

Supplementary material

Plant diversity drives positive microbial associations in the rhizosphere enhancing carbon use efficiency in agricultural soils.

Domeignoz-Horta et al. 2024

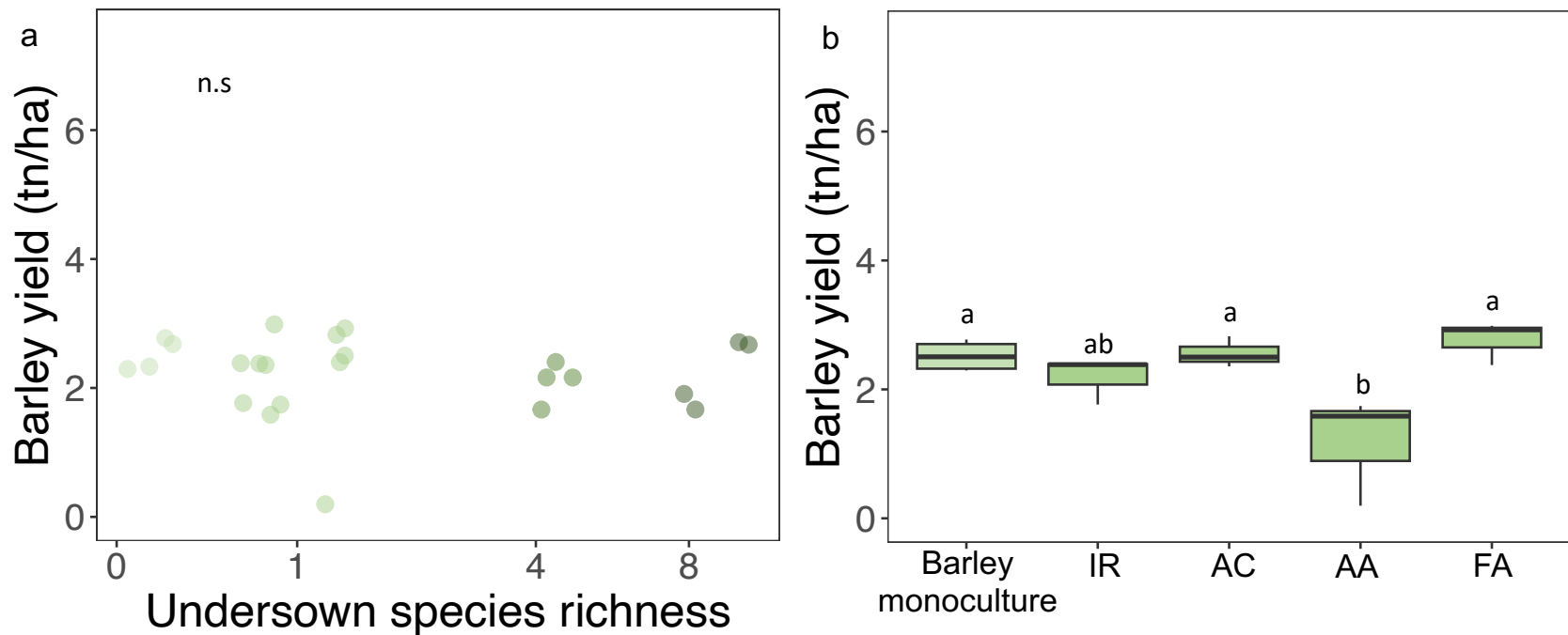
Supplementary Table 1. Root traits of undersown species in the long-term TwinWin agricultural field experiment.

| Intercropping specie | Common name | abbreviation | root depth | Nitrogen fixation |
|----------------------------|------------------|--------------|------------|-------------------|
| <i>Lolium perenne</i> | Italian ryegrass | IR | shallow | 0 |
| <i>Phleum pratense</i> | Tymothy grass | TG | shallow | 0 |
| <i>Trifolium hybridum</i> | Alsike clover | AC | shallow | 1 |
| <i>Trifolium repens</i> | White clover | WC | shallow | 1 |
| <i>Medicago sativa</i> | Alfalfa | AA | deep | 1 |
| <i>Trifolium pratense</i> | Red clover | RC | deep | 1 |
| <i>Festuca arundinacea</i> | Tall fescue | FA | deep | 0 |
| <i>Cichorium intybus</i> | Chicory | CI | deep | 0 |

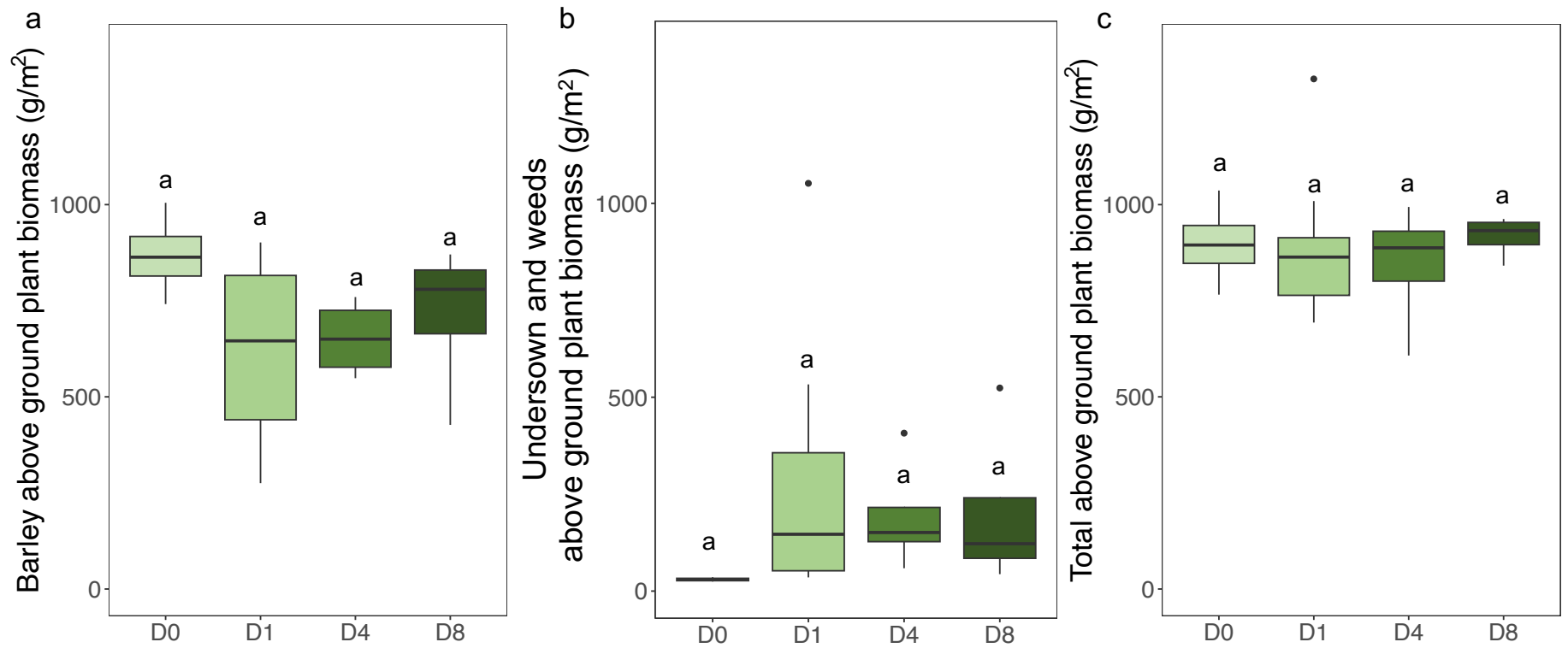
TWINWIN FIELD

| plot number: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|----------------|-----------------------------|-----------------|-----------|--------|-----------|-----------------|-----------------|-----------------------------|--------|-----------------------------|-----------------|-----------|--------|-----------------|-----------|
| Block 1 | D2= AC WC | D4= IR AC AA FA | D2= IR TG | D1= IR | D0 | D1= WC | D1= AC | BF | D0 | D8= IR AA AC WC TG RC FA CI | D1= TG | D0H | D1= RC | D2= AA RC | D1= AA |
| Block 2 | D8= IR AA AC WC TG RC FA CI | D0H | D2= FA CI | D1= AC | D0 | D0 | D4= IR TG AA FA | D1= WC | D1= FA | BF | D1= AA | D2= AA AC | D1= RC | D4= TG WC RC CI | D1= CI |
| Block 3 | D0H | D1= TG | D2= RC FA | D1= CI | D2= AC FA | D1= FA | D1= IR | D8= IR AA AC WC TG RC FA CI | D1= RC | D4= AC WC RC CI | D1= AA | D0 | D0 | BF | D2= AA IR |
| Block 4 | D8= IR AA AC WC TG RC FA CI | D1= TG | D1= WC | BF | D2= CI TG | D4= AA RC FA CI | D2= WC IR | D1= CI | D0 | D0 | D4= IR TG AC WC | D1= IR | D0H | D1= FA | D1= AC |

