Supplemental Information for:

Unraveling spatio-temporal variability of arbuscular mycorrhizal fungi in a temperate grassland plot

Kezia Goldmann, Runa S. Boeddinghaus, Sandra Klemmer, Kathleen M. Regan, Anna Heintz-Buschart, Markus Fischer, Daniel Prati, Hans-Peter Piepho, Doreen Berner, Sven Marhan, Ellen Kandeler, François Buscot and Tesfaye Wubet

Table of Contents:

Supplemental text	Page 2
Table S1	Page 3
Table S2	Page 4
Table S3	Page 5
Table S4	Page 6
Table S5	Page 6
Figure S1	Page 8
Figure S1	Page 9
Figure S3	Page 10
Figure S4	Page 11
Figure S5	Page 12
Figure S6	Page 13
Figure S7	Page 14
Figure S8	Page 15
Figure S9	Page 16
Figure S10	Page 17
Figure S11	Page 18
Figure S12	Page 19
Figure S13	Page 20
References	Page 21

Supplemental methods

We assessed the AMF beta-diversity according to the following formula (Baselga & Orme, 2012):

$$\beta_{SOR} = \beta_{SIM} + \beta_{SNE}$$

with β_{SOR} as beta-diversity, which is the sum of β_{SIM}, the turnover component i.e. taxa replacement, and $\beta_{SNE,}$ the nestedness component, i.e. taxa gain and loss.

Supplemental results

Changes in AMF community composition were more pronounced between than within subplots (Figure S10 and Figure S11). In particular, AMF composition within single subplots differed relatively little between April and May, and between May and June the within-subplot diversity was still smaller than between-subplot diversity. This effect weakened after the observed changes in June and was no longer significant for the later time points (August, October and November). The changes in AMF community composition increased from spring to summer months and decreased again slightly in autumn (Figure S10).

Supplemental Tables

Table S1: Percent distribution of overall AMF OTUs (genera) as supplement to the barplot (Figure 1) showing relative distribution of overall AMF OTUs (genera)

Genus	**April**	**May**	**June**	**August**	**October**	**November**
Glomus	69.0	73.9	71.3	73.3	76.8	77.0
Claroideoglomus	15.0	13.5	16.4	12.9	13.8	11.5
Diversispora	8.0	5.4	6.6	6.9	5.1	5.3
Archaeospora	3.0	2.7	2.5	2.6	2.2	2.7
Acaulospora	2.0	1.8	0.8	0.9	0.7	0.9
Ambispora	2.0	0.9	0.8	1.7	0.7	1.8
Paraglomus	1.0	1.8	1.6	1.7	0.7	0.9
OTUs in total	**100**	**111**	**122**	**116**	**138**	**113**

Table S2: Permutational multivariate analysis of variance based on the Sørensen distance matrix, stratified by sampling date for single environmental variables. Significance: n.s. = not significant, 0.01 < *p* < 0.05 = *, 0.001 < *p* < 0.01 =**.

explanatory variable	significance	R^2 for significant variables
soil water content	n.s.	
NH_4^+	n.s.	
N_{min}	n.s.	
EOC	n.s.	
NO_3^-	n.s.	
total plant cover	n.s.	
legume biomass	n.s.	
total plant biomass	n.s.	
silt content	**	0.020
forbs biomass	n.s.	
N_{mic}	**	0.021
pH	**	0.034
grass biomass	n.s.	
bacteria (PLFA)	*	0.011
C_{mic}	*	0.018
ratio gram+:gram- (PLFA)	n.s.	
gram- bacteria (PLFA)	**	0.011
gram+ bacteria (PLFA)	n.s.	
EON	*	0.027
root biomass	n.s.	
ratio C_{mic}:N_{mic}	**	0.025
ratio fungi:bacteria (PLFA)	n.s.	
litter biomass	n.s.	
bulk density	n.s.	
PO_4^{2-}	*	0.015
C_{total} content	*	0.008
N_{total} content	*	0.008
ratio C_{total}:N_{total}	n.s.	
fungi (PLFA)	n.s.	
bacteria 16S	n.s.	
x coordinate	**	0.024
y coordinate	**	0.019

Table S3: Permutational multivariate analysis of variance based on the Sørensen distance matrix, stratified by sampling date combining environmental variables. Significance: n.s. = not significant, 0.01 < *p* < 0.05 = *, 0.001 < *p* < 0.01 =**.

explanatory variable	significance	R^2 for significant variables
silt content	**	0.018
pH	**	0.019
N_{mic}	**	0.023
ratio C_{mic}:N_{mic}	n.s.	
$PO4^{2-}$	n.s.	
EON	*	0.013
gram- bacteria (PLFA)	n.s.	
N_{total} content	n.s.	
C_{mic}	n.s.	
C_{total} content	*	0.008
bacteria (PLFA)	n.s.	
x coordinate	**	0.024
y coordinate	**	0.019

Table S4: Variables used to identify environmental influences on AMF abundances in alphabetical order. Given are the units and the sources of first publication. At each time point, variables were analyzed in both replicates of each of the 30 subplots (n = 60).

Environmental variable	Unit	Sampling times	Source
archaea 16S	copies g^{-1} DM	6	Regan et al. (2017)
bacteria (PLFA)	µg FAME g^{-1} DM	6	Regan et al. (2014, 2015)
bacteria 16S	copies g^{-1} DM	6	Regan et al. (2017)
bulk density	g cm^3	6	Regan et al. (2014, 2015)
clay content	%	1	Regan et al. (2014, 2015)
C_{mic}	µg C g^{-1} DM	6	Regan et al. (2014, 2015)
C_{total} content	%	6	Regan et al. (2014, 2015)
EOC	µg C g^{-1} DM	6	Regan et al. (2014, 2015)
EON	µg N g^{-1} DM	6	Regan et al. (2014, 2015)
forbes biomass	g 400 cm^{-2}	6	Regan et al. (2014, 2015)
fungi (PLFA)	µg FAME g^{-1} DM	6	Regan et al. (2014, 2015)
gram- bacteria (PLFA)	µg FAME g^{-1} DM	6	Regan et al. (2014, 2015)
gram+ bacteria (PLFA)	µg FAME g^{-1} DM	6	Regan et al. (2014, 2015)
grass biomass	g 400 cm^{-2}	6	Regan et al. (2014, 2015)
legume biomass	g 400 cm^{-2}	6	Regan et al. (2014, 2015)
litter biomass	g 400 cm^{-2}	6	Regan et al. (2014, 2015)
NH_4^+	µg N g^{-1} DM	6	Regan et al. (2014, 2015)
N_{mic}	µg N g^{-1} DM	6	Regan et al. (2014, 2015)
N_{min}	µg N g^{-1} DM	6	Regan et al. (2014, 2015)
NO_3^-	µg N g^{-1} DM	6	Regan et al. (2014, 2015)
N_{total} content	%	6	Regan et al. (2014, 2015)
pH		6	Regan et al. (2014, 2015)
plant species number	count	6	Regan et al. (2014, 2015)
PO_4^{3-}	µg P g^{-1} DM	3	Regan et al. (2014, 2015)
ratio C_{mic}:N_{mic}		6	Regan et al. (2014, 2015)
ratio C_{total}:N_{total}		6	Regan et al. (2014, 2015)
ratio fungi:bacteria (PLFA)		6	Regan et al. (2014, 2015)
ratio gram+:gram- (PLFA)		6	Regan et al. (2014, 2015)
root biomass	g roots 400 cm^{-2}	6	Regan et al. (2014, 2015)
Shannon Index	–	3	Regan et al. (2014, 2015)
silt content	%	1	Regan et al. (2014, 2015)
soil water content	% DM	6	Regan et al. (2014, 2015)
total plant biomass	g 400 cm^{-2}	6	Regan et al. (2014, 2015)
total plant cover	%	4	Regan et al. (2014, 2015)

