
Figures and figure supplements

An ecological network approach for detecting and validating influential organisms for rice growth

Masayuki Ushio et al.

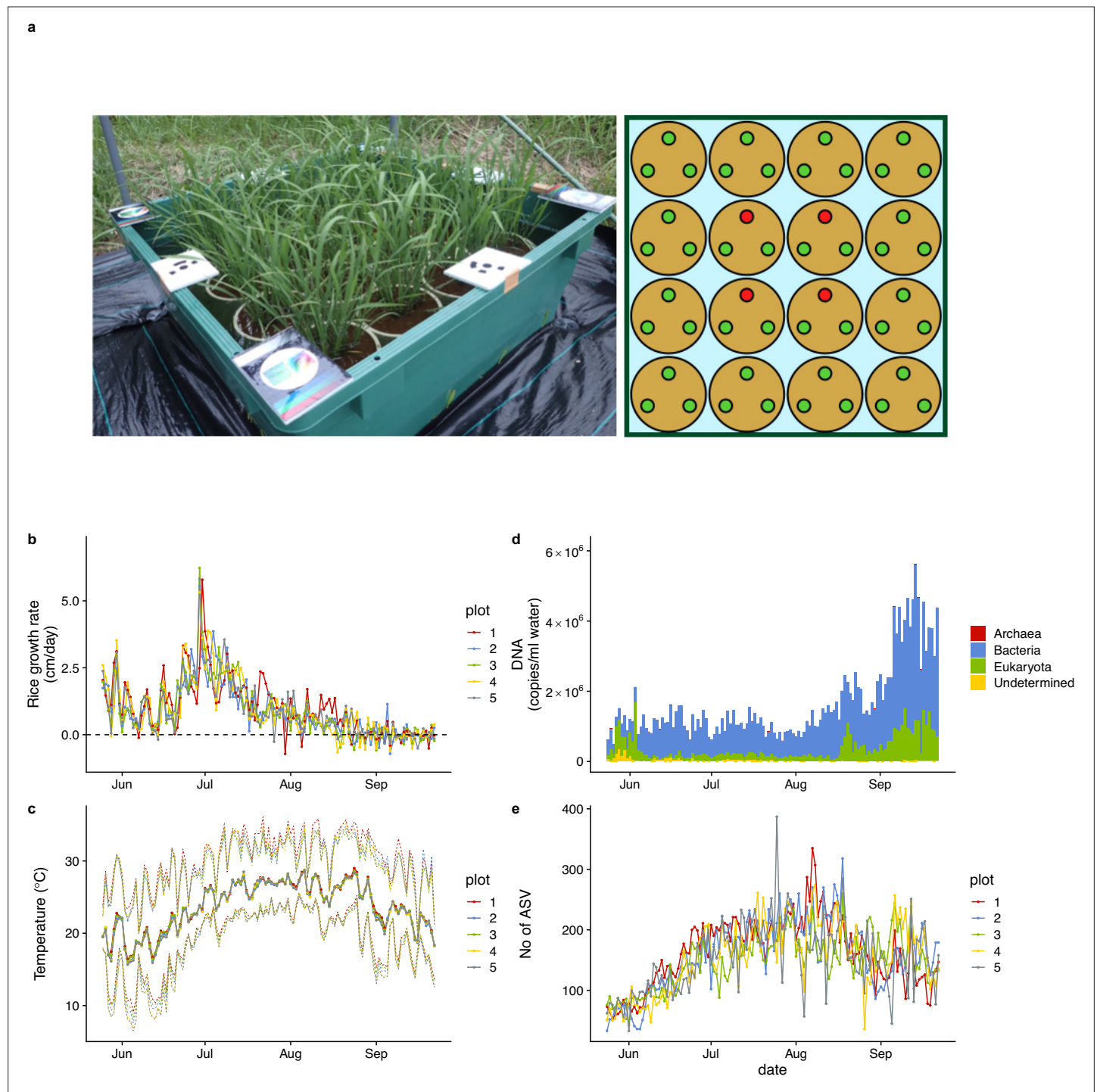


Figure 1. Rice plot, growth rate, air temperature, and ecological community dynamics. **(a)** 90 cm × 90 cm rice plot and Wagner pot alignments. Three rice individuals were grown in each pot. Heights and SAPD of the four red individuals in each plot were measured every day during the monitoring period, and the average values of the four individuals were regarded as representative values for each plot. **(b)** Rice growth rate (cm/day). **(c)** Daily mean air temperature measured at each rice plot. Upper and lower dotted lines indicate daily maximum and minimum air temperature. **(d)** Ecological community compositions and average DNA copy numbers per ml water (reported in [Ushio, 2022](#)). **(e)** The number of amplicon sequence variants (ASVs) from each water sample (reported in [Ushio, 2022](#)). For **(b)**, **(c)**, and **(e)**, different colors indicate data from different rice plots.