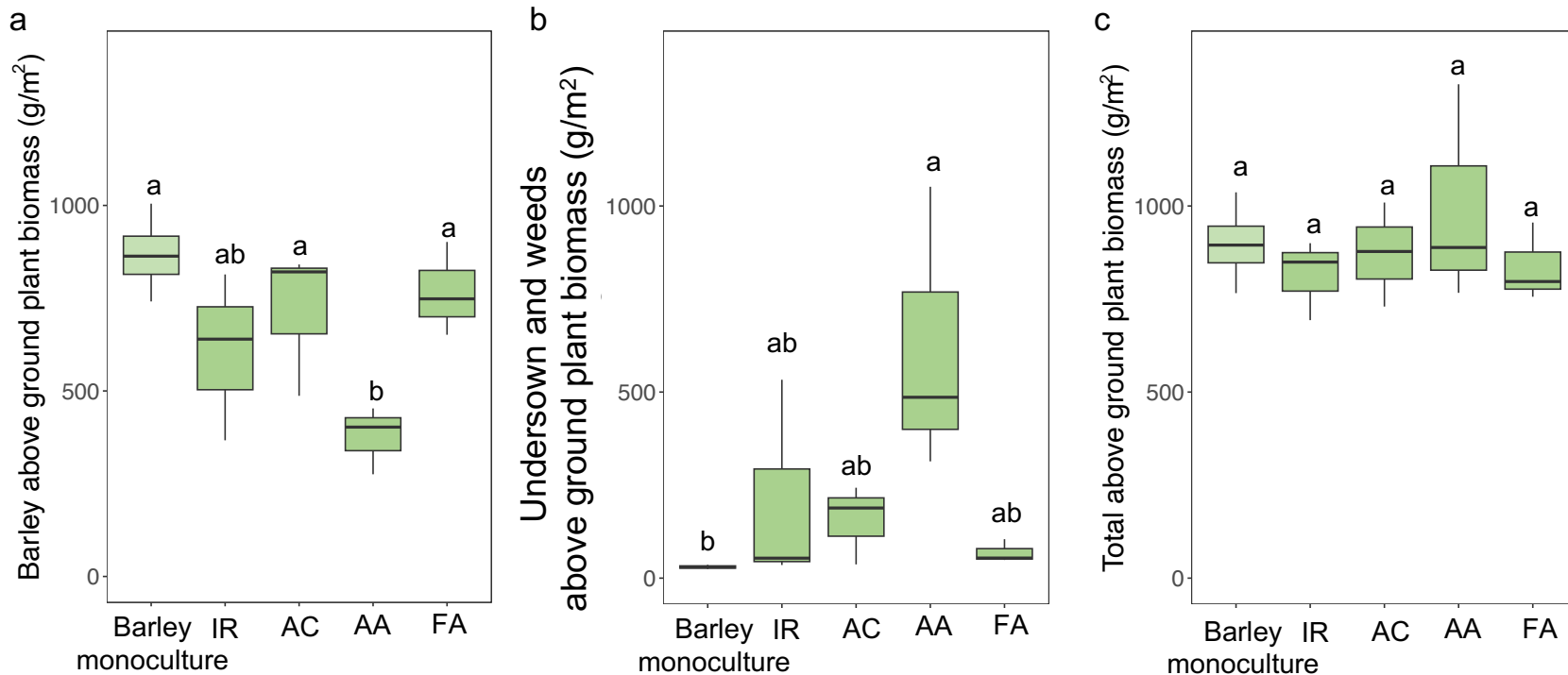
Supplementary Figure 1. TwinWin agricultural field experiment. Spatial arrangement of the 60 experimental plots along four blocks. BF: bare fellow plots; D0H: barley planted in monoculture with herbicide application; D0: barley planted in monoculture without herbicide application; D1: barley planted with one undersown specie; D2 barley planted with two undersown species; D4: barley planted with four undersown species and D8: barley planted with eight undersown species. Undersown species: *Lolium perenne* (IR), *Phleum pratense* (TG), *Trifolium hybridum* (AC), *Trifolium repens* (WC), *Medicago sativa* (AA), *Trifolium pratense* (RC), *Festuca arundinacea* (FA) and *Cichorium intybus* (CI). The plots highlighted in blue were sampled in this experiment (24 plots).



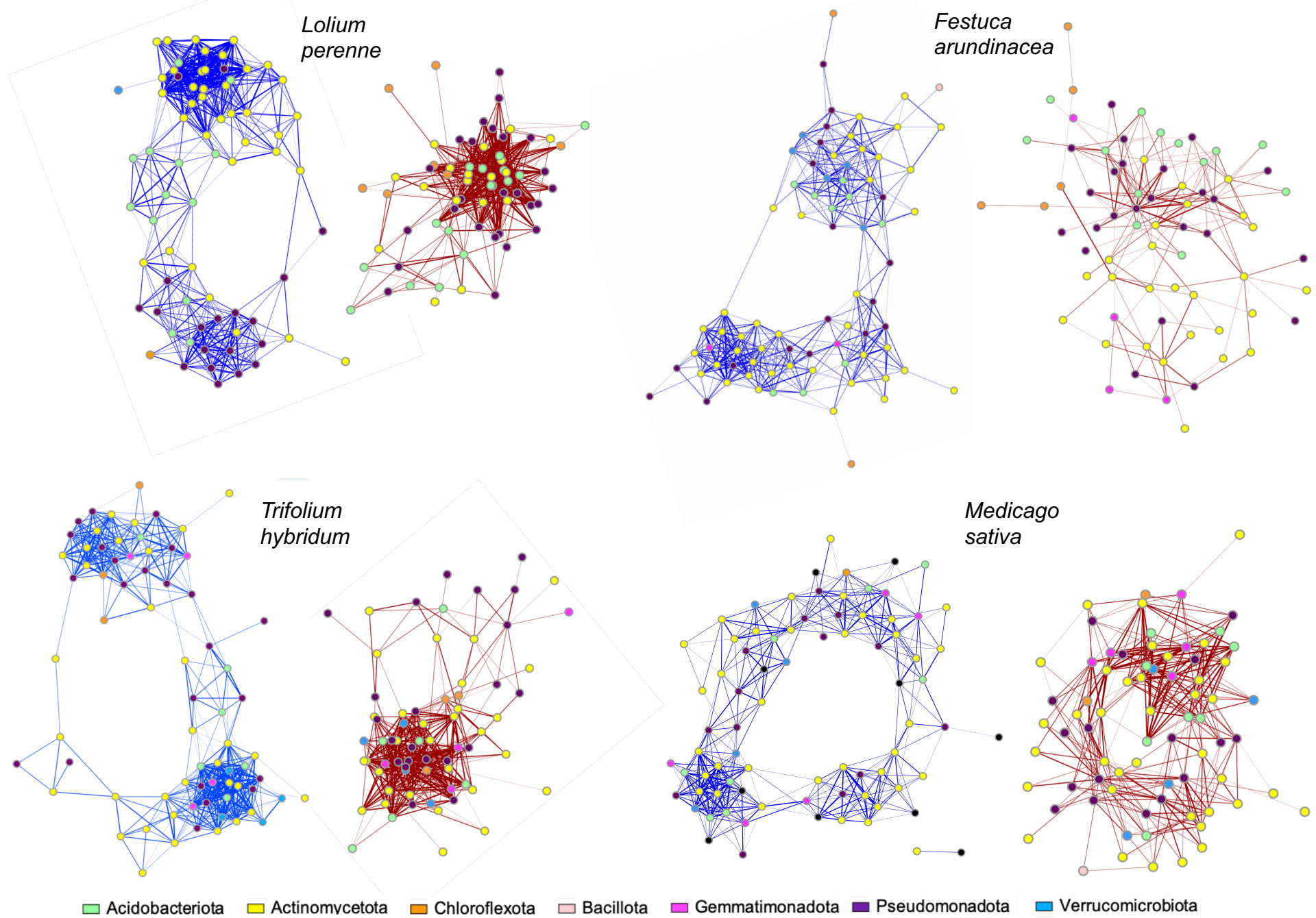
Supplementary Figure 2. Barley yield within TwinWin. Relationship between barley yield measured in 2020 and the undersown plant diversity within the TwinWin experiment (a) and barley yield at the barley monoculture and the D1 treatment. The undersown plant species at D1 are: *Lolium Perenne*: IR; *Trifolium hybridum*: AC, *Medicago sativa*: AA; *Festuca arundinacea*: FA.(b). In the boxplots, whiskers denote the minimum value or $1.5 \times$ interquartile range (whichever is more extreme), and box denotes interquartile range. The horizontal line denotes the median. Source data are provided as a Source Data file.