Table S5: Comparison of linear mixed effect models with different random structures using SAS 9.4 (SAS Institute Inc., Cary, USA). AIC = Akaike's information criterion, RCBD = randomized complete block design, POWA = anisotropic power, POW = power, EXP = exponential, ANOVA = analyses of variance, s.e.d. = standard error of difference.

Analysis	**AIC**	**Mean s.e.d.**
(1) One-way ANOVA (Regan et al. 2014, 2017)	2137.77	0.8568
(2) RCBD, random block	2090.94	0.7688
(3) RCBD, random block, random pair of sampling locations	2088.72	0.8354
(4) RCBD, random block, POWA spatial across field*	2089.31	0.8031
(5) RCBD, random block, POWA spatial within plot*	2088.46	0.8163
(6) RCBD, random block, POW=EXP spatial within plot	2085.46	0.8050
(7) RCBD, random block, POW=EXP spatial across field	2087.26	0.8020
(8) RCBD, random block, POWA spatial across field+ temporal*	2090.64	0.8472
(9) One-way ANOVA, POWA spatial across field + temporal	2096.29	0.8988
(10) One-way ANOVA, POWA spatial across field+ temporal + nugget	2074.57	1.7337

* Nugget did not yield improvement

Supplemental Figures

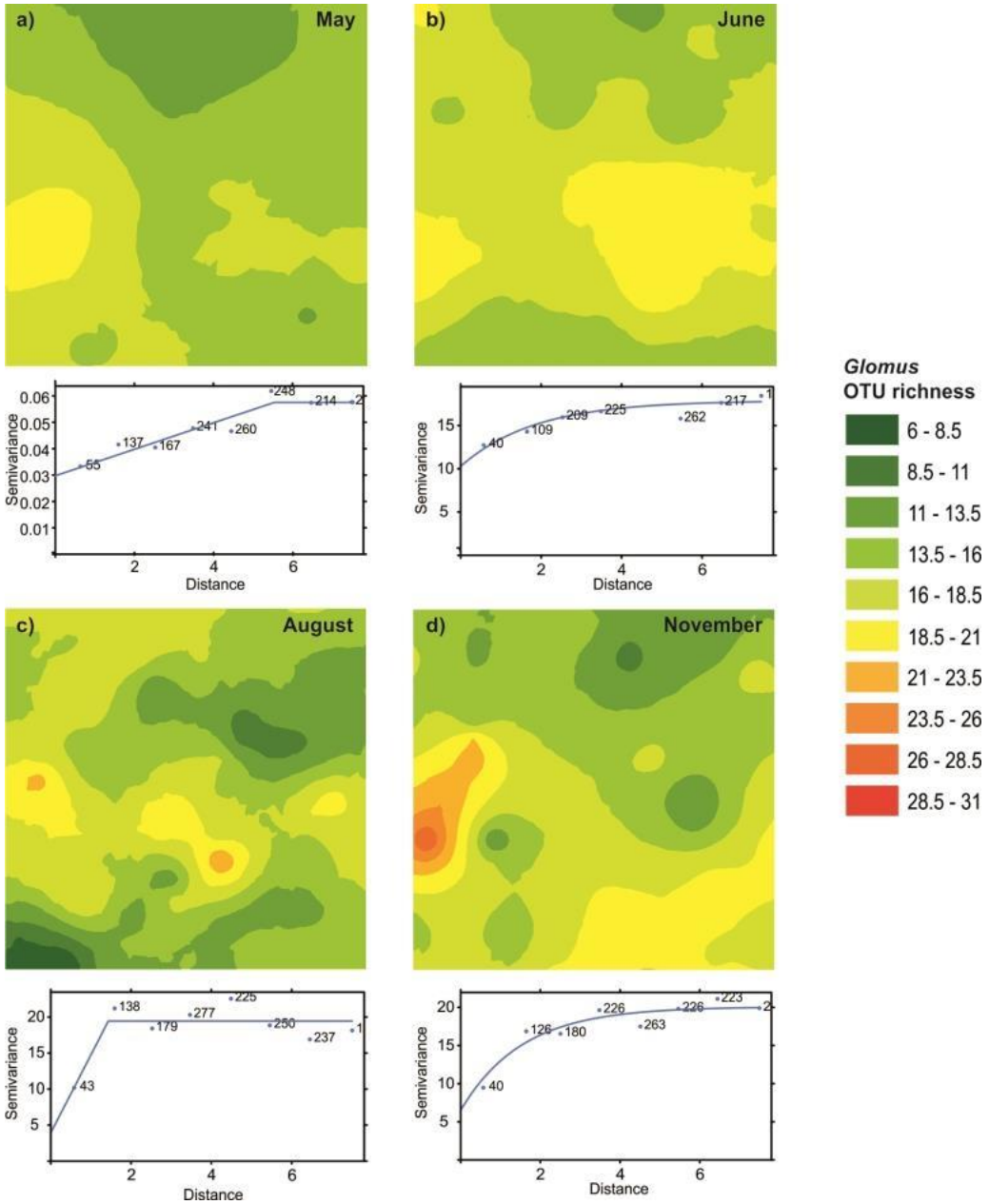

Figure S1: Spatial analysis of *Glomus* OTU richness grouped together per sampling date: a) May, b) June, c) August and d) November. Spatial patterns within the data were analyzed and calculated as semivariogram models and visualized as kriged maps using these models. Dimensions of all maps are 10 m x 10 m.

