhizosphere and **B**) root of RKN-inoculated and non-inoculated samples at different dpi's. Each point represented an individual ASV assigned to corresponding taxa names. The position along the x-axis represents the direction of fold change. The red line showed the threshold of significant differential ASVs ( $|\log_2(FC)| \geq 2$ ). Taxa names of 20 most differentially abundant taxa were shown in each compared set. Differentially enriched ASVs in non-inoculated and RKN-inoculated samples are shown in blue and yellow dots, respectively, while gray dots represent non-significant ASVs.



**Figure S10.** Volcano plot visualization of differentially abundant abundant protists/nematode taxa (at ASV levels) in **A)** rhizosphere and **B)** root of RKN-inoculated and non-inoculated samples at different dpi's. Each point represented an individual ASV assigned to corresponding taxa names. The position along the x-axis represents the direction of fold change. The red line showed the threshold of significant differential ASVs ( $|\log_2(FC)| \geq 2$ ). Taxa names of 20 most differentially abundant taxa were shown in each compared set. Differentially enriched ASVs in non-inoculated and RKN-inoculated samples are shown in blue and yellow dots, respectively, while gray dots represent non-significant ASVs.

**Supplementary Table S2:** Mean, median and range of reads per compartment for bacterial, fungal and eukaryote libraries.

|                  | Compartment    | Mean     | Median   | Min   | Max    | Total   |
|------------------|----------------|----------|----------|-------|--------|---------|
| Bacteria (16S)   | Non-inoculated | 47464.1  | 50067.5  | 16125 | 70352  | 4271767 |
|                  | RKN-inoculated | 50851.3  | 50767.5  | 15693 | 79352  | 4576616 |
| Fungi (ITS)      | Non-inoculated | 106056.5 | 127972.5 | 4168  | 197541 | 9545089 |
|                  | RKN-inoculated | 100277   | 130952   | 3718  | 182357 | 9024969 |
| Eukaryotes (18S) | Non-inoculated | 38163.3  | 29890    | 2834  | 130224 | 3396534 |
|                  | RKN-inoculated | 36657.9  | 20407    | 2042  | 142304 | 3299213 |

**Supplementary Table S3:** Percent abundance of Tylenchida in eukaryotes community and *M. incognita* in Tylenchida in samples of RKN-inoculated setups, at different sampling time points

| Percent abundance of Tylenchida          |               |             |                |      |         |            |             |            |                       |
|------------------------------------------|---------------|-------------|----------------|------|---------|------------|-------------|------------|-----------------------|
| OTU                                      | Sample        | %Abundance  | Treatment      | Time | SamTime | SampleType | Family      | Genus      |                       |
| 2603 ASV_47                              | RKNBKT13dpi   | 1.323733286 | RKN-inoculated | T1   | 3dpi    | BK         | Chromadorea | Tylenchida |                       |
| 2604 ASV_47                              | RKNRAST13dpi  | 1.390742223 | RKN-inoculated | T1   | 3dpi    | RAS        | Chromadorea | Tylenchida |                       |
| 2610 ASV_47                              | RKNRTST430dpi | 75.13035108 | RKN-inoculated | T4   | 30dpi   | RTS        | Chromadorea | Tylenchida |                       |
| 2608 ASV_47                              | RKNRAST560dpi | 1.87012987  | RKN-inoculated | T5   | 60dpi   | RAS        | Chromadorea | Tylenchida |                       |
| 2599 ASV_47                              | RKNRTST560dpi | 17.05138727 | RKN-inoculated | T5   | 60dpi   | RTS        | Chromadorea | Tylenchida |                       |
| Percent abundance of <i>M. incognita</i> |               |             |                |      |         |            |             |            |                       |
| OTU                                      | Sample        | Abundance   | RKN-inoculated | Time | SamTime | SampleType | Family      | Genus      | Species               |
| 121 ASV_47                               | RKNBKT0       | 15.51724138 | RKN-inoculated | T0   | 3dpi    | BK         | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 123 ASV_47                               | RKNBKT1       | 13.06306306 | RKN-inoculated | T1   | 3dpi    | BK         | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 131 ASV_47                               | RKNRAST1      | 43.90756303 | RKN-inoculated | T1   | 3dpi    | RAS        | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 127 ASV_47                               | RKNRTST1      | 80.06134969 | RKN-inoculated | T1   | 3dpi    | RTS        | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 125 ASV_47                               | RKNBKT2       | 30.46357616 | RKN-inoculated | T2   | 7dpi    | BK         | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 133 ASV_47                               | RKNRAST2      | 3.149606299 | RKN-inoculated | T2   | 7dpi    | RAS        | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 129 ASV_47                               | RKNRTST2      | 27.86259542 | RKN-inoculated | T2   | 7dpi    | RTS        | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 126 ASV_47                               | RKNBKT4       | 36.58536585 | RKN-inoculated | T4   | 30dpi   | BK         | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 122 ASV_47                               | RKNRAST4      | 4.354354354 | RKN-inoculated | T4   | 30dpi   | RAS        | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 135 ASV_47                               | RKNRTST4      | 77.10513204 | RKN-inoculated | T4   | 30dpi   | RTS        | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 128 ASV_47                               | RKNBKT5       | 64.74719101 | RKN-inoculated | T5   | 60dpi   | BK         | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 124 ASV_47                               | RKNRAST5      | 44.11177645 | RKN-inoculated | T5   | 60dpi   | RAS        | Chromadorea | Tylenchida | Meloidogyne_incognita |
| 132 ASV_47                               | RKNRTST5      | 79.53939736 | RKN-inoculated | T5   | 60dpi   | RTS        | Chromadorea | Tylenchida | Meloidogyne_incognita |