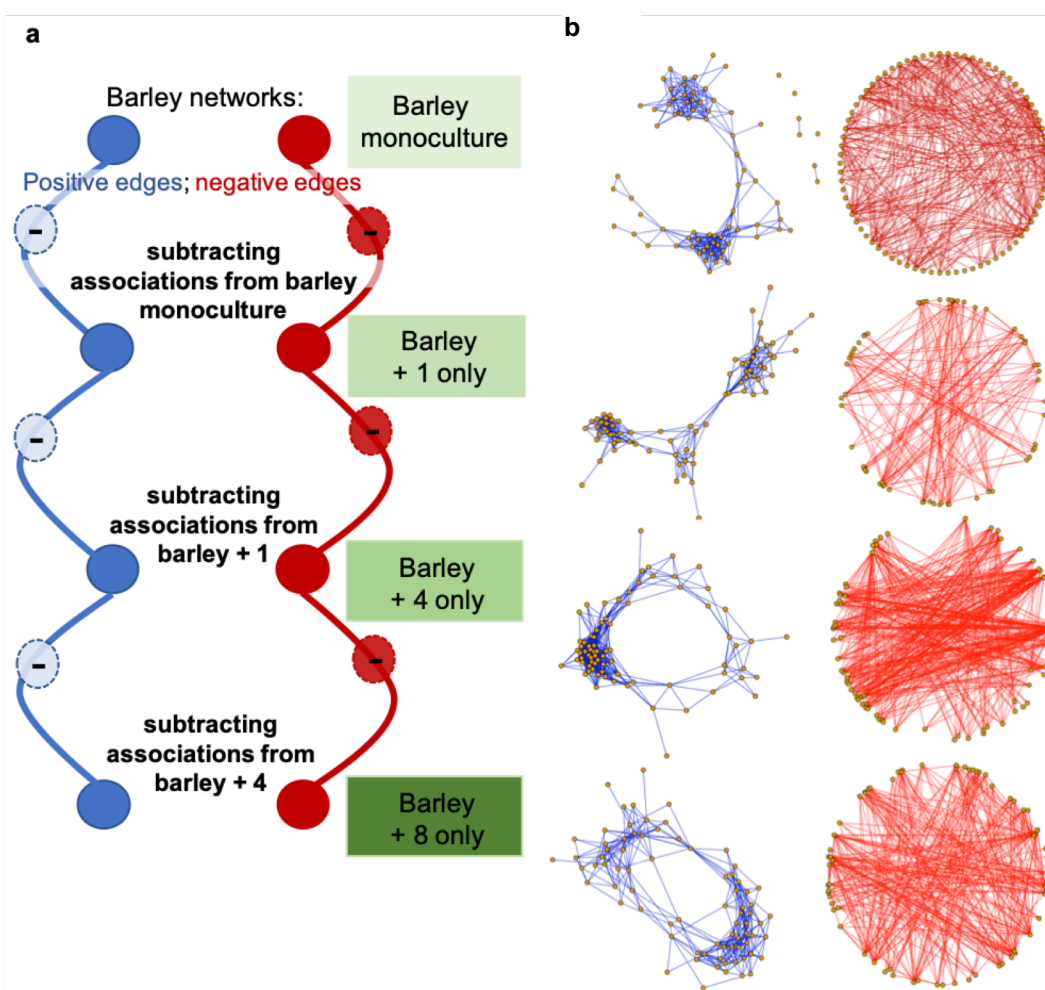
Supplementary Figure 3. Above ground plant biomass sampled in August 2020 within the TwinWin experiment at the plot level. Barley above ground biomass (a), undersown and weed above ground biomass (b), and total above ground plant biomass (c). In the boxplots, whiskers denote the minimum value or $1.5 \times$ interquartile range (whichever is more extreme), and box denotes interquartile range. The horizontal line denotes the median. Source data are provided as a Source Data file.



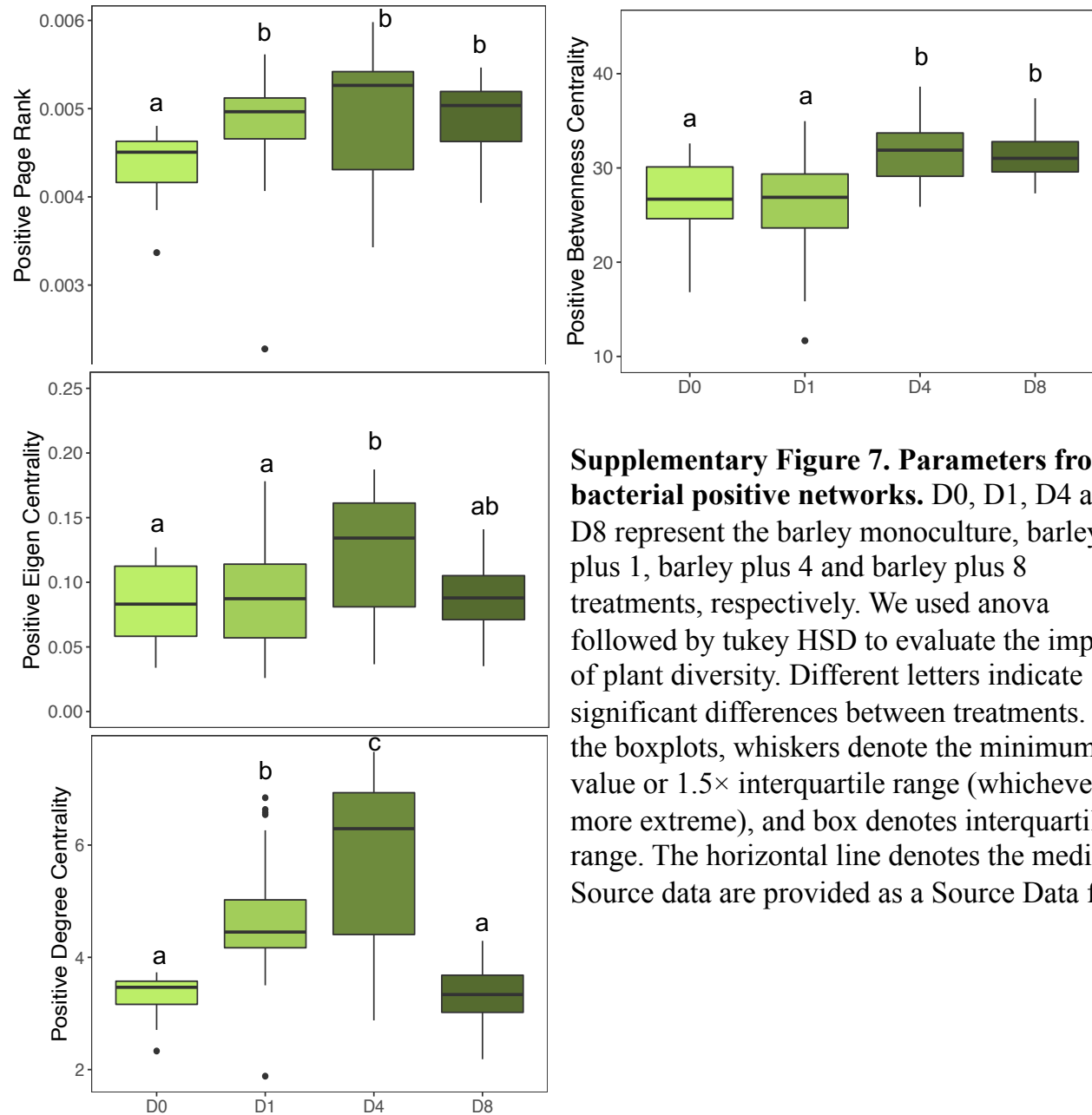
Supplementary Figure 4. Above ground plant biomass from barley monoculture and D1 treatment (Barley plus 1 undersown species) measured during summer 2020. Barley above ground plant biomass (a), undersown and weeds above ground plant biomass (b) and total plant above ground biomass (c). The undersown plant species are: *Lolium Perenne*: IR; *Trifolium hybridum*: AC, *Medicago sativa*: AA; *Festuca arundinacea*: FA. In the boxplots, whiskers denote the minimum value or 1.5× interquartile range (whichever is more extreme), and box denotes interquartile range. The horizontal line denotes the median. Source data are provided as a Source Data file.