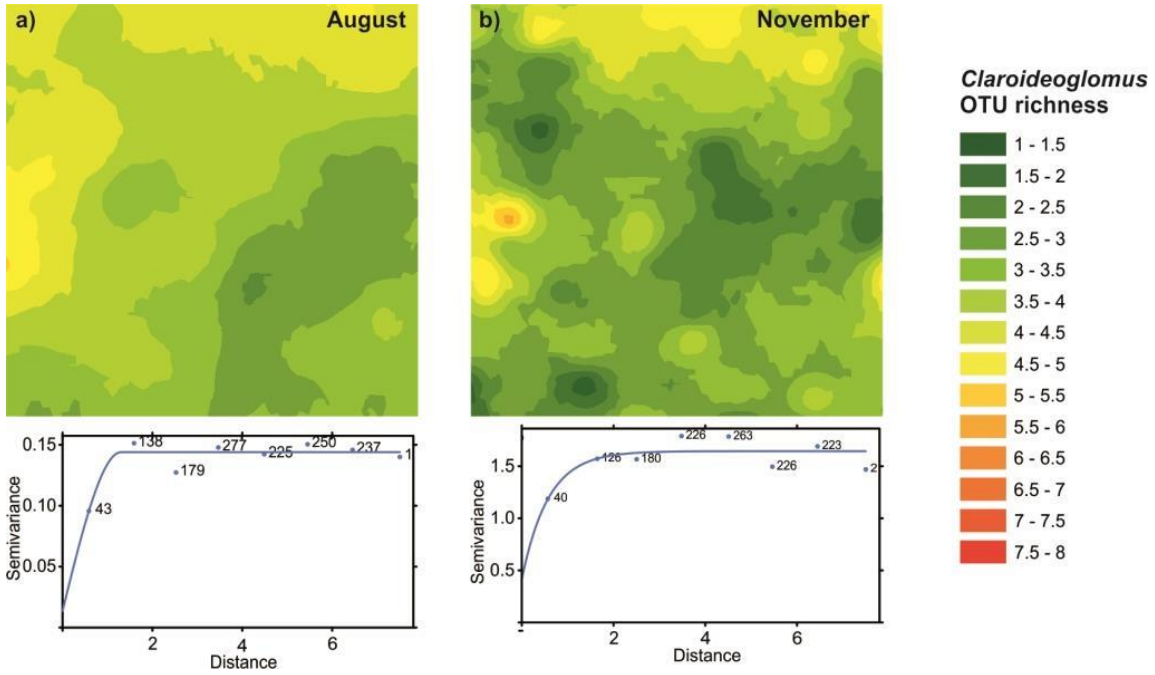

Figure S2: Spatial analysis of *Claroideoglomus* OTU richness grouped together per sampling date: a) August, b) November. Spatial patterns within the data were analyzed and calculated as semivariogram models and visualized as kriged maps using these models. Dimensions of all maps are 10 m x 10 m.

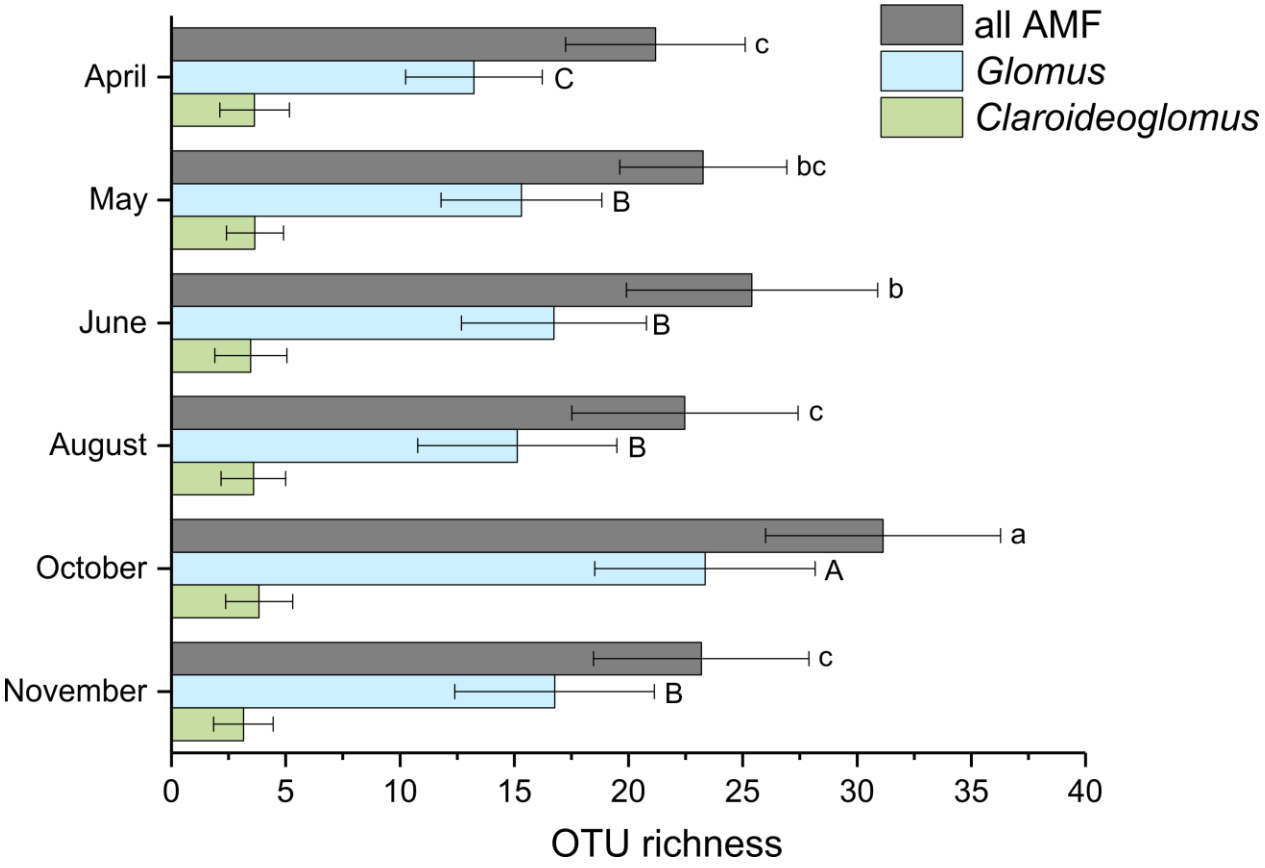

Figure S3 Seasonal distribution of total AMF OTUs and additionally for the OTUs assigned to the genera Glomus and Claroideoglomus, displayed as bar graphs; differences in significance ($p<0.05$) are indicated by different letters above bars/boxplots. Given are mean values of subplots, whiskers indicate standard deviation.