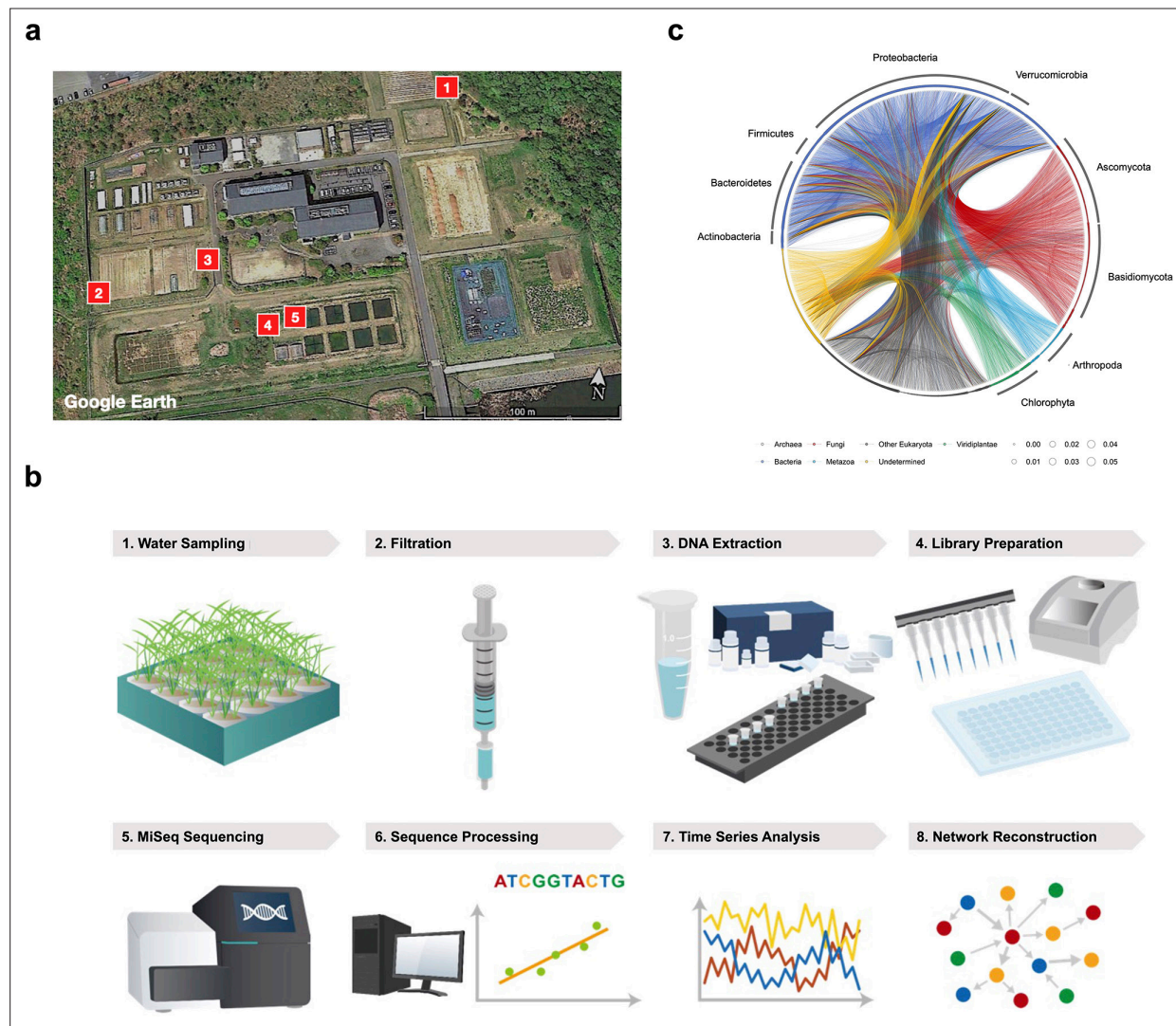


Figure 1—figure supplement 1. Monitoring framework of ecological community (as in *Ushio, 2022*). **(a)** Locations of the five rice plots in 2017. Map image was modified from Google Earth. **(b)** Research framework to generate quantitative environmental DNA time series and network reconstruction. **(c)** Ecological community network reconstructed by non-linear time series. These figures were reproduced from *Ushio, 2022*.

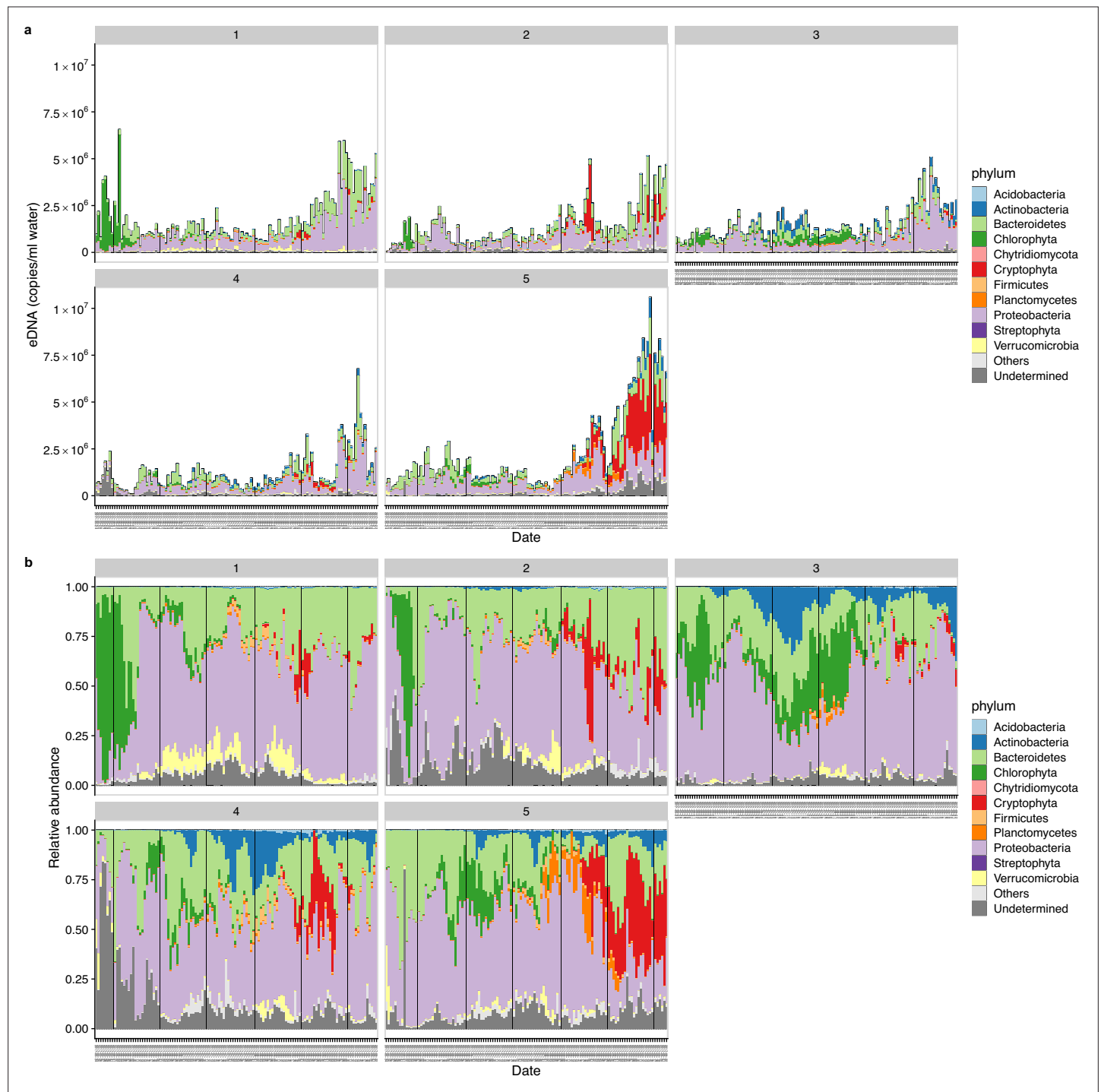


Figure 1—figure supplement 2. Environmental DNA (eDNA)-based monitoring of ecological communities in 2017. **(a)** eDNA concentrations of ecological communities. The number on each panel indicates each plot. **(b)** Relative abundance of ecological communities. The data was reported in *Ushio, 2022*.