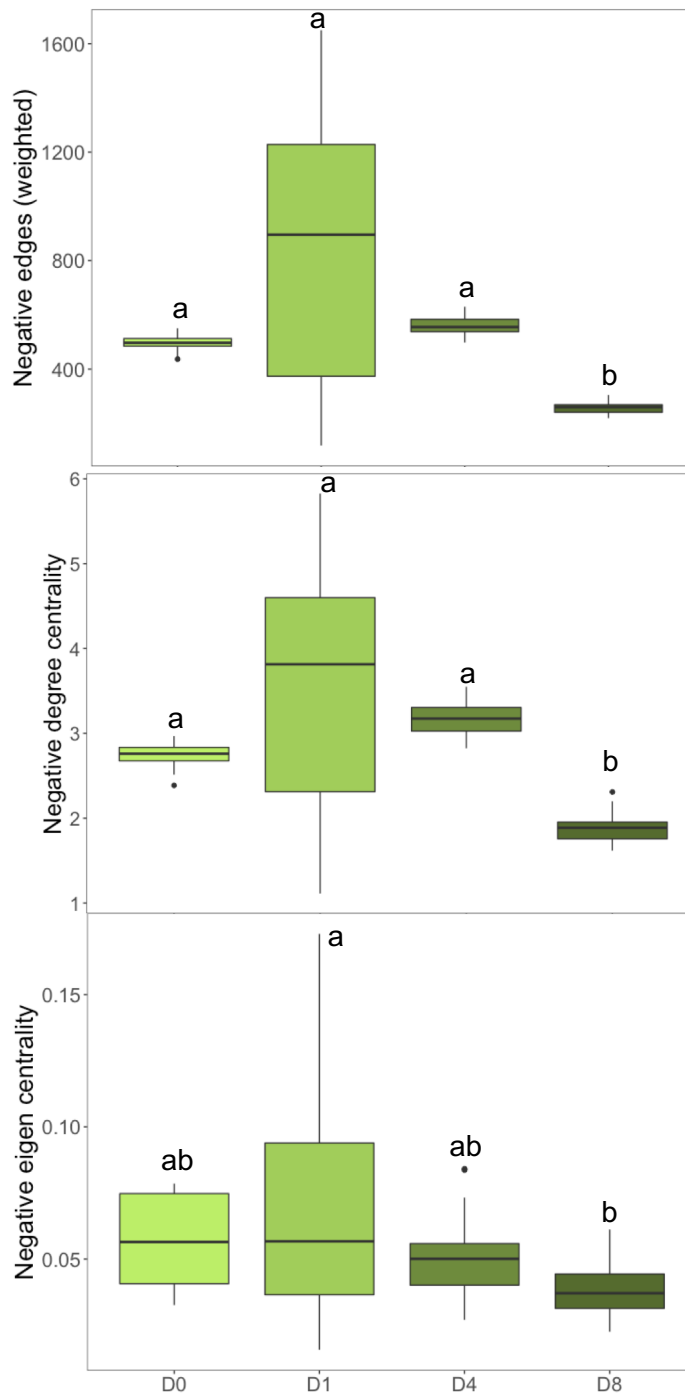
Supplementary Figure 5. Bacterial network analysis for each undersown species at the first diversity level (D1; Barley plus 1). Networks shown in blue are positive or networks shown in red are negative within each undersown species.



Supplementary Figure 6. Network analysis design and findings. Network analysis approach using arithmetic subtraction to evaluate the impact of undersown diversity on microbial associations within the rhizosphere of barley subtracting the associations observed at adjacent lower diversity level (a). Networks shown in blue if positive or in red if negative within each plant diversity treatment after subtraction from previous diversity treatment. From top to down: barley monoculture, barley + 1, barley + 4 and barley + 8, respectively (b).

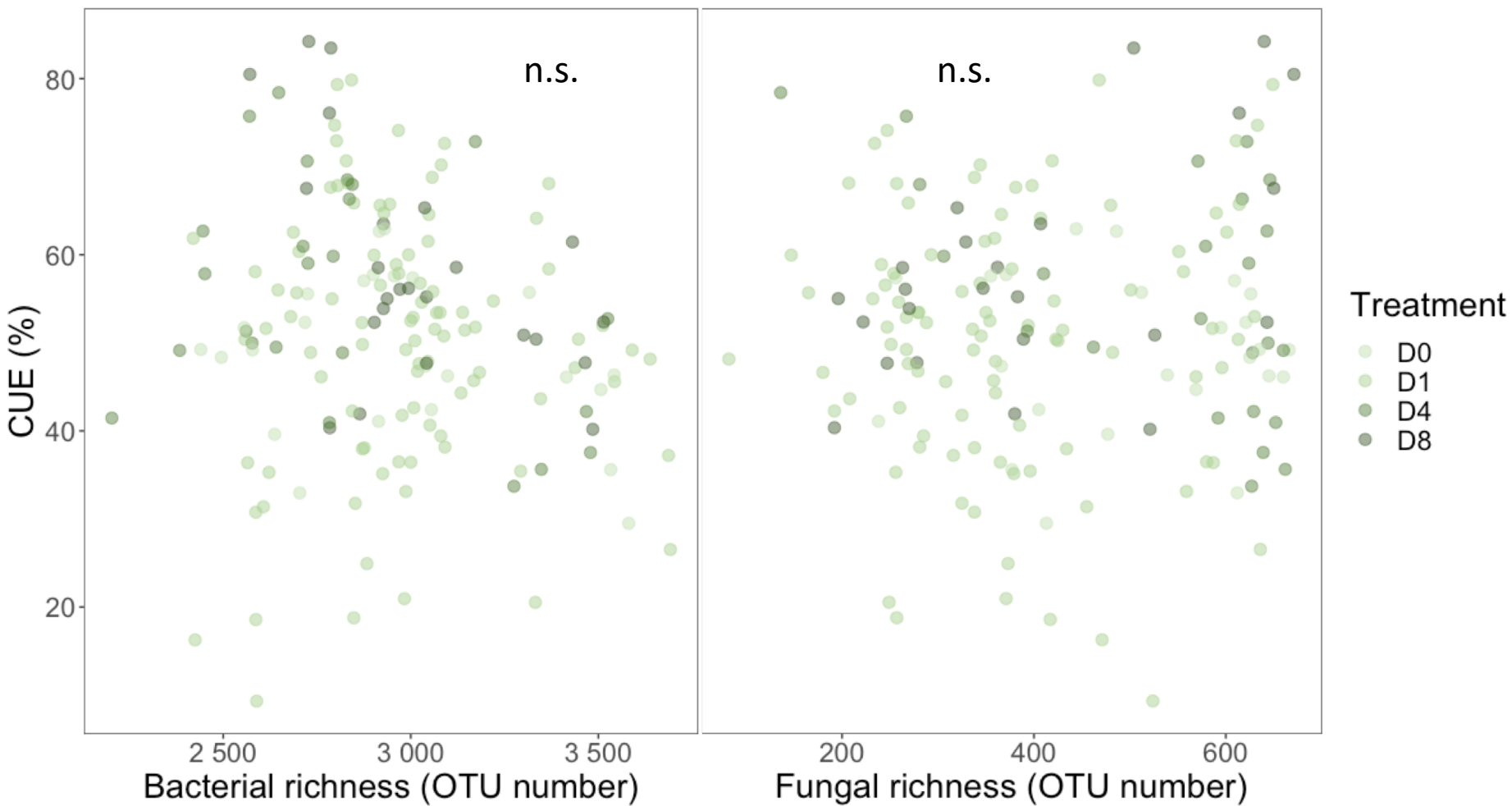


Supplementary Figure 7. Parameters from bacterial positive networks. D0, D1, D4 and D8 represent the barley monoculture, barley plus 1, barley plus 4 and barley plus 8 treatments, respectively. We used anova followed by tukey HSD to evaluate the impact of plant diversity. Different letters indicate significant differences between treatments. In the boxplots, whiskers denote the minimum value or 1.5× interquartile range (whichever is more extreme), and box denotes interquartile range. The horizontal line denotes the median. Source data are provided as a Source Data file.

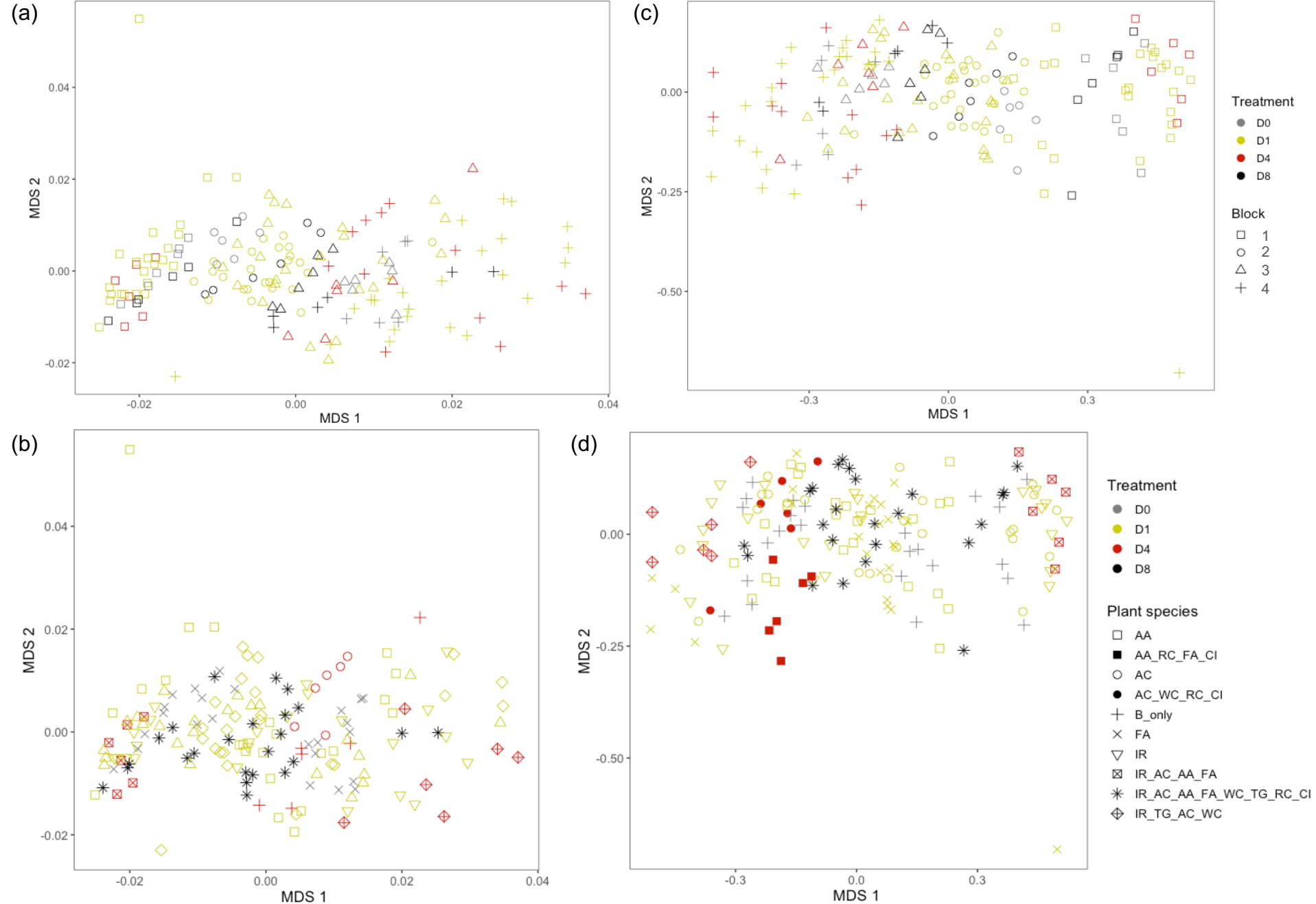


Supplementary Figure 8. Parameters from bacterial negative networks.