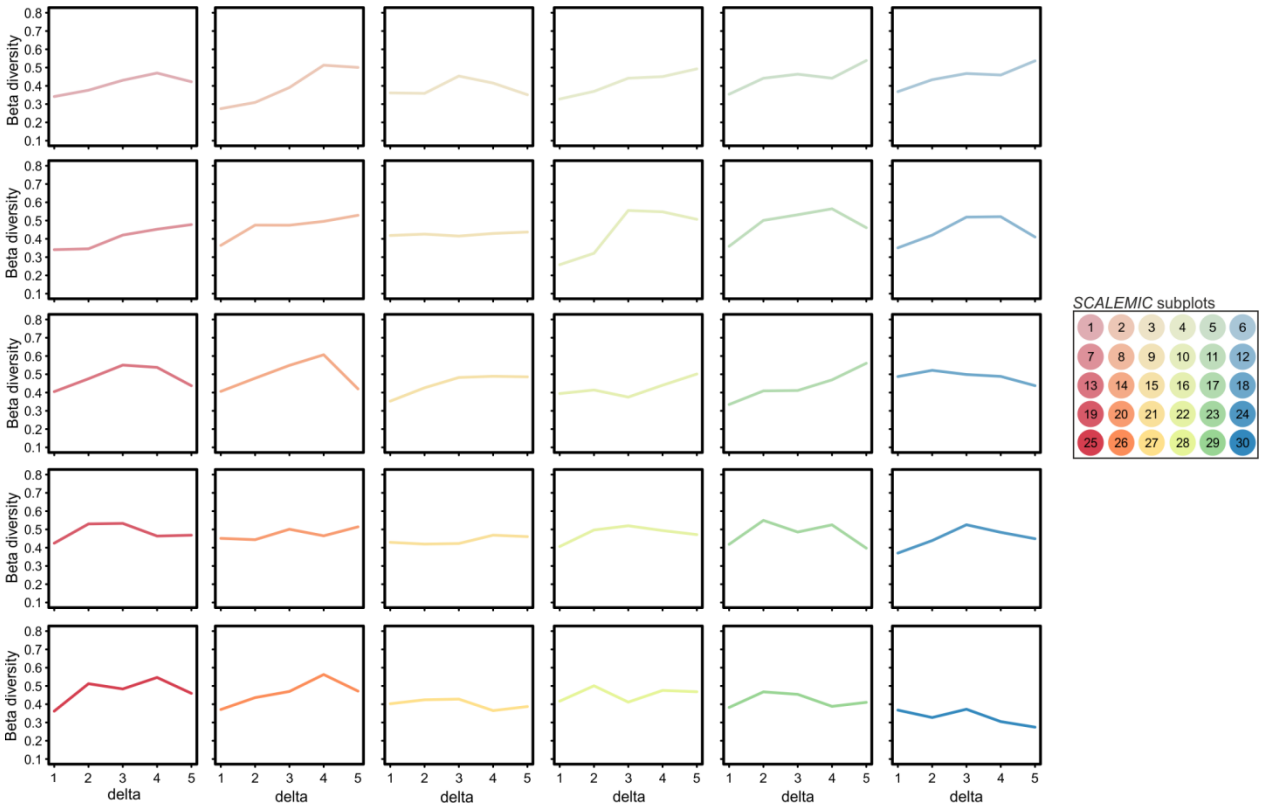

Figure S4: Patterns of variability within AMF beta-diversity for all subplots from one studied time point to the next (delta 1: April to May, delta 2: May to June, delta 3: June to August, delta 4: August to October and delta 5: October to November); lines represent the overall beta-diversity (β_{SOR}) observed in the partial data sets, computed using the R-package *betapart* (Baselga & Orme, 2012).

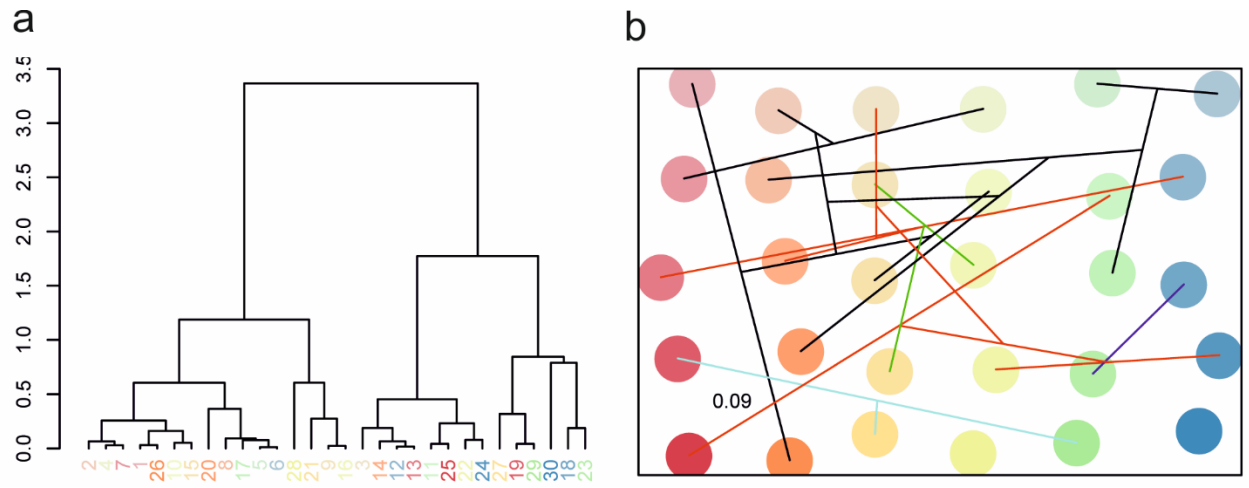

Figure S5: Correlation of the development of AMF beta-diversity (β_{SOR}) from one time point to the next (see Figure S3), represented as a) dendrogram; b) dendrogram overlaid on a jittered representation of the subplots. Crossing lines indicate that no correlation between spatial subplot position and development of AMF beta-diversity could be determined; displayed value: mean Mantel correlation coefficient of Euclidean distance of x- and y-values and the correlation between AMF beta-diversity developments of the subplots.