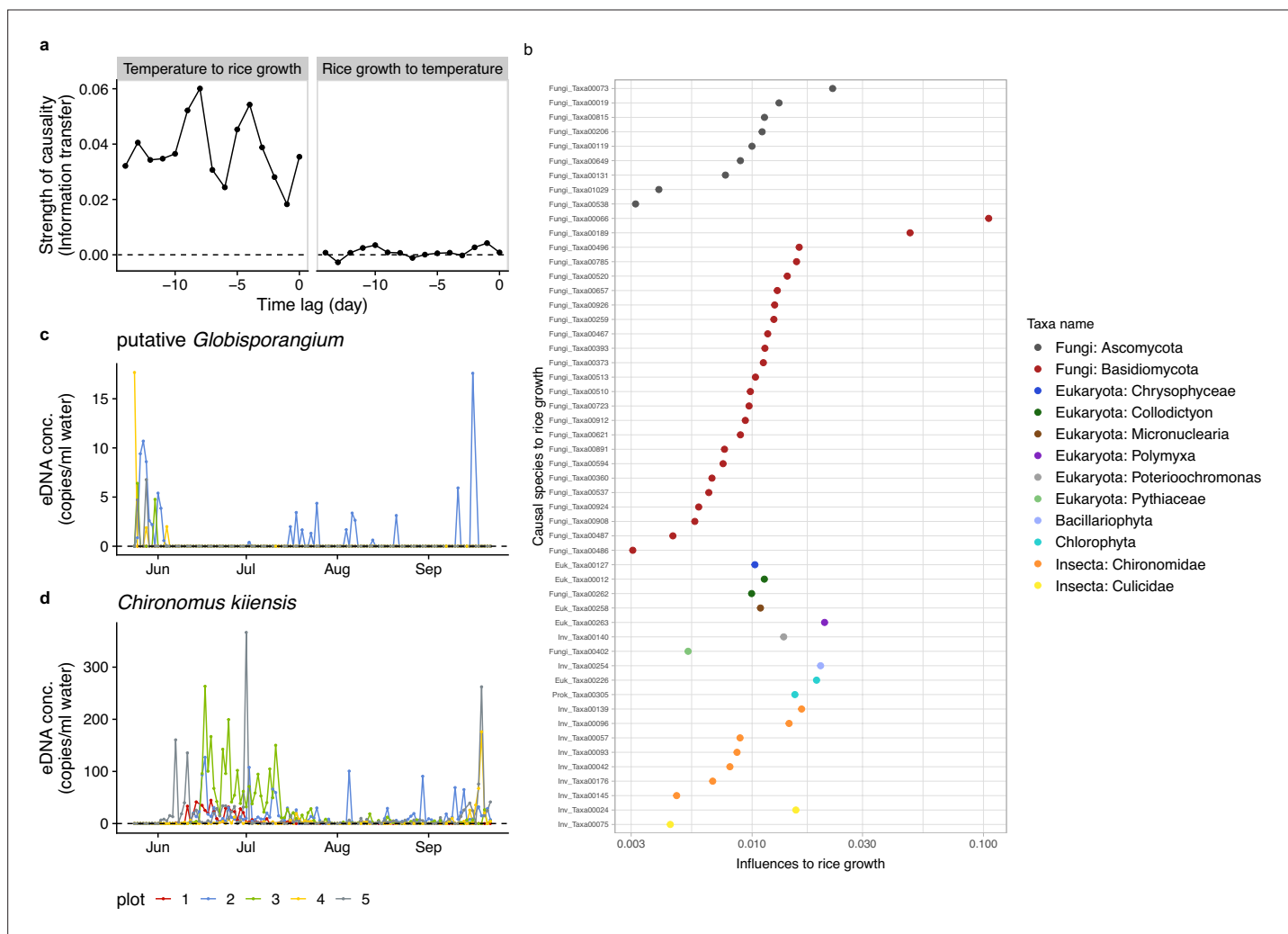


Figure 2. Information transfer between rice growth and ecological community members. **(a)** An example of the results of the unified information-theoretic causality (UIC) analysis. Information transfer between air temperature and rice growth rates was quantified. Much higher information transfer was detected from air temperature to rice growth (left panel) compared with the opposite direction (right panel). **(b)** Strength of causal influence from ecological community members to rice growth. Transfer entropy (TE) quantified by the UIC method was used as an index of causal influence. Colors indicate taxa assigned to ASVs. y-axis indicates ASV ID. Note that the prefix (e.g. 'Fungi_') of the IDs corresponds a major target group of the primer and does not necessarily indicate a taxonomic group assigned to the ASV (see **Supplementary file 1**). **(c)** eDNA dynamics of putative *Globisporangium nunn* (Fungi_Taxa00402 in **Supplementary file 1**). **(d)** eDNA dynamics of *Chironomus kiiensis* (total DNA copy numbers of five midge ASVs). For **(c)** and **(d)**, different colors indicate data from different rice plots.