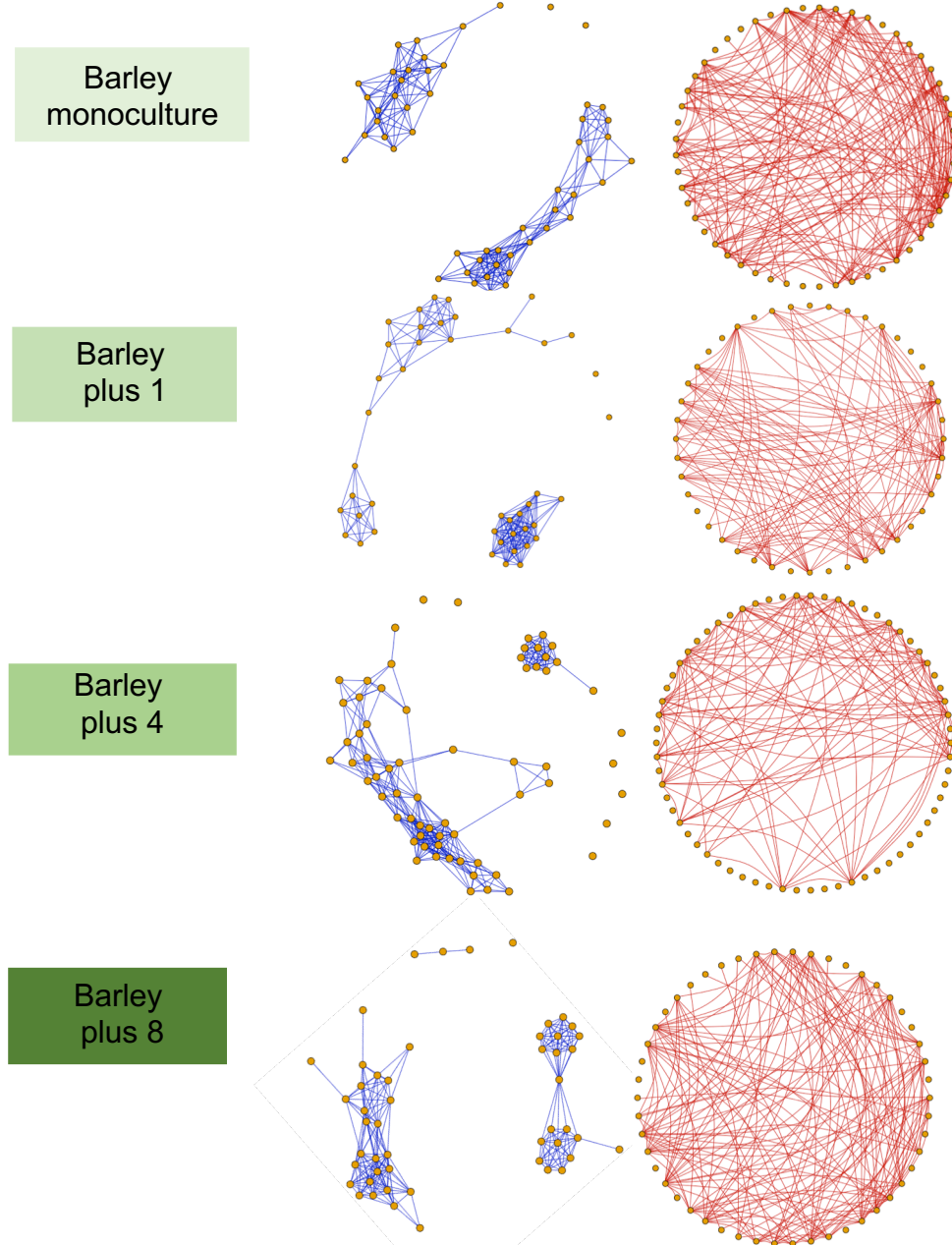
D0, D1, D4 and D8 represent the barley monoculture, barley plus 1, barley plus 4 and barley plus 8 treatments, respectively. We used anova followed by tukey HSD to evaluate the impact of plant diversity. Different letters indicate significant differences between treatments. In the boxplots, whiskers denote the minimum value or $1.5 \times$ interquartile range (whichever is more extreme), and box denotes interquartile range. The horizontal line denotes the median. Source data are provided as a Source Data file.



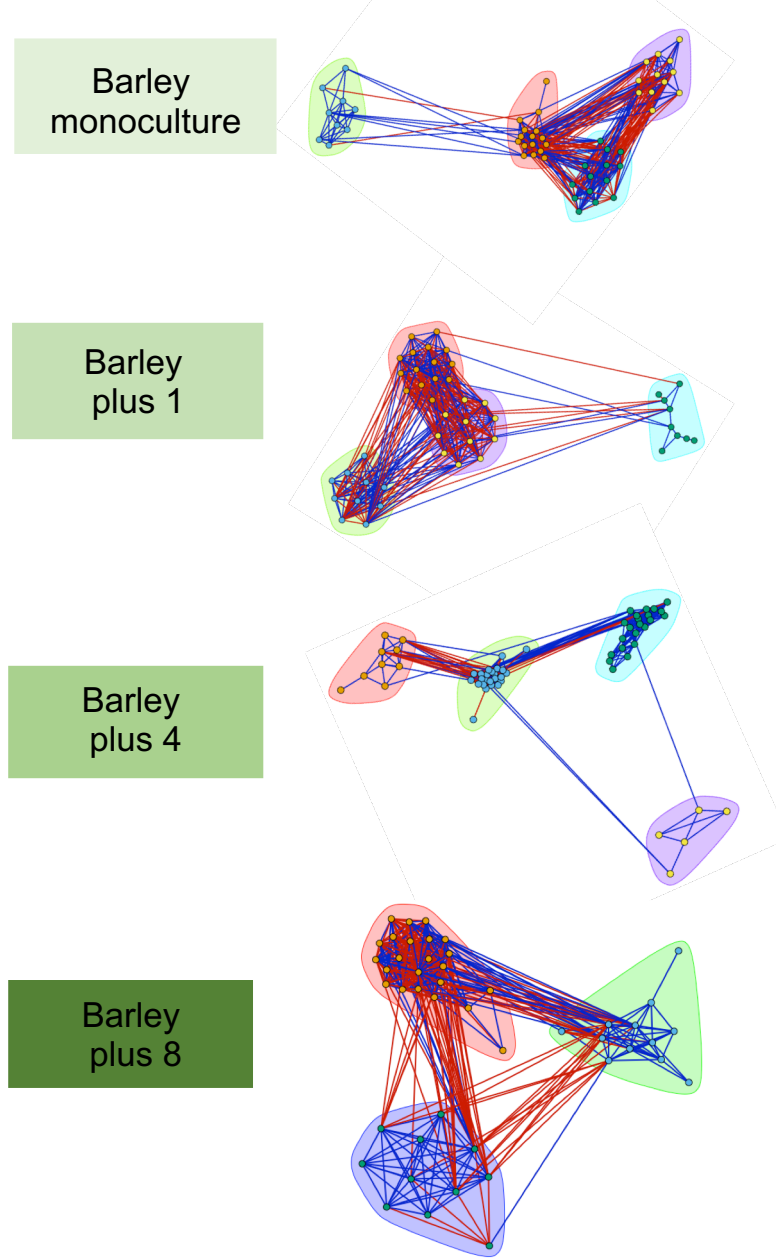
Supplementary Figure 9. Relationship between CUE and bacterial and fungal richness (OTU numbers). Source data are provided as a Source Data file.



Supplementary Figure 10. Bacterial community composition. A) community composition of the more abundant species ($\geq 5\%$ relative abundance) per block and treatment; b) community composition of the more abundant species ($\geq 5\%$ relative abundance) per treatment and plant species identity; c) total bacteria community composition per block and treatment; d) total bacteria community composition per treatment and plant species identity.