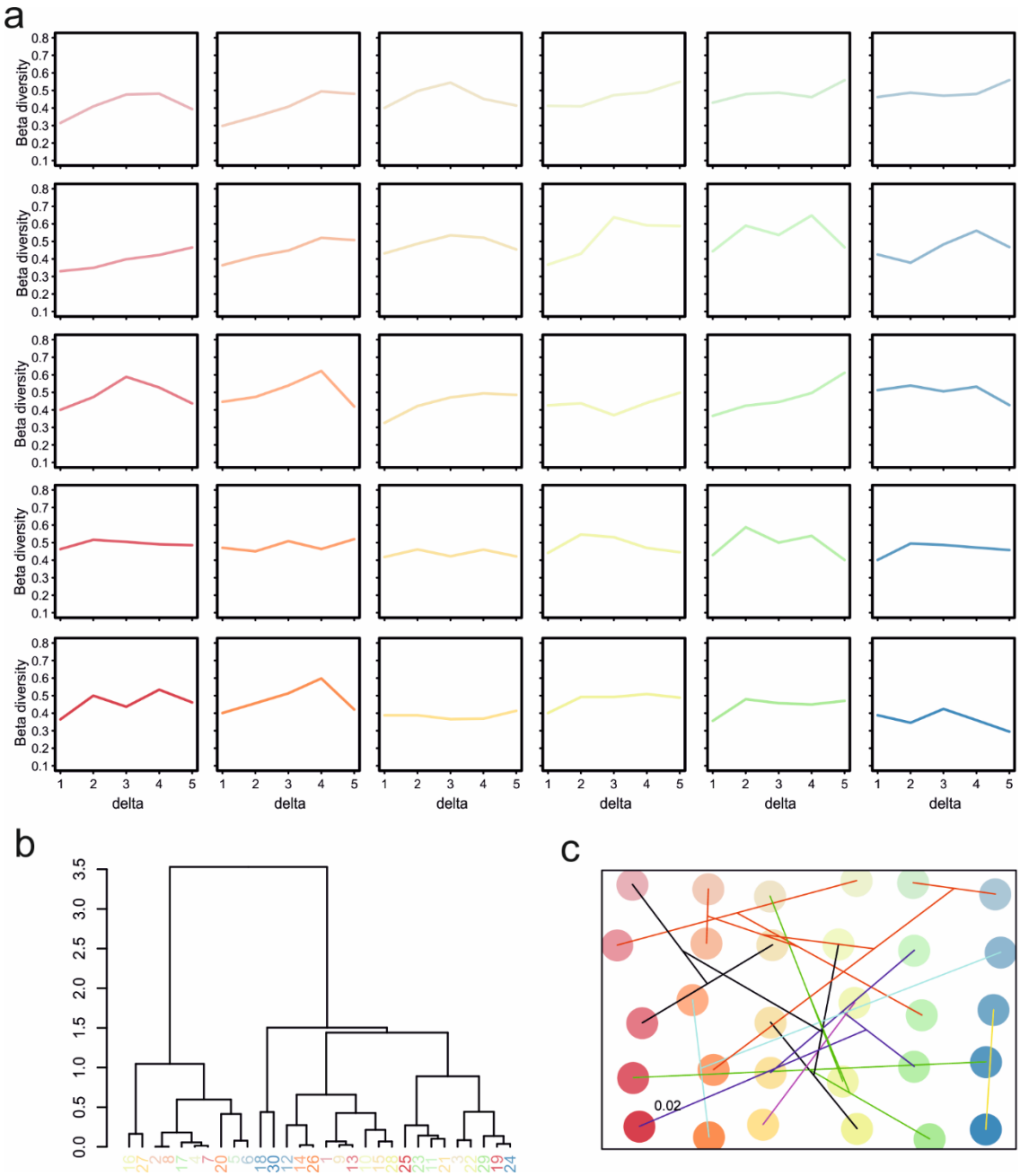

Figure S6: a) Patterns of variability within *Glomus* beta-diversity for all subplots from one studied time point to the next (delta 1: April to May, delta 2: May to June, delta 3: June to August, delta 4: August to October and delta 5: October to November); lines represent the overall beta-diversity (β_{SOR}) observed in the partial data sets, computed using the R-package *betapart* (Baselga & Orme, 2012); b) Dendrograms displaying the correlation between *Glomus* beta-diversity profiles; c) overlaid on jittered spatial representations of the subplots (displayed value: Mantel coefficient of correlation of Euclidean distance of x- and y-values and the *Glomus* beta-diversity development of the subplot).

