

SUPPLEMENTARY MATERIALS

Waste-derived organic soil amendments for a sustainable vineyard management: linking microbiome responses to soil biochemistry

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Supplementary Tables

Supplementary Tab. S1 – Potential soil enzymatic activities. Means \pm standard error of soil potential β -glucosidase (β -glu, $\mu\text{gN gds}^{-1} \text{h}^{-1}$), dehydrogenase (Dehy, $\mu\text{gINTF gds}^{-1} \text{h}^{-1}$), phosphatase (Phos, $\mu\text{gP gds}^{-1} \text{h}^{-1}$), laccase (Lac, $\mu\text{molABTS gds}^{-1} \text{min}^{-1}$), and tyrosinase (Tyr, $\mu\text{moldopachrome gds}^{-1} \text{h}^{-1}$) activities. Different lower-case letters indicate significant differences (p-value < 0.05).

Potential enzymatic activities					
Treatment	β -glu	Dehy	Phos	Lac	Tyr
CK	67.5 \pm 5.8	41.8 \pm 3.8 ^c	91.9 \pm 14.1 ^c	2.32 \pm 0.08	1.32 \pm 0.14 ^{abc}
MF	72.0 \pm 6.2	58.4 \pm 4.5 ^{abc}	99.2 \pm 13.2 ^c	2.16 \pm 0.03	0.98 \pm 0.18 ^{bc}
MOWC1	63.0 \pm 1.8	45.8 \pm 3.6 ^{bc}	154 \pm 21 ^{bc}	2.31 \pm 0.20	1.20 \pm 0.30 ^{abc}
MOWC2	77.8 \pm 9.7	65.5 \pm 5.9 ^{ab}	267 \pm 29 ^a	2.23 \pm 0.06	0.89 \pm 0.20 ^c
SP1	77.6 \pm 4.4	59.5 \pm 5.2 ^{abc}	183 \pm 19 ^b	2.25 \pm 0.16	1.43 \pm 0.13 ^{ab}
SP2	69.4 \pm 4.0	78.6 \pm 5.8 ^a	193 \pm 14 ^b	2.15 \pm 0.15	1.12 \pm 0.23 ^{abc}
SSC1	58.8 \pm 1.8	47.3 \pm 4.0 ^{bc}	189 \pm 28 ^b	2.29 \pm 0.08	1.48 \pm 0.18 ^a
SSC2	73.6 \pm 2.2	58.9 \pm 5.9 ^{abc}	211 \pm 14 ^{ab}	2.29 \pm 0.12	1.52 \pm 0.24 ^a
<i>p-value</i>	0.118	< 0.001	< 0.001	0.624	0.001

CK = Control, MF = Mineral Fertilization, MOWC = Municipal Organic Waste Compost, SSC = Sewage Sludge Compost, SP = Sludge Plasters.

Supplementary Tab. S2. Bacterial α -diversity. Average bacterial α -diversity indices (based on 16S rRNA gene sequencing) in all samples and in separate groups of samples, at 0-20 cm and 30-50 cm depth.

	Shannon index			Faith PD index			Chao1 index		
	0-20 cm depth	30-50 cm depth	p value ^a	0-20 cm depth	30-50 cm depth	p value ^a	0-20 cm depth	30-50 cm depth	p value ^a
All samples	7.7	7.8	0.8	61.0	62.0	0.6	454.7	456.6	0.9
CK	7.5	7.4	0.7	51.9	50.0	0.9	378.9	349.4	0.7
MF	7.4	7.6	1.0	51.9	59.5	0.7	365.1	433.0	0.3
MOWC1	7.7	8.1	0.3	63.7	70.6	0.2	464.7	546.9	0.1
MOWC2	8.1	8.1	0.9	69.8	67.3	0.9	532.9	501.5	0.7
SP1	7.7	7.6	0.7	63.1	57.7	0.3	454.7	412.7	0.3
SP2	7.4	7.6	0.5	53.9	59.1	0.3	364.9	429.5	0.3
SSC1	8.0	7.9	0.7	65.0	60.0	0.5	508.3	445.9	0.3
SSC2	8.2	8.0	0.9	68.6	71.8	0.7	568.1	533.8	0.3

^aWilcoxon rank-sum test.