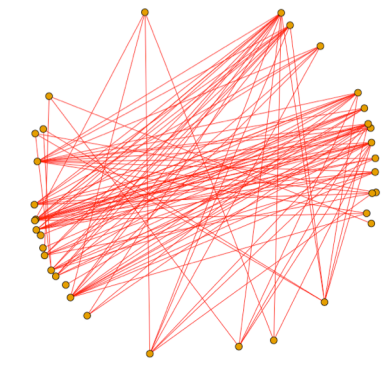
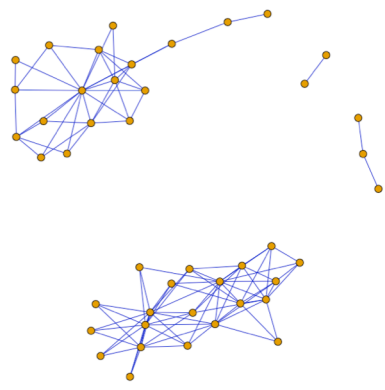


Supplementary Figure 11. Fungal networks. Networks shown in blue represent positive associations or negative associations are shown in red within each plant diversity treatment.

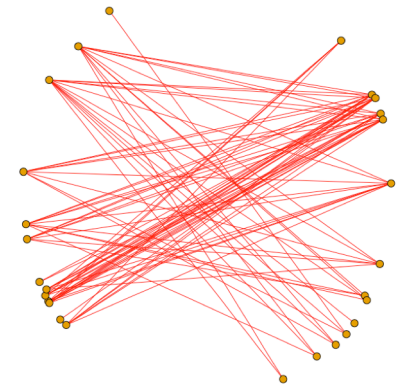
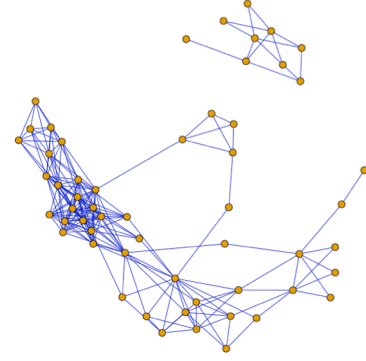


Supplementary Figure 12. Cluster analysis of positive and negative fungal networks. Cluster analysis show four clusters at the first three levels of diversity and three clusters for barley plus 8.

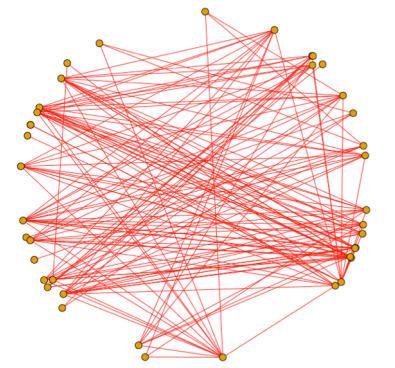
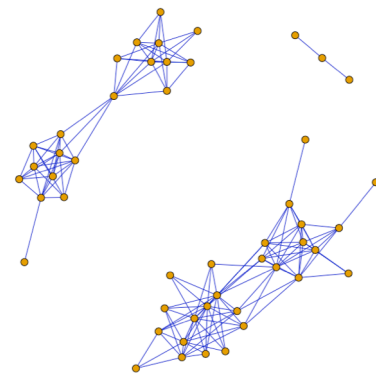
Barley
plus 1 only



Barley
plus 4 only

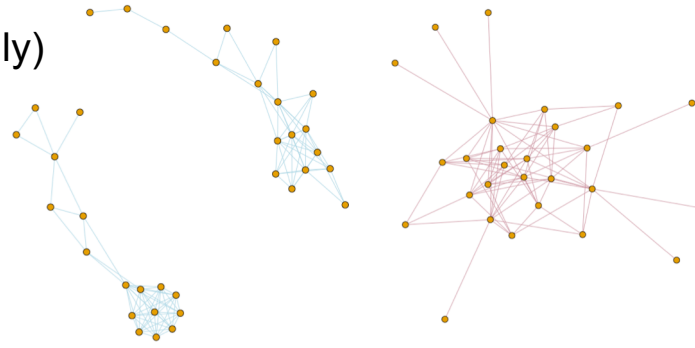


Barley
plus 8 only

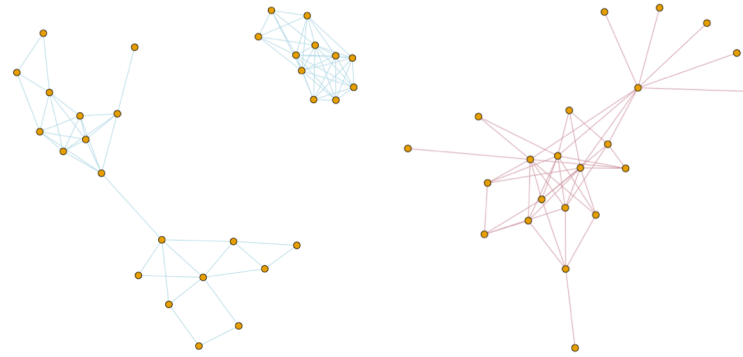


Supplementary Figure 13. Fungal networks after arithmetic subtraction. Network analysis approach to evaluate the impact of undersown diversity on microbial associations within the rhizosphere of barley subtracting the associations observed at adjacent lower diversity levels: Barley plus 1 – Barley monoculture; Barley plus 4 – Barley plus 1 and Barley plus 8 - Barley plus 4, respectively.

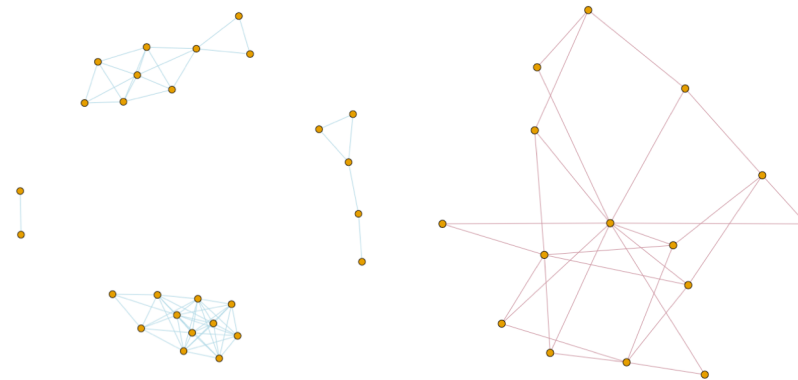
(D1 / Barley only)



(D4 / D1)



(D8 / D4)

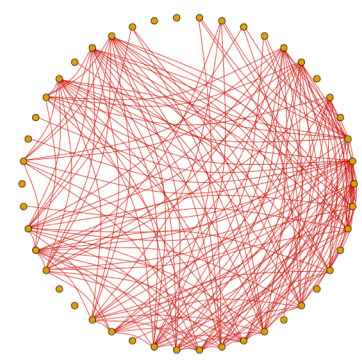
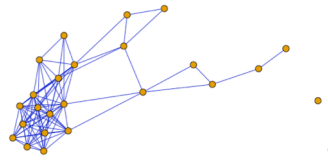


Supplementary Figure 14. Fungal networks intersections.

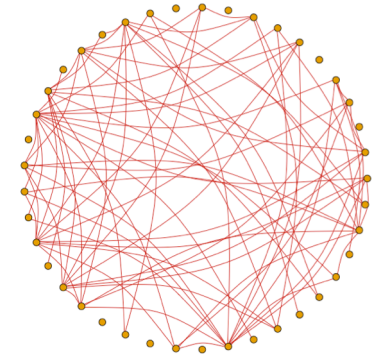
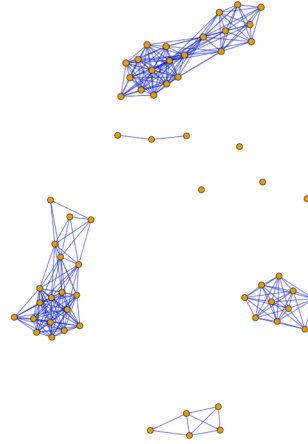
Intersections from subtracting the networks of previous adjacent diversity treatment showing the nodes and edges that co-occur at two subsequent diversity levels.

Supplementary Figure 15. Fungal network analysis for each undersown species at the first diversity level (D1; Barley plus 1). Networks shown in blue are positive or networks shown in red are negative within each undersown specie.