**Supplementary Table S4:** Summary of permutational analysis of variance (PERMANOVA) using the “adonis” test on Bray-Curtis distance matrices for bacterial, fungal and eukaryotes community dissimilarity assessment using 1,000 permutations. The whole dataset including BK was used for this analysis.

| Dataset | Factors     | Bacteria (16S) R <sup>2</sup> | Fungi (ITS) R <sup>2</sup> | Eukaryotes (18S) R <sup>2</sup> |
|---------|-------------|-------------------------------|----------------------------|---------------------------------|
| Whole   | Compartment | 0.22***                       | 0.35***                    | 0.07***                         |
|         | treatment   | 0.01*                         | 0.01*                      | 0.01**                          |

|        |                         |         |         |          |
|--------|-------------------------|---------|---------|----------|
|        | Compartment x treatment | ns      | ns      | 0.02***  |
| 0 dpi  | Compartment             | 0.68*** | 0.21*** | 0.10 **  |
|        | treatment               | ns      | 0.05*   | 0.12***  |
|        | Compartment x treatment | ns      | ns      | 0.17***  |
| 3 dpi  | Compartment             | 0.68*** | 0.29*** | 0.15***  |
|        | treatment               | 0.03*   | 0.09*** | 0.13***  |
|        | Compartment x treatment | 0.03    | ns      | 0.13***  |
| 7 dpi  | Compartment             | 0.70*** | 0.27*** | 0.08**   |
|        | treatment               | 0.04*   | 0.05**  | 0.12***  |
|        | Compartment x treatment | ns      | 0.08**  | 0.21***  |
| 30 dpi | Compartment             | 0.27*** | 0.34*** | 0.27 *** |
|        | treatment               | 0.15*** | 0.07*** | 0.16***  |
|        | Compartment x treatment | 0.07**  | 0.09**  | 0.17***  |
| 60 dpi | Compartment             | 0.38*** | 0.40*** | 0.23 *** |
|        | treatment               | 0.16*** | 0.10*** | 0.09***  |
|        | Compartment x treatment | 0.15*** | 0.14*** | 0.12***  |

Significance of test indicated as \*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ . The ns denotes not statistically significant, - not determined and  $R^2$  is the proportion of variation explained.

**Supplementary Table S5:** Beta diversity based pairwise comparison between compartments of RKN inoculated (RKN) and non-inoculated (Non-inoculated) samples using pairwise.adonis function

| Community | DPI | Factors (Compartment)                    | $R^2$ (All dataset) | $R^2$ (Without BK) |
|-----------|-----|------------------------------------------|---------------------|--------------------|
| Bacteria  | T0  | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.12*               | -                  |
|           |     | Non-inoculated_RAS_vs_RKN-inoculated_RAS | ns                  | ns                 |
|           |     | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.17*               | 0.18*              |
|           | T1  | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.22**              | -                  |
|           |     | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.17*               | 0.18*              |
|           |     | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.20**              | 0.20               |

|       |    |                                          |        |        |
|-------|----|------------------------------------------|--------|--------|
|       | T2 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.32** | -      |
|       |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | ns     | ns     |
|       |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | ns     | ns     |
|       | T4 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.35** | -      |
|       |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.21** | 0.21** |
|       |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.38** | 0.37** |
|       | T5 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.37** | -      |
|       |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.37** | 0.39** |
|       |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.62** | 0.59** |
| Fungi | T0 | Non-inoculated_BK_vs_RKN-inoculated_BK   | ns     | -      |
|       |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.12*  | 0.12*  |
|       |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.20*  | 0.20*  |
|       | T1 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.36** | -      |
|       |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | ns     | ns     |
|       |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.18*  | 0.18   |
|       | T2 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.22** | -      |
|       |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | ns     | 0.13*  |
|       |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | ns     | ns     |
|       | T4 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.25*  | -      |
|       |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.18*  | 0.18*  |
|       |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.25** | 0.25** |

|            |    |                                          |         |         |
|------------|----|------------------------------------------|---------|---------|
|            | T5 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.19**  | -       |
|            |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.20**  | 0.20**  |
|            |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.57**  | 0.57**  |
| Eukaryotes | T0 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.28*** | -       |
|            |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.35*** | 0.35**  |
|            |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.31*** | 0.31**  |
|            | T1 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.27**  | -       |
|            |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.40**  | 0.40**  |
|            |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.23**  | 0.23**  |
|            | T2 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.47**  | -       |
|            |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.25**  | 0.25**  |
|            |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.36**  | 0.36*** |
|            | T4 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.26**  | -       |
|            |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.26**  | 0.26**  |
|            |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.70**  | 0.70**  |
|            | T5 | Non-inoculated_BK_vs_RKN-inoculated_BK   | 0.24**  | -       |
|            |    | Non-inoculated_RAS_vs_RKN-inoculated_RAS | 0.29**  | 0.29**  |
|            |    | Non-inoculated_RTS_vs_RKN-inoculated_RTS | 0.26**  | 0.26**  |

Significance of test indicated as \*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ . The ns denotes not statistically significant, - not determined and  $R^2$  is the proportion of variation explained.