PD, phylogenetic diversity.

CK, control; MF, mineral fertilizer; MOWC, municipal organic waste compounds-based amendment; SP, sludge plasters-based amendment; SSC, sewage sludge compost-based amendment.

Supplementary Table S3. Fungal α -diversity. Average fungal α -diversity indices (based on ITS sequencing) in all samples and in separate groups of samples, at 0-20 cm and 30-50 cm depth.

	Shannon index			Chao1 index		
	0-20 cm depth	30-50 cm depth	p value ^a	0-20 cm depth	30-50 cm depth	p value ^a
All samples	5.3	5.4	0.3	195.0	204.9	0.3
CK	5.0	5.6	0.5	184.9	203.6	0.7
MF	5.2	4.7	0.7	180.3	183.4	0.7
MOWC1	5.1	5.9	0.3	164.7	246.3	0.1
MOWC2	4.9	5.7	0.3	186.4	218.0	0.9
SP1	5.1	4.9	0.7	176.0	172.8	0.5
SP2	4.9	4.8	0.7	176.5	176.1	0.7
SSC1	6.1	5.8	1	252.0	226.1	0.7
SSC2	6.0	5.7	0.7	239.3	213.0	0.5

^aWilcoxon rank-sum test.

CK, control; MF, mineral fertilizer; MOWC, municipal organic waste compounds-based amendment; SP, sludge plasters-based amendment; SSC, sewage sludge compost-based amendment.

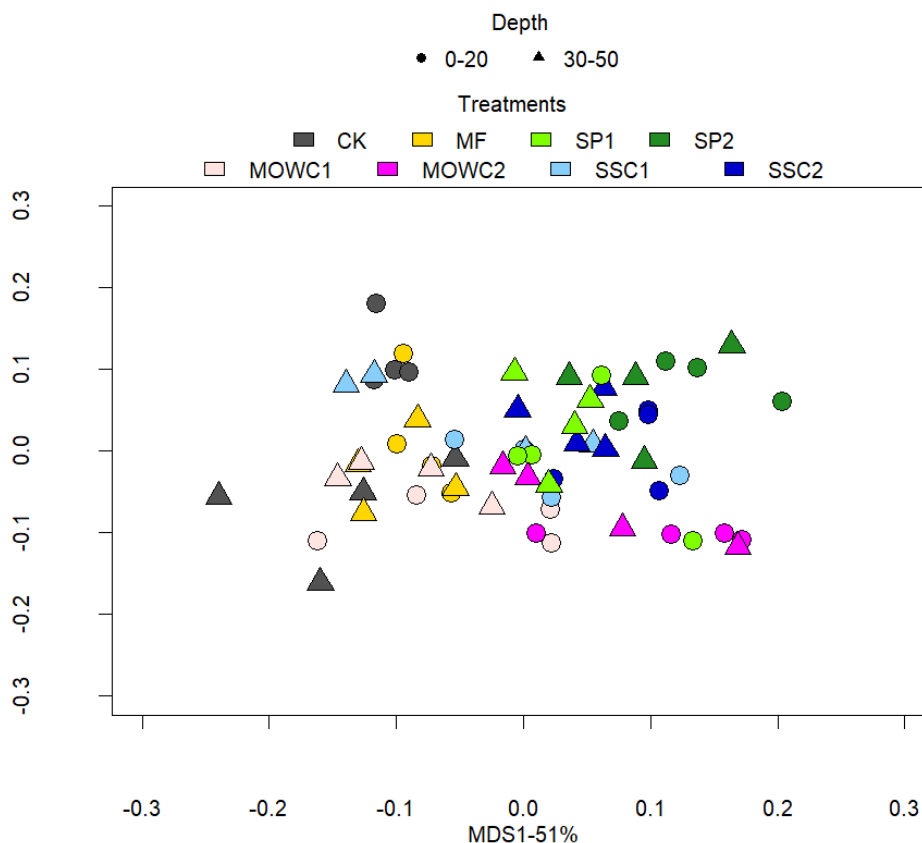
Supplementary Table S4. Bacterial phyla abundance. Mean relative abundance of the most represented bacterial phyla (mean relative abundance >2%) in the different groups of soil samples.