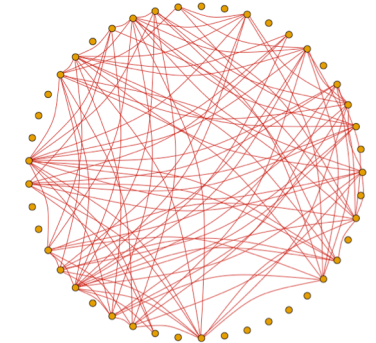
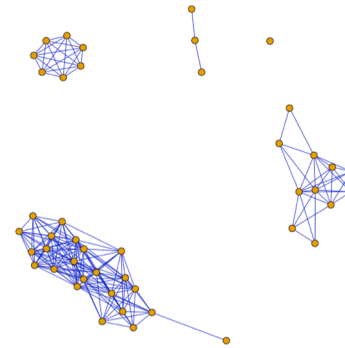
Lolium perenne



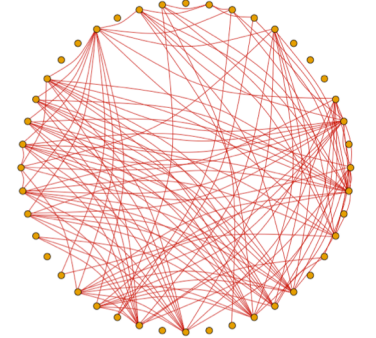
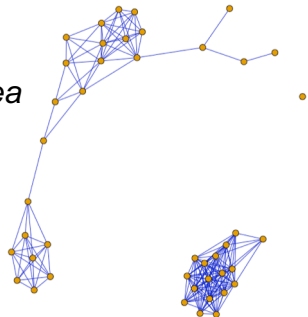
Trifolium hybridum