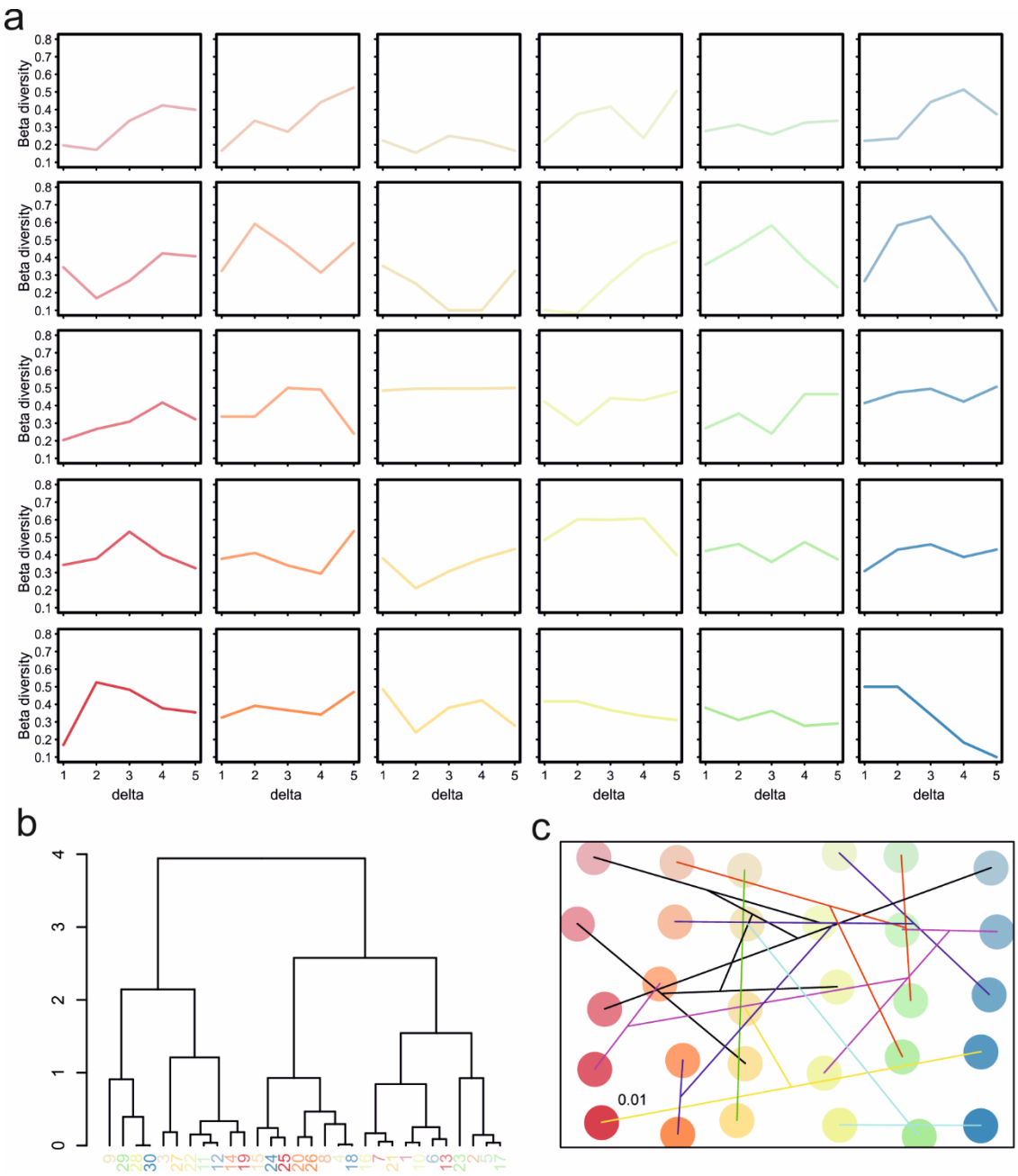

Figure S7: a) Patterns of variability within *Claroideoglomus* beta-diversity for all subplots from one studied time point to the next (delta 1: April to May, delta 2: May to June, delta 3: June to August, delta 4: August to October and delta 5: October to November); lines represent the overall beta-diversity (β_{SOR}) observed in the partial data sets, computed using the R-package *betapart* (Baselga & Orme, 2012); b) Dendrograms displaying the correlation between *Claroideoglomus* beta-diversity profiles; c) overlaid on jittered spatial representations of the subplots (displayed value: Mantel coefficient of correlation of Euclidean distance of x- and y-values and the *Claroideoglomus* beta-diversity development of the subplot).

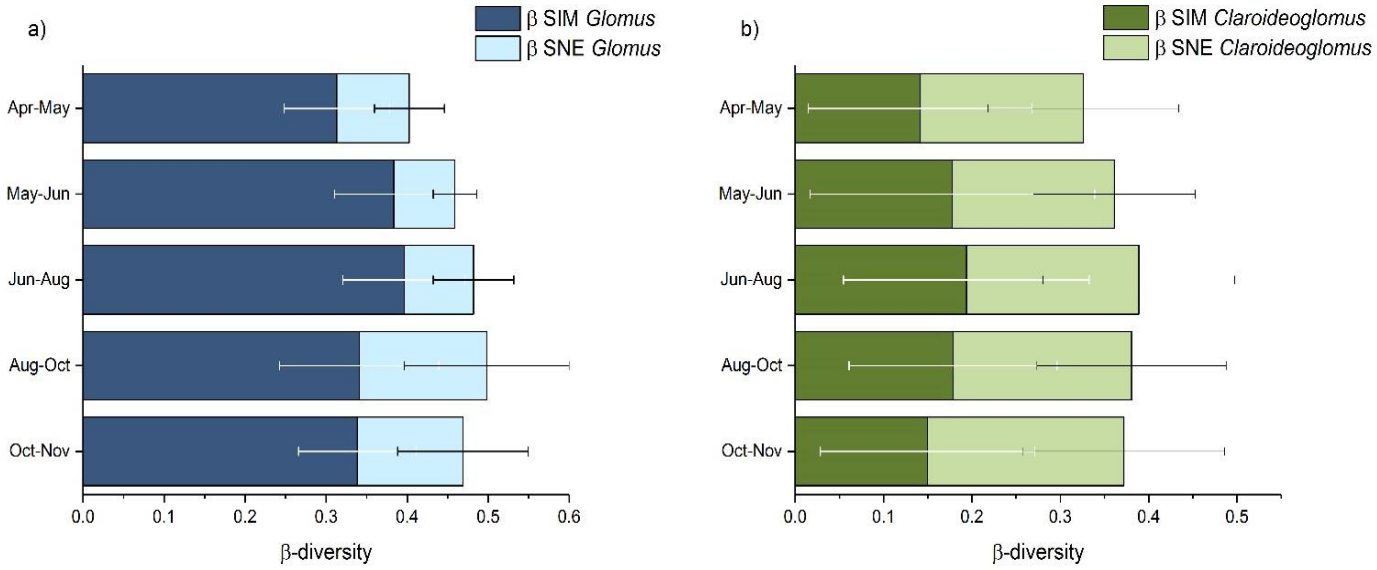

Figure S8: Patterns of variability within assemblages of a) *Glomus* and b) *Claroideoglomus* across the studied plot from one studied time point to the next. The stacked bars represent overall beta-diversity (β_{SOR}) observed in the partial data sets, computed using the R-package betapart (Baselga & Orme, 2012); darker sections of the bars represent the contribution of the turnover of the represented genus (β_{SIM}), whereas the lighter sections account for the nestedness of the represented genus (β_{SNE}); error bars represent variability between subplots.