Phylum	Mean Relative Abundance (%)							
	CK	MF	MOWC1	MOWC2	SP1	SP2	SSC1	SSC2
Acidobacteriota	22.0	19.1	17.1	13.8	10.8	8.6	19.1	14.4
Actinobacteriota	23.0	26.1	20.9	12.8	21.0	18.0	17.5	18.8
Bacteroidota	4.1	4.2	8.4	15.6	10.3	12.9	9.0	10.9
Chloroflexi	3.0	2.5	2.5	2.5	3.0	2.4	3.0	2.9
Firmicutes	2.5	2.6	2.2	1.8	2.0	2.1	1.8	2.3
Gemmatimonadota	4.5	4.4	4.1	3.8	4.7	4.9	4.3	4.1
Myxococcota	2.1	1.8	2.9	3.8	1.7	1.4	3.0	2.4
Planctomycetota	2.9	2.8	2.6	2.5	2.3	2.9	3.1	2.8
Proteobacteria	27.0	24.7	30.9	35.5	37.8	40.7	29.3	32.5
Verrucomicrobiota	5.4	5.9	4.3	4.6	3.5	3.2	5.8	4.9

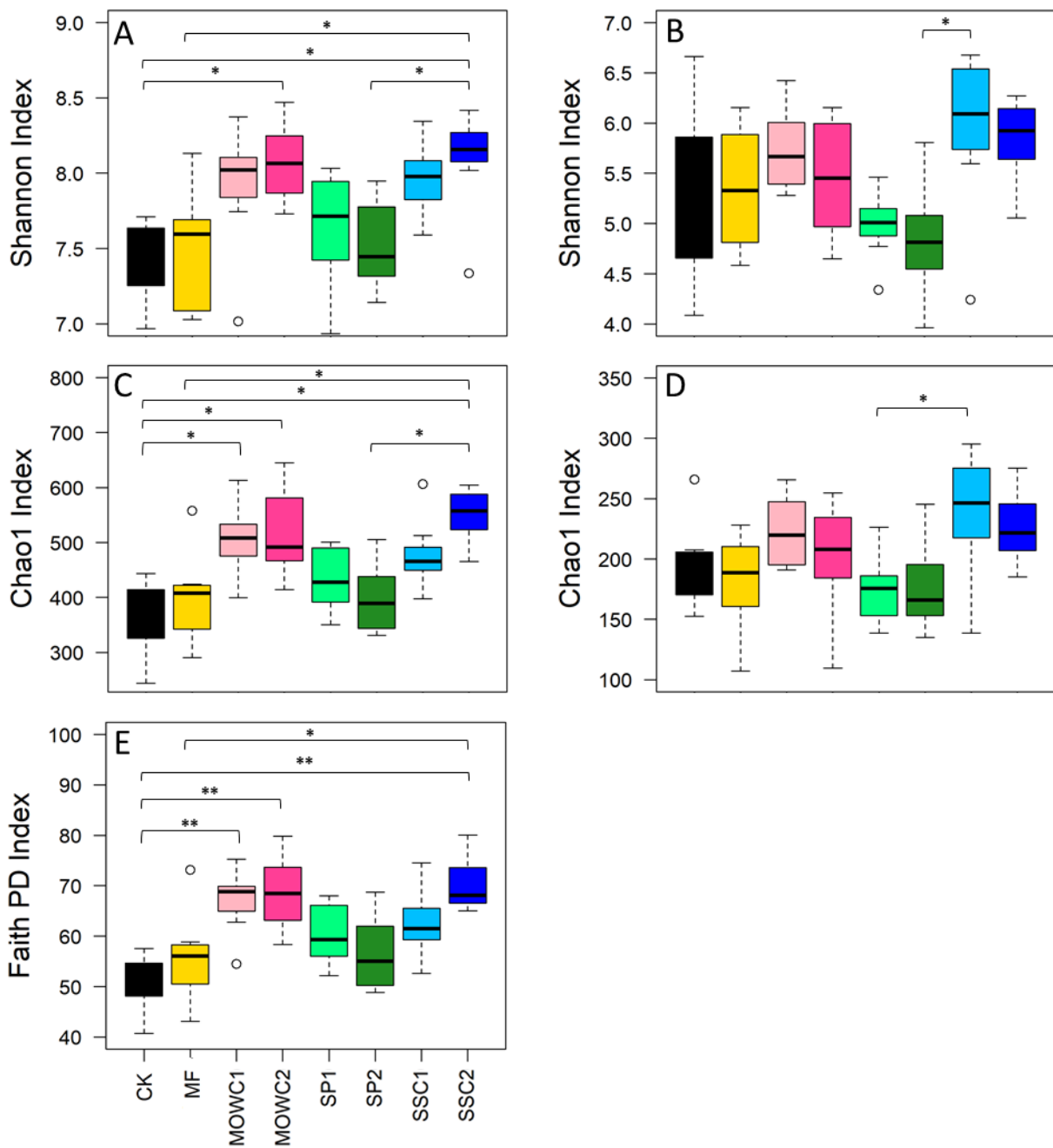
Supplementary Table S5. Putative hub nodes for the soil microbial community network. Putative hub nodes for the soil microbial community network, identified by defining a combination of highest values of closeness centrality, degree and betweenness centrality, as proposed by Agler et al. (2016).