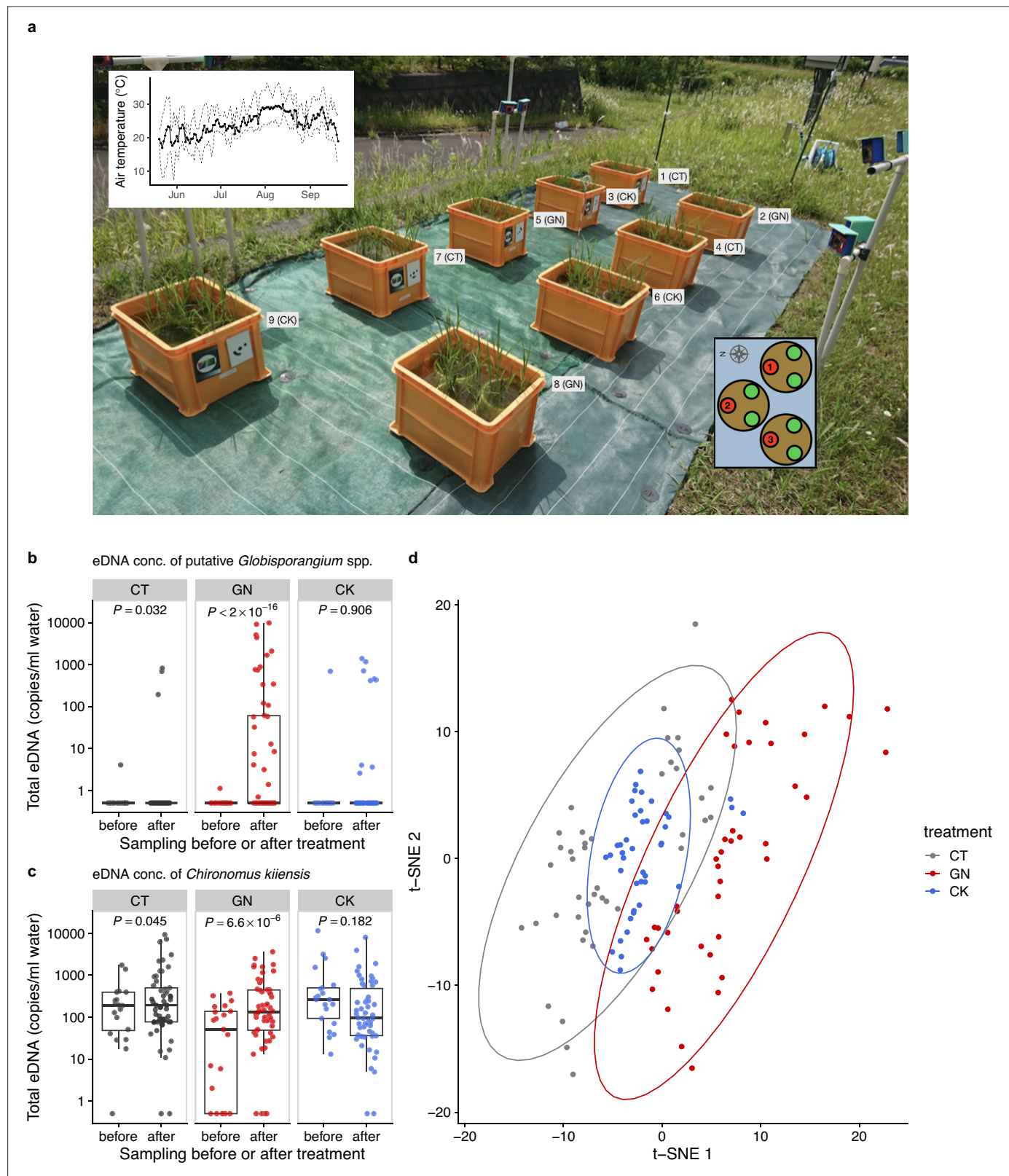


Figure 3. The manipulation experiment performed in 2019 and ecological community compositions before and after the manipulation. **(a)** Setting of the manipulation experiment in 2019. The number and characters next to each plot indicate the plot number and treatment. The left-top inset shows daily mean air temperature (thick line) and daily maximum and minimum air temperature (dashed lines). The right-bottom inset shows three individuals (red and green points) in each Wagner pot, and the number in each red individual indicates the pot location number. Heights and SPAD

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Figure 3 continued

of the red individuals were measured. Total eDNA copy numbers of **(b)** putative *Globisporangium* spp. and **(c)** midge (*Chironomus kiiensis*) in the rice plots. 'before' and 'after' indicate 'from 18 June to 24 June' and 'from 25 June to 12 July', respectively. **(d)** Overall community compositions after the manipulation. Gray, red, and blue indicate CT (control), GN (*Globisporangium nunn* added), and CK (*Chironomus kiiensis* removed) treatments, respectively.