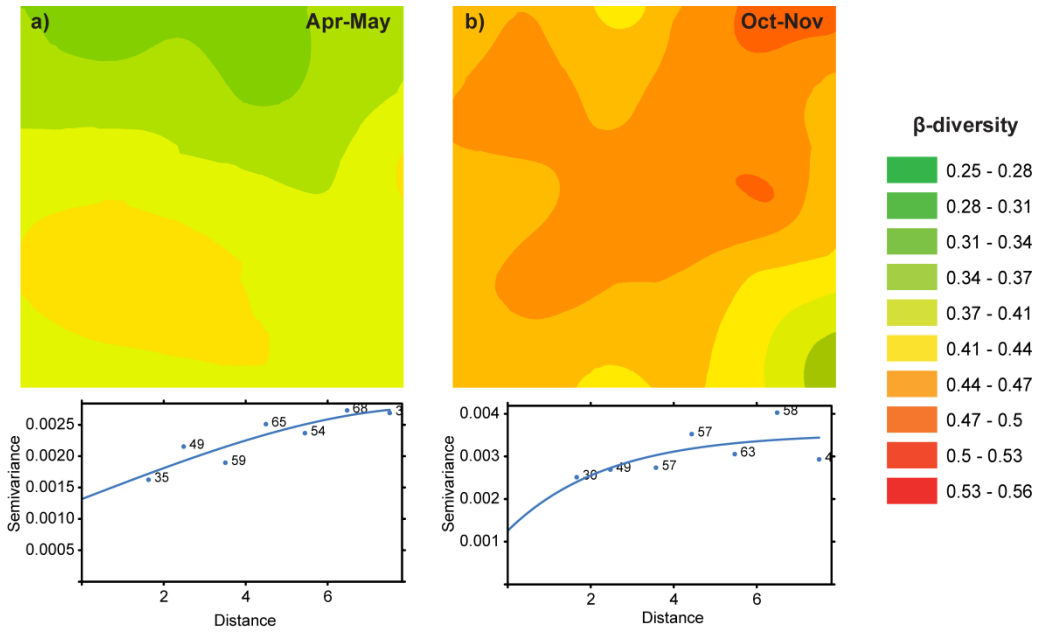

Figure S9 Spatial analysis of AMF beta-diversity with all AMF OTUs grouped together from one time point to the next: a) April-May, b) October-November. Spatial patterns within the data were analyzed and calculated as semivariogram models (lower panel) and visualized as kriged maps (upper panel) using these models. Dimensions of all maps are 10 m x 10 m.

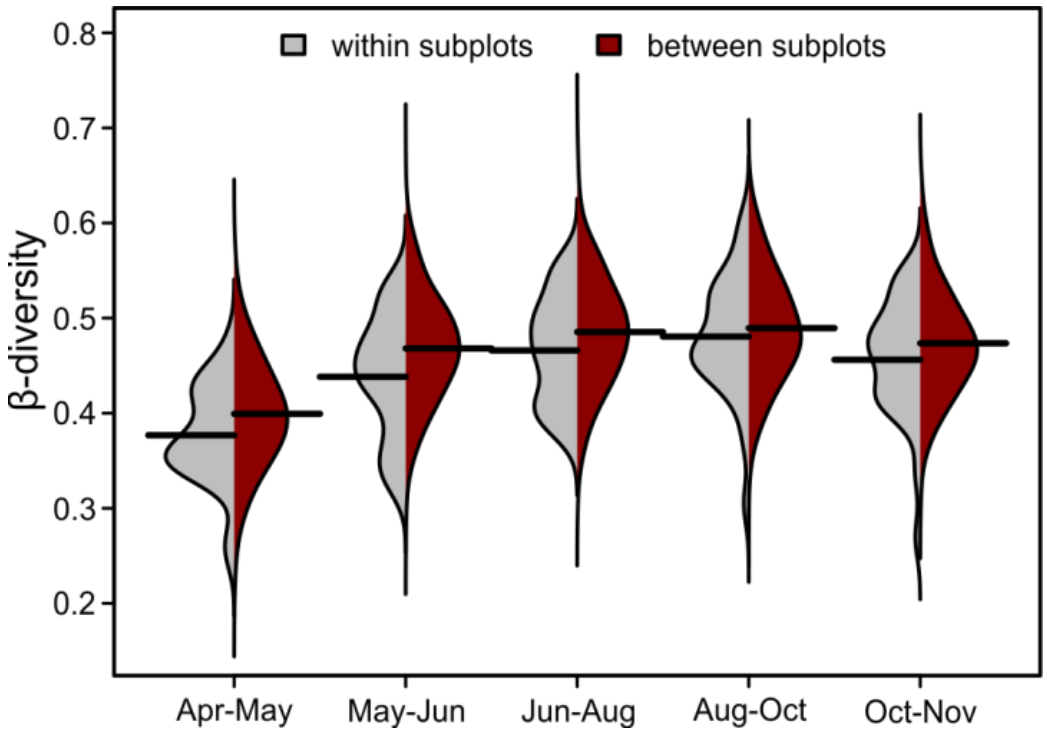

Figure S10 Histograms and comparisons of AMF beta-diversity (β_{SOR}) within and between subplots from one time point to the next; April-May (p = 0.03644), May-June (p = 0.021), June-August (p = 0.08424), August-October (p = 0.5145) and October-November (p = 0.2616); p-values below 0.05 indicate significant differences in AMF beta-diversity within and between subplots.

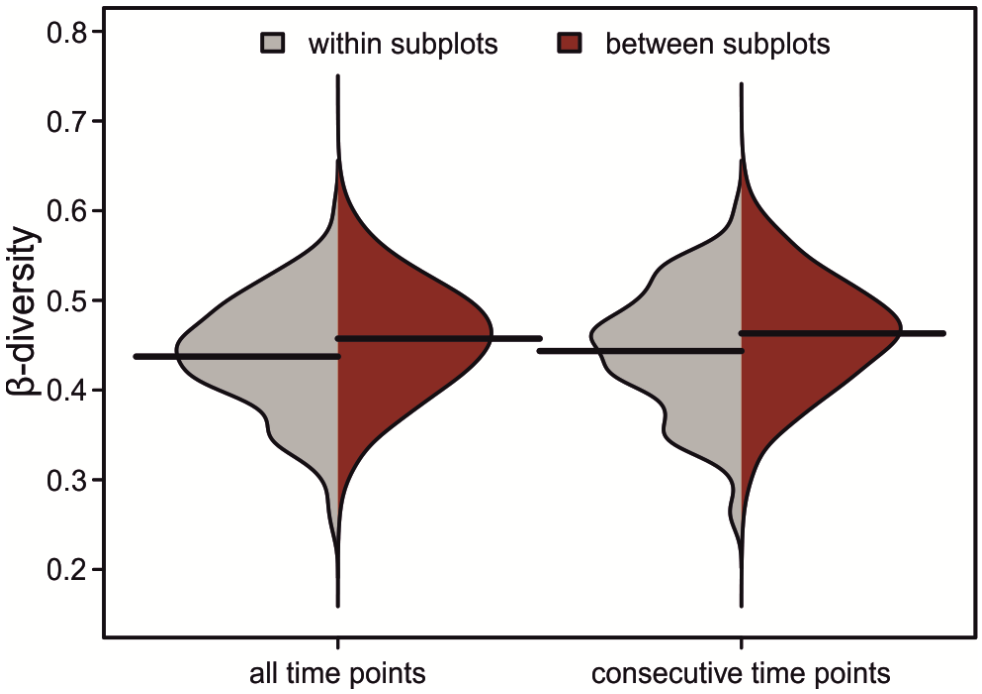

Figure S11: Histograms and comparisons of AMF beta-diversity (β_{SOR}) within and between subplots over all time points ($p < 0.001$) and from one time point to the next ($p = 0.0015$); *p*-values below 0.05 indicate significant differences in AMF beta-diversity within and between subplots.