	Closeness Centrality	Degree	Betweenness Centrality
<i>Rhizobiaceae</i>	0.70	42	0.04
<i>Gaiellales unclassified</i>	0.69	43	0.04
<i>Gaiellaceae</i>	0.69	42	0.04
<i>Pyrinomonadaceae</i>	0.69	41	0.04
<i>Xanthomonadaceae</i>	0.69	41	0.04

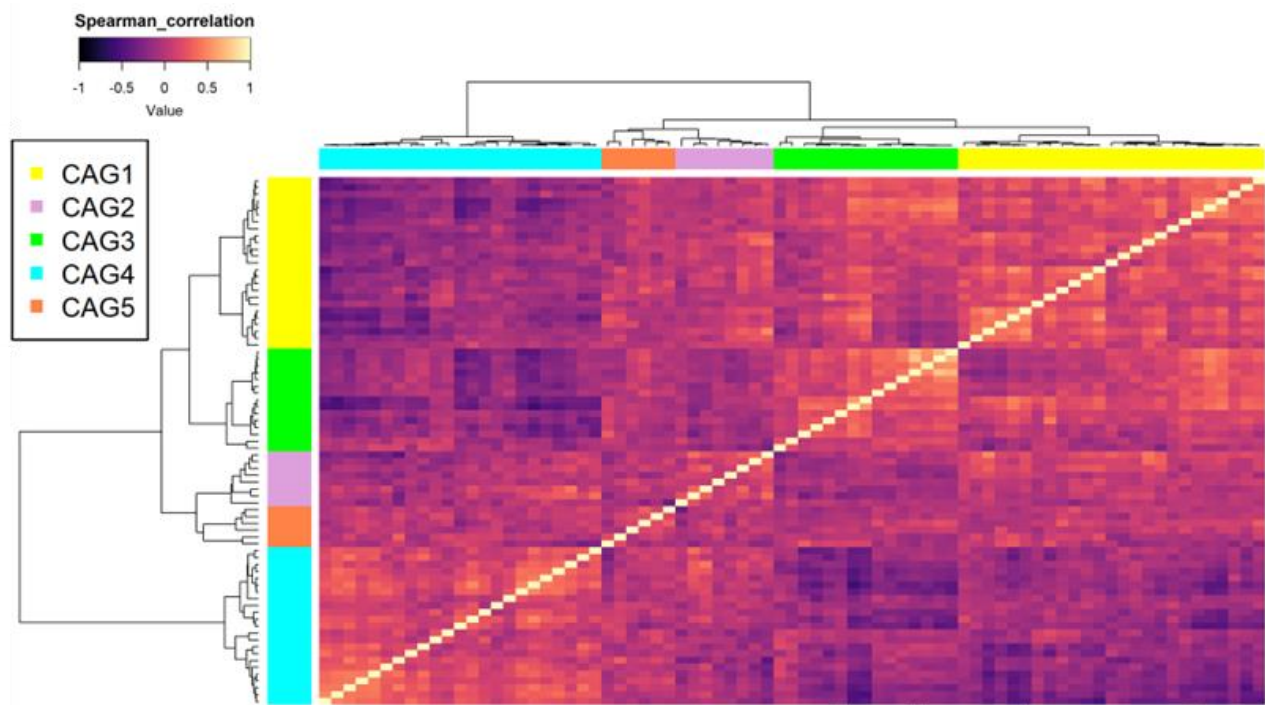
Supplementary Figures



Supplementary Fig. S1. Impact of sampling depth on soil biochemical properties. PCoA is based on Bray-Curtis dissimilarity matrix in soil collected at 0-20 cm (circles) and 30-50 cm (triangles). Different colors indicate different matrices and doses as reported in the legend. First and second coordination axes (MDS1 and MDS2) are plotted for each analysis. Percentages of variance in the dataset accounted for by MDS1 and MDS2 are reported.