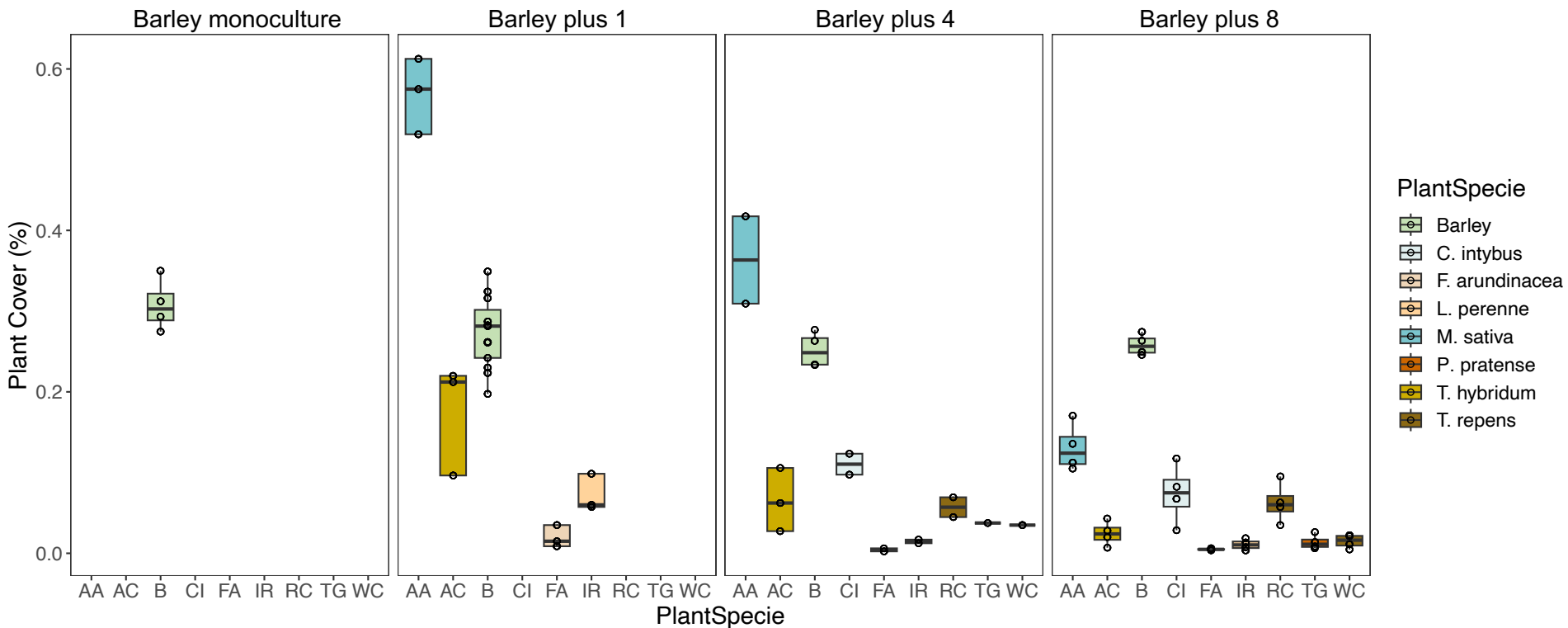


Medicago sativa

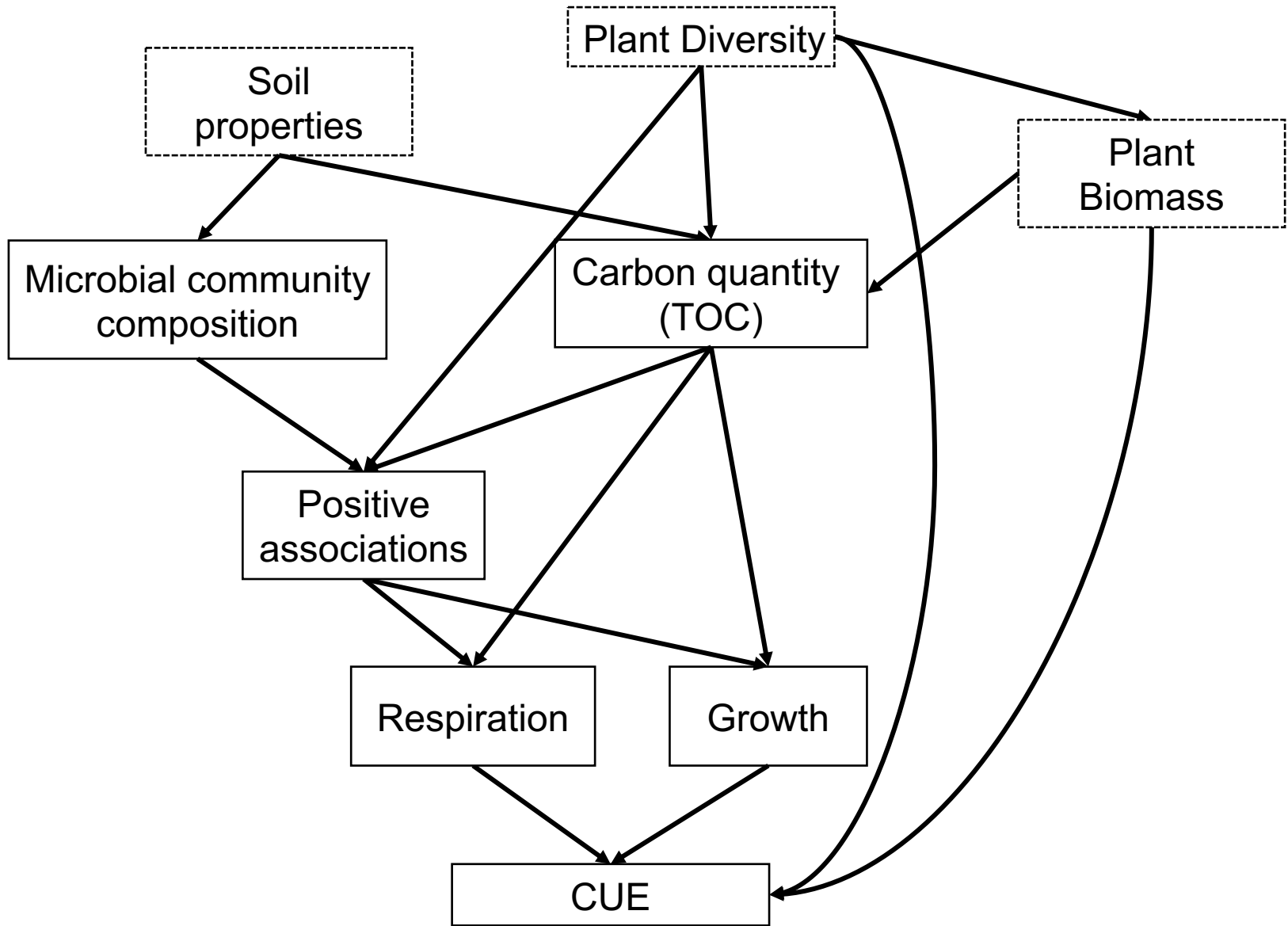


Festuca arundinacea

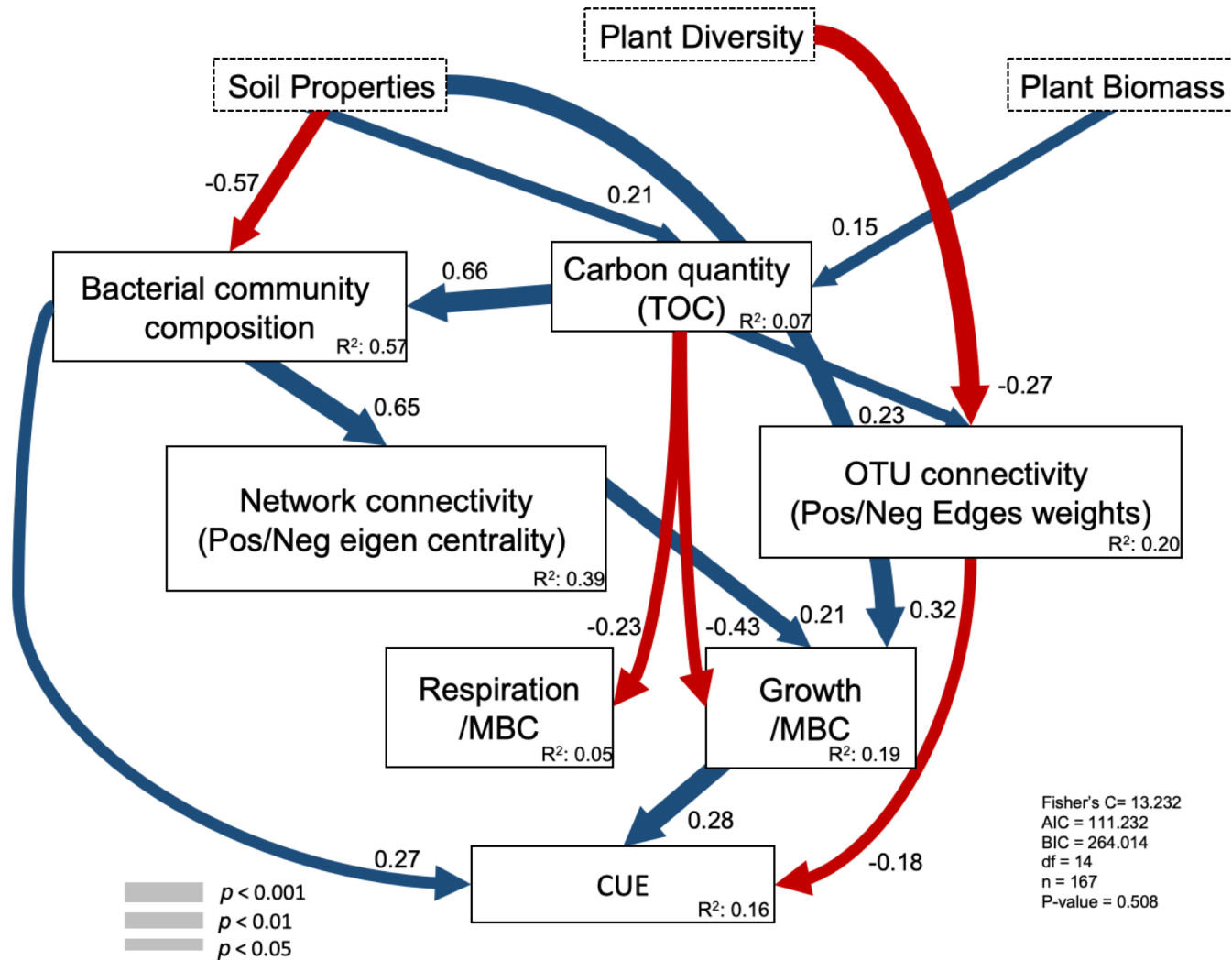




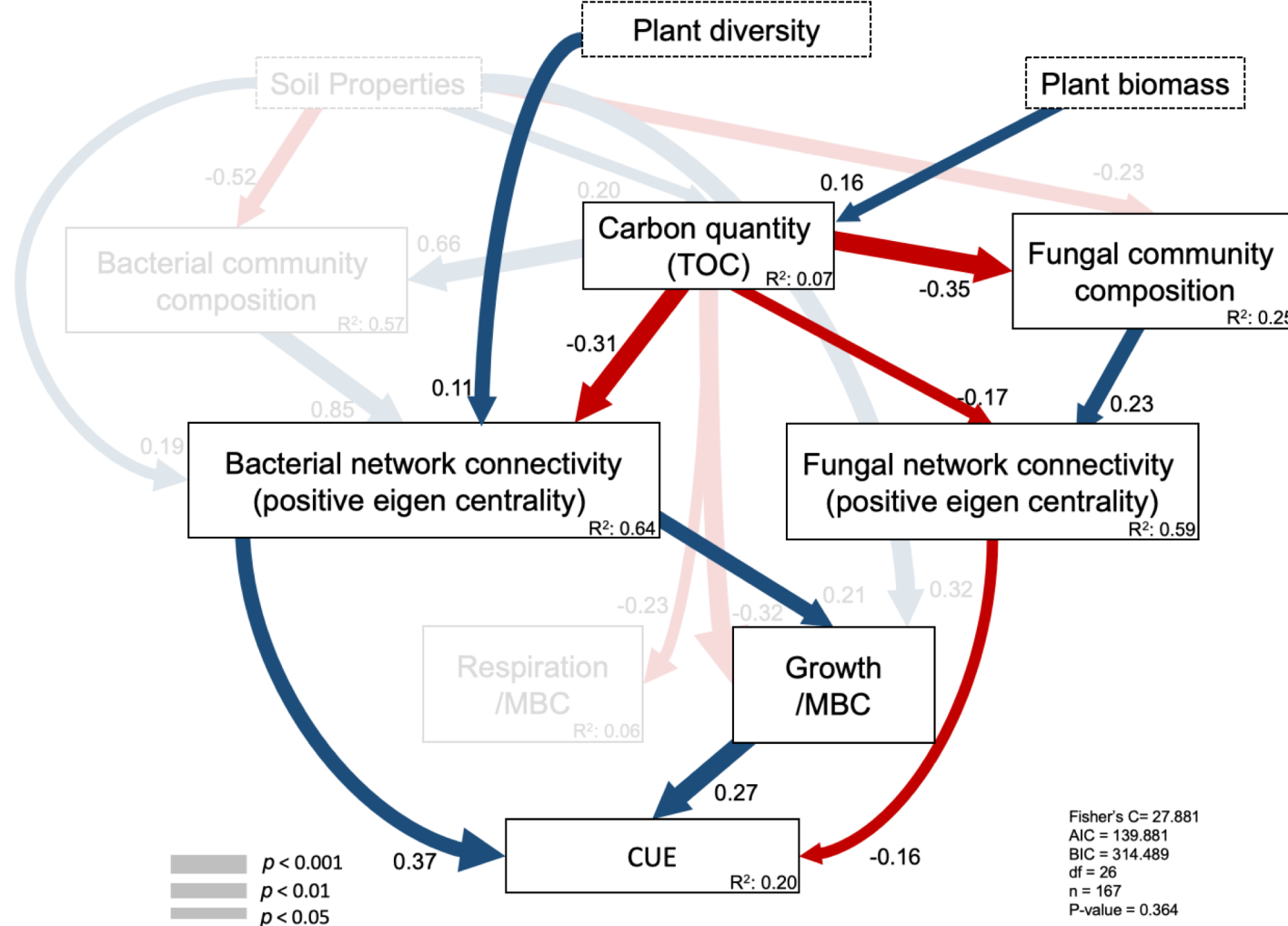
Supplementary Figure 16. Percent plant cover in the TwinWin experiment during August 2020 in the sampled plots for the experiment. The plant species are: *Medicago sativa*: AA; *Trifolium hybridum*: AC; *Hordeum vulgare* (barley): B; *Cichorium intybus*: CI; *Festuca arundinacea*: FA; *Lolium Perenne*: IR; *Trifolium pratense*: RC; *Phleum pratense*: TG; *Trifolium repens*: WC. In the boxplots, whiskers denote the minimum value or $1.5 \times$ interquartile range (whichever is more extreme), and box denotes interquartile range. The horizontal line denotes the median. Source data are provided as a Source Data file.



Supplementary Figure 17. Full hypothesized path model.



Supplementary Figure 18. Structural equation model incorporating the ratio between positive and negative network parameters. Path width corresponds to degree of significance as shown in the lower right and standard coefficient for each path is shown on a circle within each path. The amount of variance explained by the model (R^2) is shown for each response variable. Measures of overall model fit are shown in the lower left. Soil properties: composite variable of soil properties (i.e. pH, Calcium (g/kg soil), C/N ratio and Cation exchange capacity (cmol/kg soil)), Plant diversity: Simpson's plant diversity index calculated based on species present in a plot and their relative abundances; Plant biomass: individual barley (*Hordeum vulgare*) plants above ground biomass; Bacterial community composition: NMDS axis 1 of bacterial community structure; Ratio between positive and negative bacterial network connectivity: positive/negative eigen centrality; Respiration/MBC: mass specific respiration; Growth/MBC: mass specific growth; CUE: carbon use efficiency. Global goodness-of-fit: Fisher's C. Source data are provided as a Source Data file.



Supplementary Figure 19. Structural equation model including both bacterial and fungal network parameters. Path width corresponds to degree of significance as shown in the lower right and standard coefficient for each path is shown on a circle within each path. The amount of variance explained by the model (R²) is shown for each response variable. Measures of overall model fit are shown in the lower left. Soil properties: composite variable of soil properties (i.e. pH, Calcium (g/kg soil), C/N ratio and Cation exchange capacity (cmol/kg soil)), Plant diversity: Simpson's plant diversity index calculated based on species present in a plot and their relative abundances; Plant biomass: individual barley (*Hordeum vulgare*) plants above ground biomass; Bacterial community composition: NMDS axis 1 of bacterial community structure; Fungal community composition: NMDS axis 1 of fungal community structure; Positive bacterial and fungal network connectivity: positive eigen centrality from positive bacterial and fungal networks, respectively; Respiration/MBC: mass specific respiration; Growth/MBC: mass specific growth; CUE: carbon use efficiency. Global goodness-of-fit: Fisher's C. Source data are provided as a Source Data file.