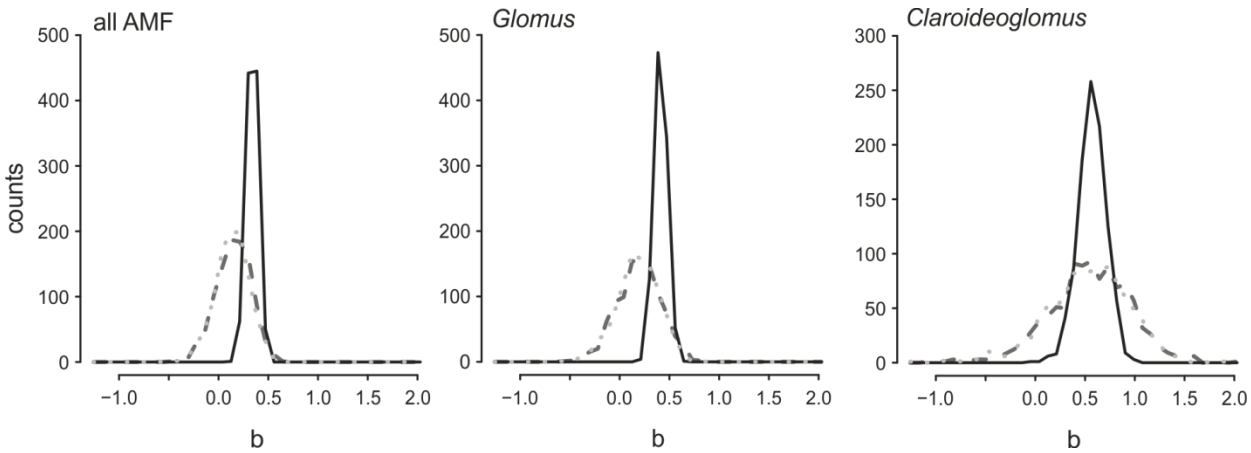

Figure S12: Histograms illustrate the distribution of regression coefficients (b) that account for the fitting of 1000 different rarefactions (black line), compared to null-models based on 1000 draws of species identities (based on their relative probability of occurrence among samples) of the whole dataset (grey dashed line) and timepoint-specific datasets (light grey dotted line).

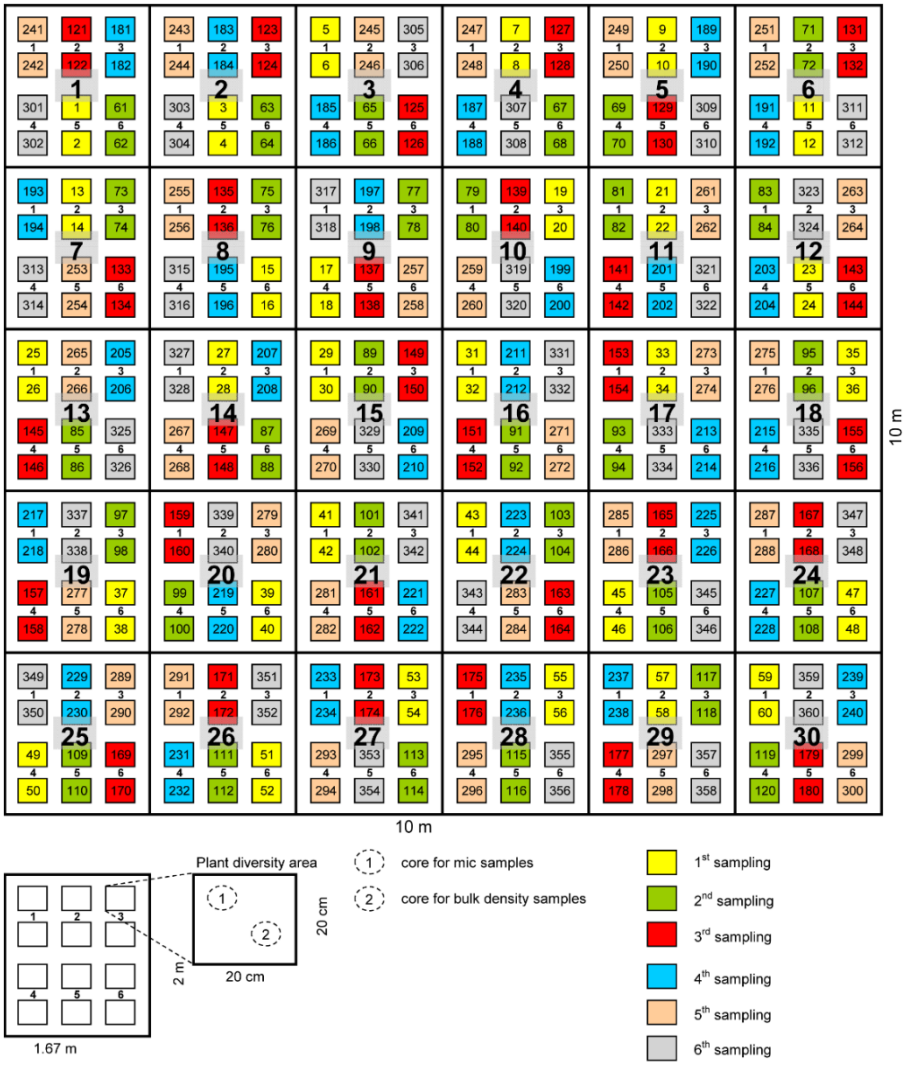

Figure S13: Sampling design of *SCALEMIC Experiment* (taken from Regan et al. (2014))

References

Baselga, A., and Orme, C.D.L. (2012) betapart: an R package for the study of beta diversity. Methods in Ecology and Evolution 3: 808-812.

Regan, K.M., Nunan, N., Boeddinghaus, R.S., Baumgartner, V., Berner, D., Boch, S. et al. (2014) Seasonal controls on grassland microbial biogeography: Are they governed by plants, abiotic properties or both? Soil Biol Biochem 71: 21-30.

Regan, K.M., Nunan, N., Boeddinghaus, R.S., Baumgartner, V., Berner, D., Boch, S. et al. (2015) Corrigendum to "Seasonal controls on grassland microbial biogeography: are they governed by plants, abiotic properties or both?"[Soil Biology and Biochemistry 71 (April 2014)]. Soil Biology & Biochemistry 86: 2e214.

Regan, K., Stempfhuber, B., Schloter, M., Rasche, F., Prati, D., Philippot, L. et al. (2017) Spatial and temporal dynamics of nitrogen fixing, nitrifying and denitrifying microbes in an unfertilized grassland soil. Soil Biology and Biochemistry 109: 214-226.