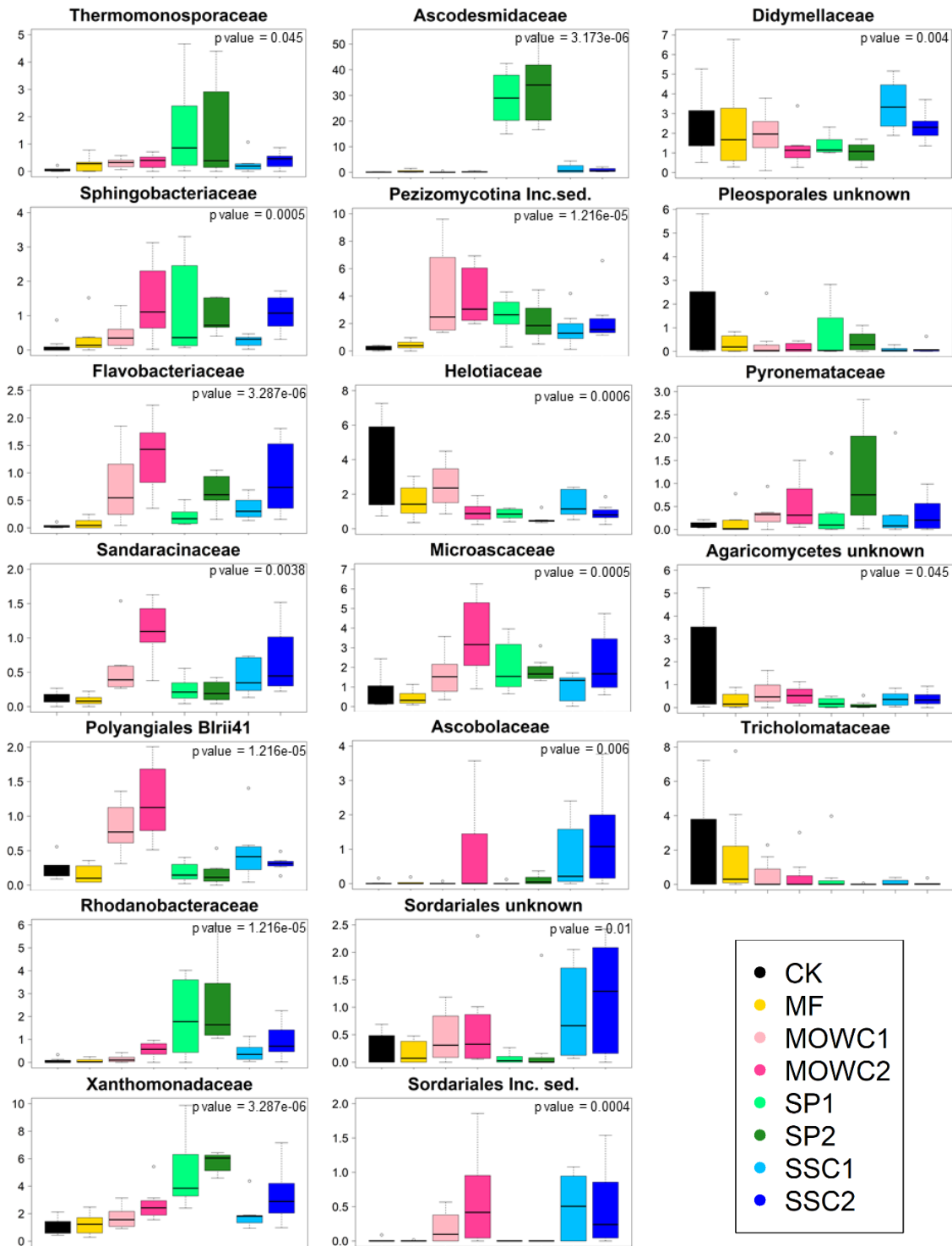
Supplementary Fig. S2. Alpha diversity distributions for bacterial (A, C, E) and fungal (B, D, F) communities in the different groups of samples. Box-and-whiskers distribution of Shannon (A, B), Chao1 (C, D) and Faith's Phylogenetic Diversity (PD) indices (E, F) in soil samples amended using mineral amendment (MF, yellow), MOWC1 and MOWC2 (light and dark pink), SP1 and SP2 (light and dark green), SSC1 and SSC2 (light and dark blue), and not amended (CK, black). Significant differences between datasets are indicated, as calculated using Tukey post hoc test after Kruskal–Wallis test for multiple comparisons (*, $p < 0.05$; **, $p < 0.005$).



Supplementary Fig. S3. Heatmap of samples divided into co-abundant groups (CAGs) identified by hierarchical clustering with the Kendall correlation. Heatmap of soil microbial community, both prokaryotic and eukaryotic, at the family level. The assignment of CAGs is based on heatmap representing Kendall correlations between bacterial and fungal families clustered using hierarchical clustering approach, with Spearman correlation coefficient and Ward's linkage method. Color scale for Spearman correlation is provided (top left), where white represents the highest positive correlation and dark blue the lowest negative correlation. Only families whose relative abundance was higher than 1% in at least 10% of samples were included in the analysis. The obtained clusters were represented by dendrograms at the top and left margins of the heatmap. Each CAG is colored according to the color legend in the left.

Prokaryotes

Eukaryotes



Supplementary Fig. S4. Relative abundance distribution in the differently amended soils of prokaryotic (left column) and eukaryotic families (network nodes) with >2 overabundance values in at least one of the amendment conditions. Box-and-whiskers distribution in soil samples amended using mineral amendment (MF, yellow), MOWC1 and MOWC2 (light and dark pink), SP1 and SP2 (light and dark green), SSC1 and SSC2 (light and dark blue), and not amended (CK, black). Significant differences among samples groups are indicated (fdr corrected p values), as calculated using Kruskal–Wallis test.