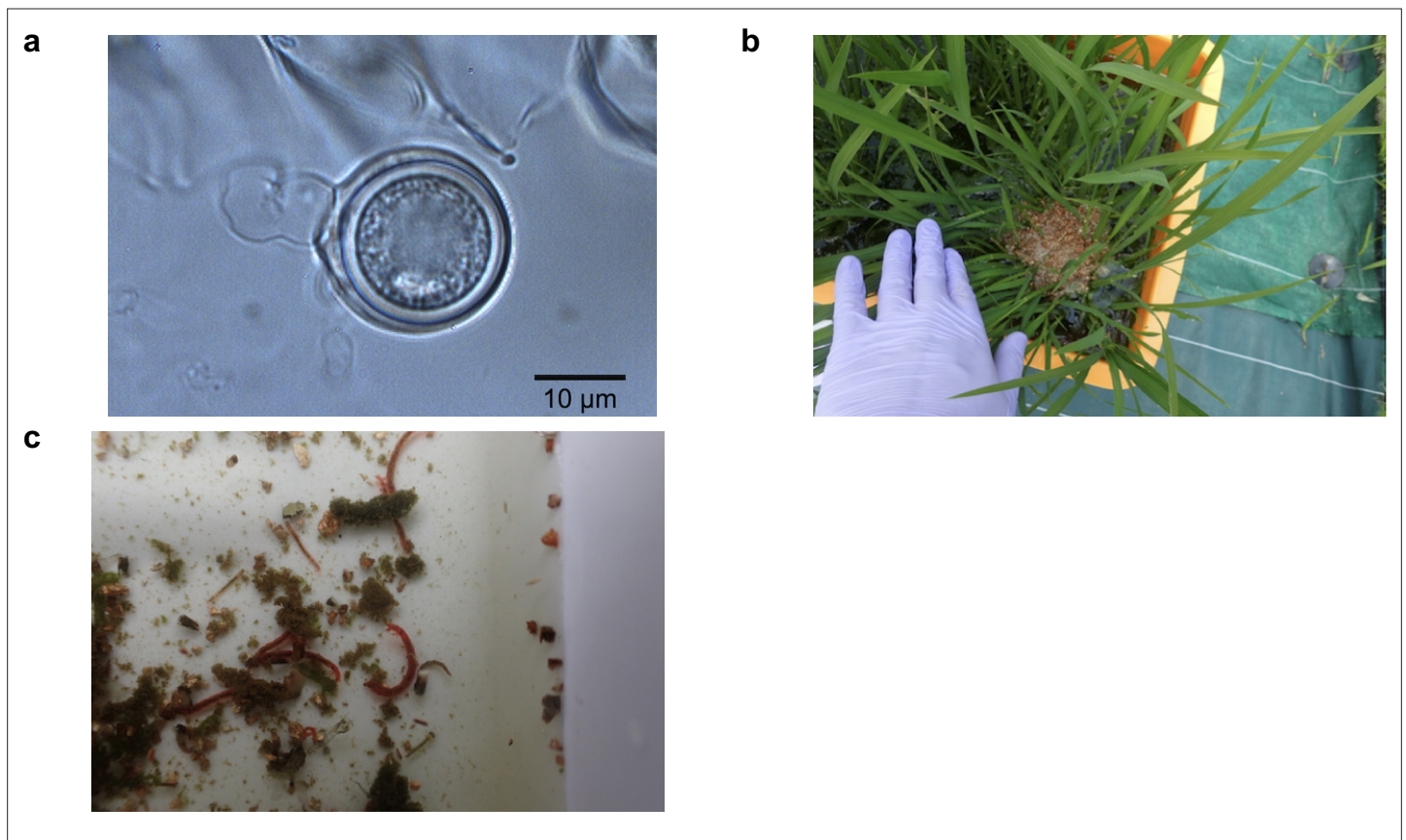


Figure 3—figure supplement 1. *Globisporangium nunn* and Midge (*Chironomus kiiensis*) used in the manipulation experiment in 2019. **(a)** Microscopic image of *G. nunn* (photo by M. Tojo). **(b)** *G. nunn* was incubated in vermiculite and added to the rice plots. For the control and midge-removed treatments, vermiculite without *G. nunn* was added. **(c)** Larvae of midge (*C. kiiensis*) naturally colonized in the rice plots (the larvae in the photo are mostly 0.5–2 cm). The larvae were manually removed by a commercial net with $\phi 1$ mm mesh.

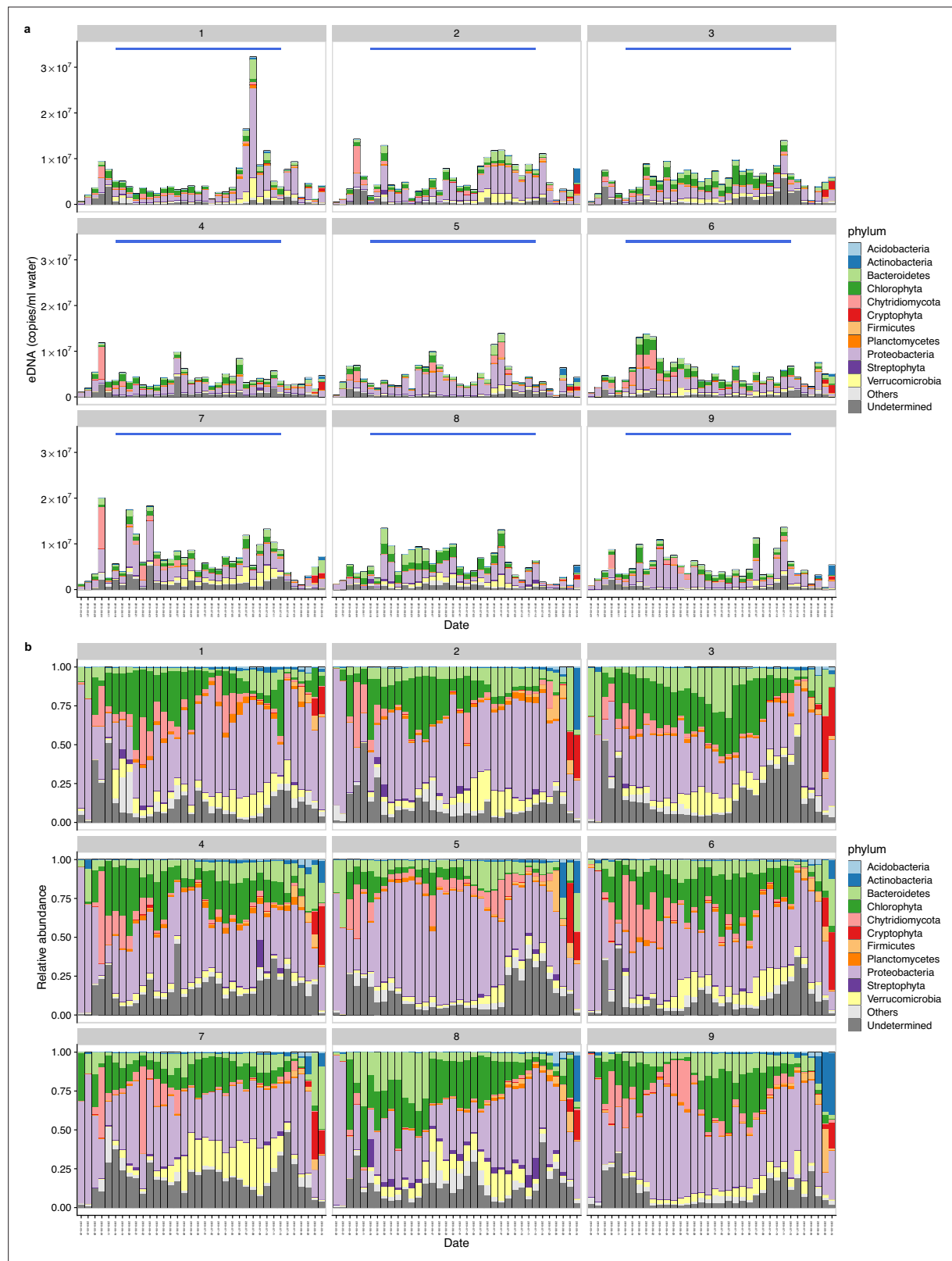


Figure 3—figure supplement 2. Environmental DNA (eDNA)-based monitoring of ecological communities in 2019. **(a)** eDNA concentrations of ecological communities. The number on each panel and characters in the parenthesis indicates the plot number and treatment, respectively. The blue line in each panel indicates the intensive monitoring period (i.e. daily monitoring from 18 June to 12 July). **(b)** Relative abundance of ecological communities.

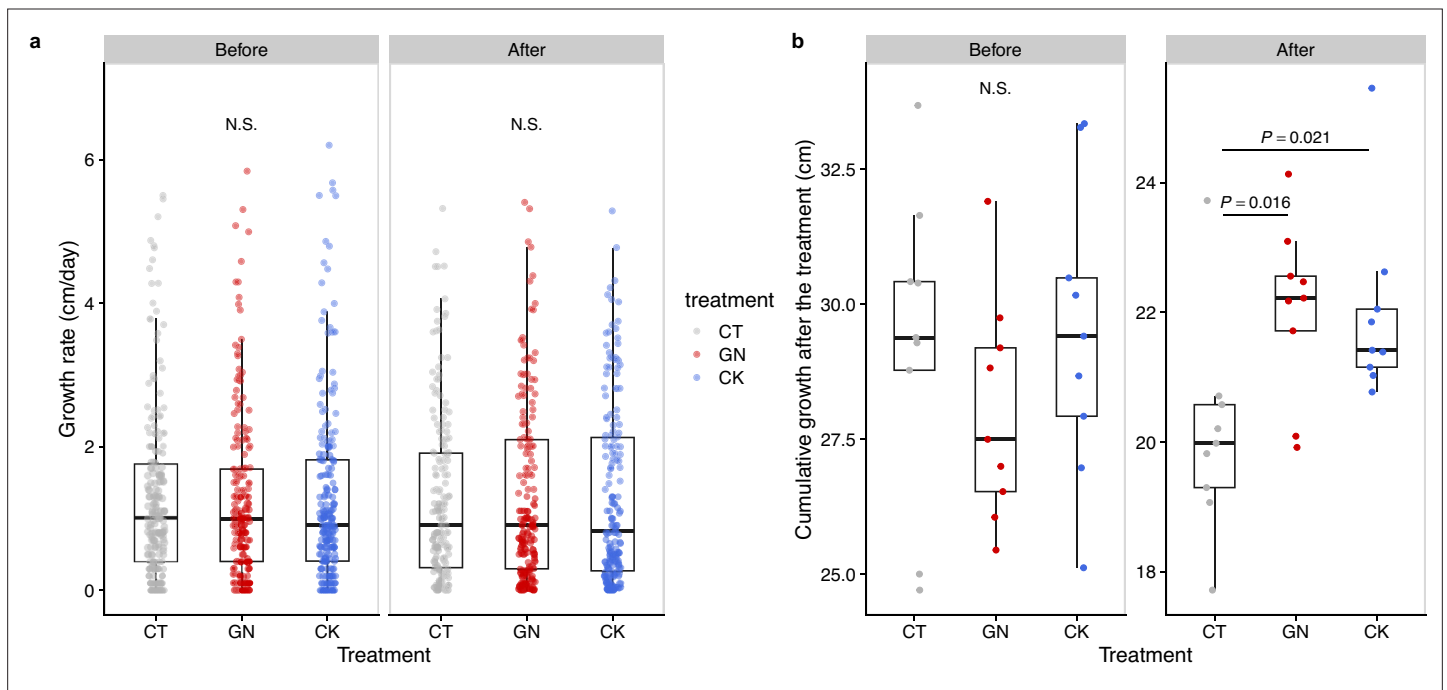


Figure 4. Rice growth rate and cumulative growth before and after the manipulation experiment in 2019. (a) Growth rates and (b) cumulative growth of the rice individuals in the three treatments (CT = control; GN = *Globisporangium nunn* added; CK = *Chironomus kiiensis* removed) before and after the manipulation (cumulative growth was calculated by summing up height growth before the third manipulation or during 10 days after the third manipulation).

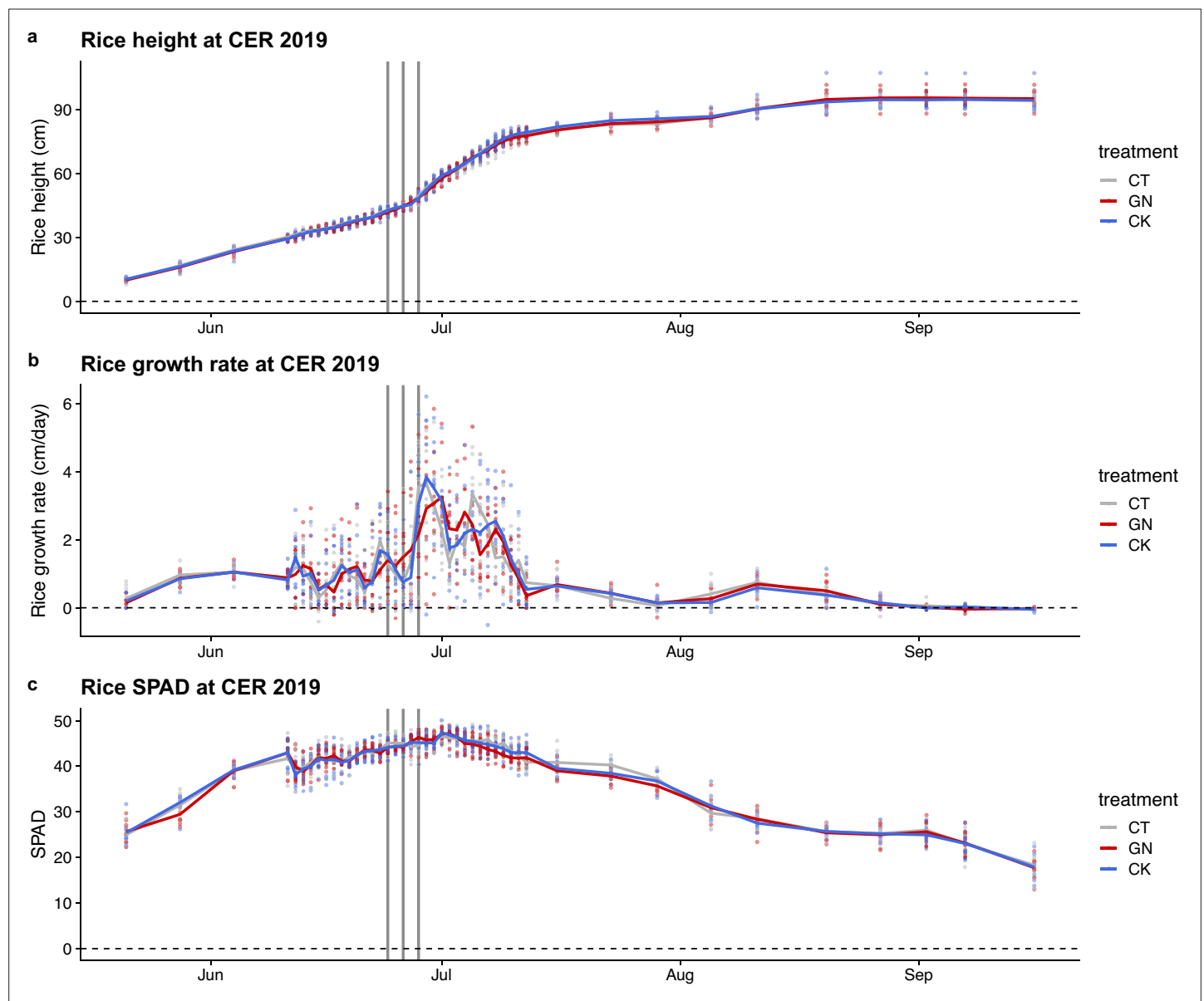


Figure 4—figure supplement 1. Rice growth trajectory in 2019. (a) Rice height, (b) growth rate, and (c) SPAD values of the rice during the growing season in 2019. Points indicate observed values, lines indicate mean trends, and gray lines indicate the field manipulation experiments. Colors indicate the treatments.

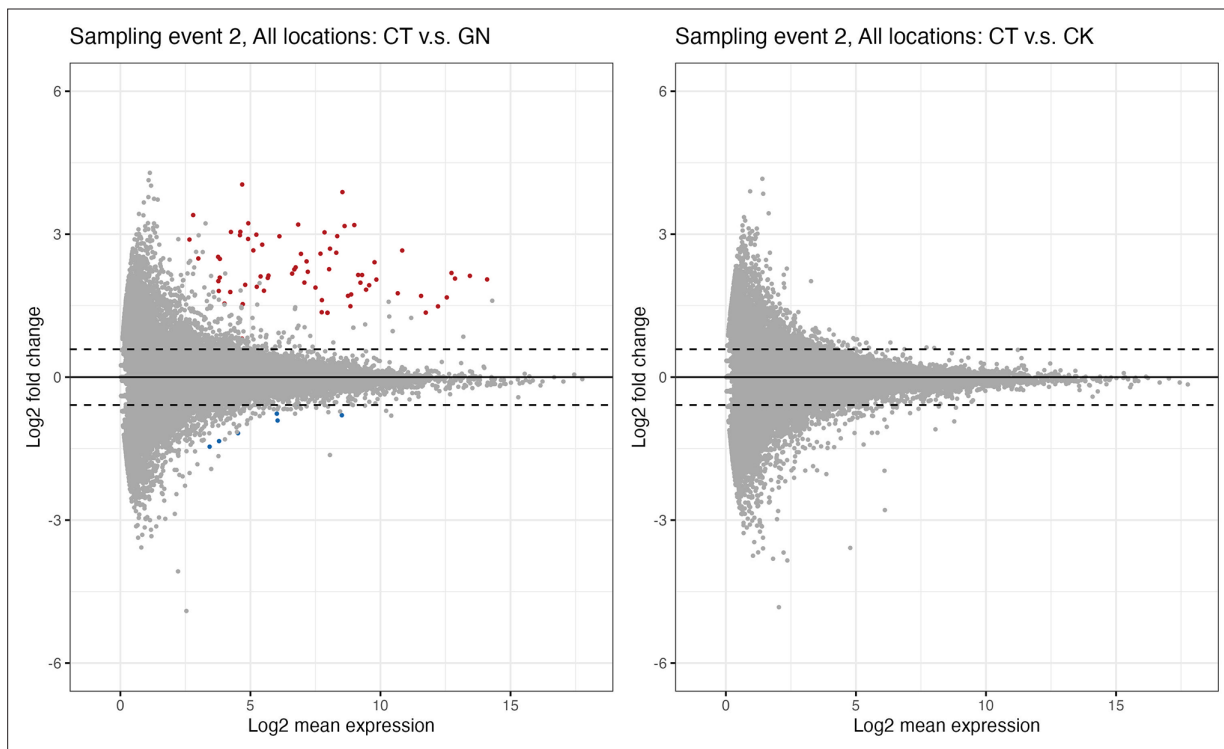


Figure 5. Differential expression genes analysis. (a) *Globisporangium nunn*-added and (b) *Chironomus kiiensis*-removed treatment. Red and blue points indicate significant up- and down-regulated genes, respectively. Upper and lower dashed lines indicate $\log_2(1.5)$ and $-\log_2(1.5)$, respectively.

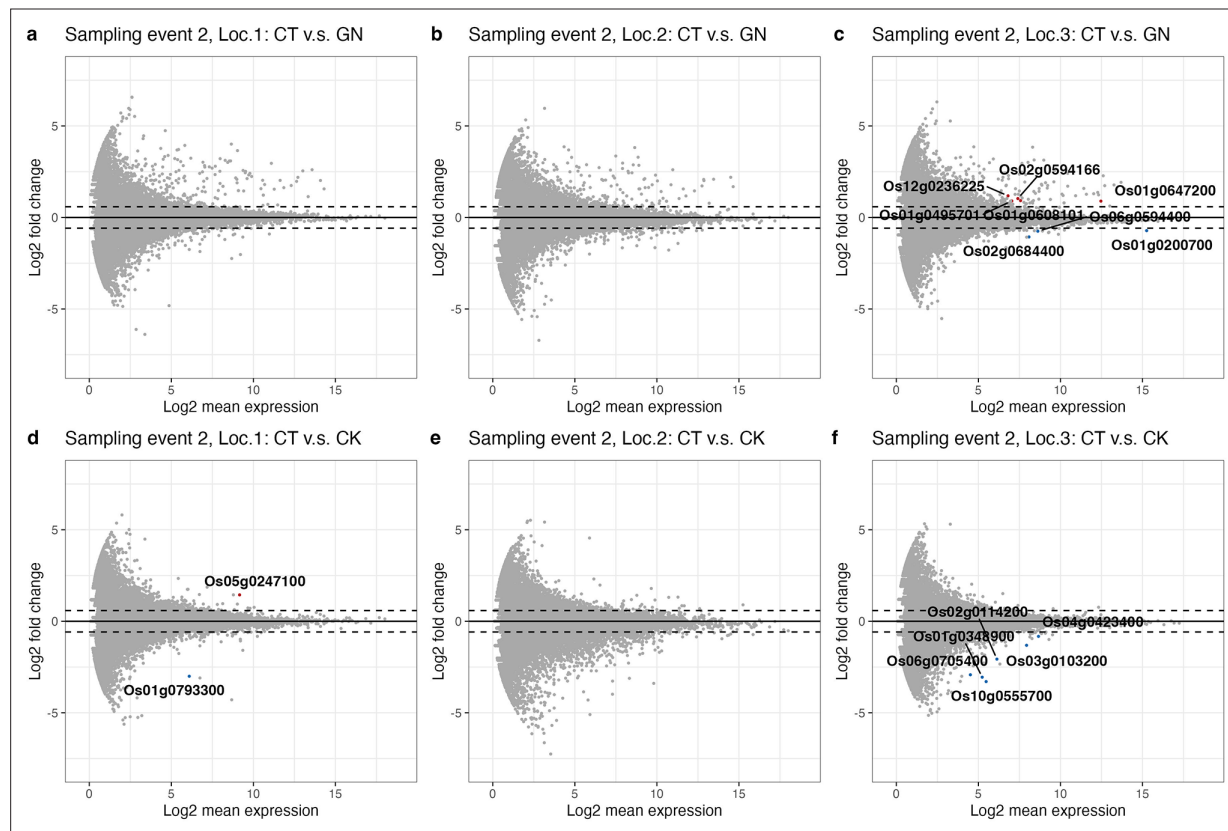


Figure 5—figure supplement 1. Differential expression gene (DEG) patterns for each Wagner pot (Pot location-specific analysis). DEG for (a) location 1, (b) location 2, and (c) location 3 for the control (CT) versus *Globisporangium nunn*-added (GN) treatments. (d-f) DEG analysis for the control versus *Chironomus kiiensis*-removed (CK) treatments for rice grown at locations 1–3. Red and blue points indicate significant up- and down-regulated genes, respectively. Upper and lower dashed lines indicate $\log_2(1.5)$ and $-\log_2(1.5)$, respectively.

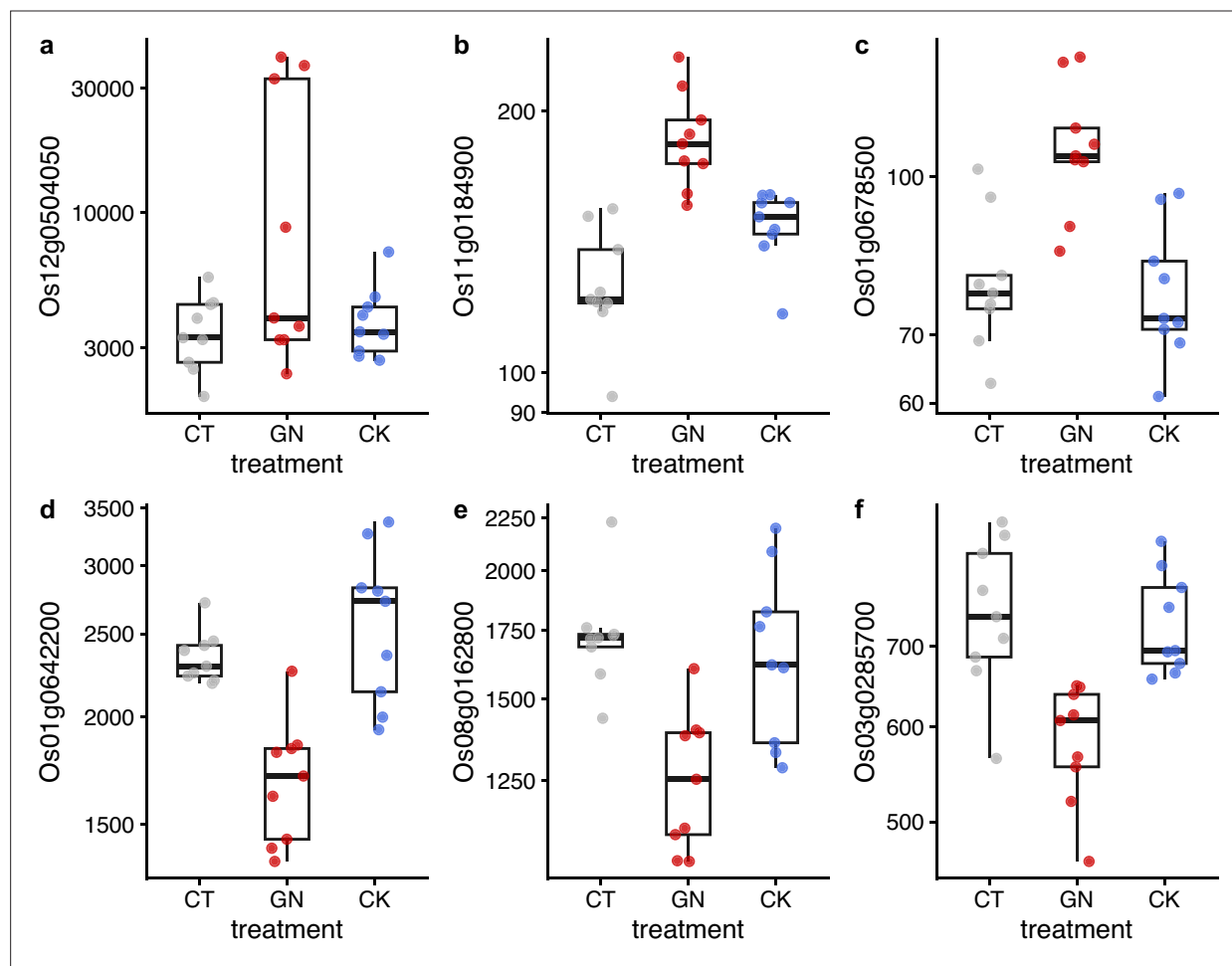


Figure 6. Examples of differentially expressed genes after the manipulation experiment. Results of (a) Os12g0504050, (b) Os11g0184900, (c) Os08g0162800, (d) Os01g0642200, (e) Os03g0285700, and (f) Os01g0678500 are presented. y-axis represents DESeq2-normalized read counts. Gray, red, and blue indicate CT (control), GN (*Globisporangium nunn* added), and CK (*Chironomus kiiensis* removed) treatments, respectively. The gene expressions of the GN treatment in all six genes are statistically clearly different from those of the other two treatments ($p < 0.0001$) except for the panel GN vs. CK in c ($p = 0.00014$) and GN vs. CK in (d) ($p = 0.0087$).