

**Supplementary Information for:**

**Metabarcoding analysis of the bacterial and fungal communities during the maturation of Preparation 500, used in biodynamic agriculture, suggests a rational link between horn and manure.**

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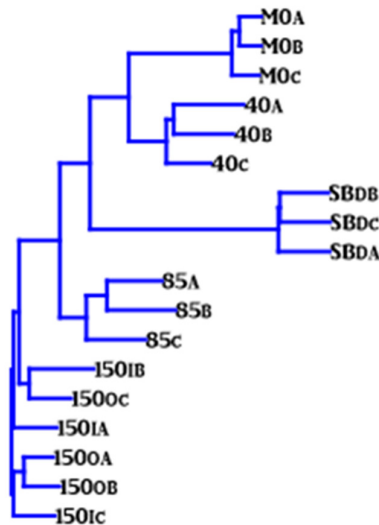
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<b>16S</b>	M0a	M0b	M0c	40a	40b	40c	85a	85b	85c	150ia	150ib	150ic	150oa	150ob	150oc	SBda	SBdb	SBdc
M0a																		
M0b	0,295																	
M0c	0,354	0,328																
40a	0,534	0,497	0,518															
40b	0,546	0,521	0,560	0,282														
40c	0,556	0,529	0,508	0,268	0,251													
85a	0,668	0,655	0,630	0,319	0,382	0,326												
85b	0,659	0,647	0,659	0,376	0,331	0,333	0,275											
85c	0,701	0,668	0,652	0,361	0,351	0,331	0,262	0,279										
150ia	0,760	0,697	0,718	0,449	0,459	0,442	0,395	0,440	0,401									
150ib	0,718	0,690	0,692	0,445	0,375	0,411	0,384	0,327	0,376	0,323								
150ic	0,697	0,653	0,637	0,407	0,396	0,354	0,369	0,368	0,360	0,314	0,286							
150oa	0,731	0,738	0,682	0,465	0,453	0,421	0,349	0,355	0,375	0,310	0,308	0,348						
150ob	0,771	0,712	0,704	0,484	0,507	0,469	0,381	0,439	0,415	0,385	0,331	0,338	0,313					
150oc	0,794	0,745	0,755	0,484	0,500	0,448	0,383	0,394	0,423	0,324	0,358	0,340	0,315	0,333				
SBda	0,906	0,905	0,885	0,794	0,791	0,813	0,732	0,707	0,735	0,758	0,721	0,751	0,705	0,695	0,699			
SBdb	0,901	0,908	0,881	0,805	0,814	0,805	0,756	0,697	0,753	0,752	0,700	0,733	0,695	0,656	0,671	0,330		
SBdc	0,909	0,901	0,867	0,781	0,808	0,812	0,745	0,709	0,765	0,771	0,717	0,751	0,718	0,703	0,684	0,301	0,270	

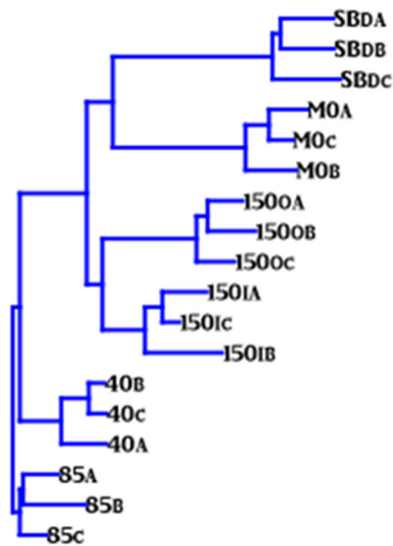
**Fig. S1. Whittaker beta diversity pairwise distance matrix of the bacterial community data.** M0: manure time zero; 40: sampling at time 40 days, 85: sampling at time 85 days; 150i: sampling at 150 days, inner core; 150o: sampling at 150 days, outer layer; SBd: soil of the Biodynamic farm. The three replicates are distinguished by the ending letters a,b,c. The color-based conditional formatting from green to red depicts the degrees of distance, where dark green indicates less distant and dark red indicates more distant communities.

ITS	M0a	M0b	M0c	40a	40b	40c	85a	85b	85c	150ia	150ib	150ic	150oa	150ob	150oc	SBda	SBdb	SBdc
M0a																		
M0b	0,254																	
M0c	0,255	0,262																
40a	0,489	0,529	0,497															
40b	0,405	0,432	0,367	0,359														
40c	0,388	0,447	0,417	0,258	0,308													
85a	0,439	0,479	0,436	0,447	0,395	0,347												
85b	0,425	0,450	0,423	0,389	0,333	0,303	0,316											
85c	0,457	0,444	0,410	0,484	0,413	0,395	0,388	0,351										
150ia	0,445	0,494	0,467	0,400	0,376	0,351	0,310	0,338	0,400									
150ib	0,398	0,387	0,409	0,438	0,368	0,377	0,323	0,319	0,354	0,301								
150ic	0,341	0,392	0,368	0,412	0,358	0,367	0,376	0,353	0,409	0,291	0,276							
150oa	0,358	0,410	0,349	0,474	0,318	0,339	0,350	0,328	0,390	0,309	0,279	0,269						
150ob	0,395	0,432	0,372	0,487	0,424	0,407	0,398	0,416	0,452	0,381	0,360	0,311	0,259					
150oc	0,406	0,420	0,406	0,455	0,424	0,369	0,362	0,369	0,400	0,394	0,343	0,348	0,338	0,324				
SBda	0,421	0,441	0,401	0,632	0,521	0,569	0,506	0,465	0,500	0,517	0,416	0,410	0,438	0,425	0,391			
SBdb	0,465	0,515	0,472	0,592	0,541	0,538	0,514	0,481	0,484	0,488	0,429	0,434	0,452	0,427	0,439	0,252		
SBdc	0,492	0,510	0,477	0,643	0,587	0,617	0,507	0,513	0,467	0,519	0,468	0,450	0,470	0,432	0,420	0,284	0,289	

**Fig. S2. Whittaker beta diversity pairwise distance matrix of the fungal community data.** M0: manure time zero; 40: sampling at time 40 days, 85: sampling at time 85 days; 150i: sampling at 150 days, inner core; 150o: sampling at 150 days, outer layer; SBd: soil of the Biodynamic farm. The three replicates are distinguished by the ending letters a,b,c. The color-based conditional formatting from green to red depicts the degrees of distance as in Fig. S3.



**Fig. S3. Cluster analysis ordination of the bacterial data.** The Neighbor Joining dendrogram based on the Bray-Curtis dissimilarity of the 16S amplicons data is shown.



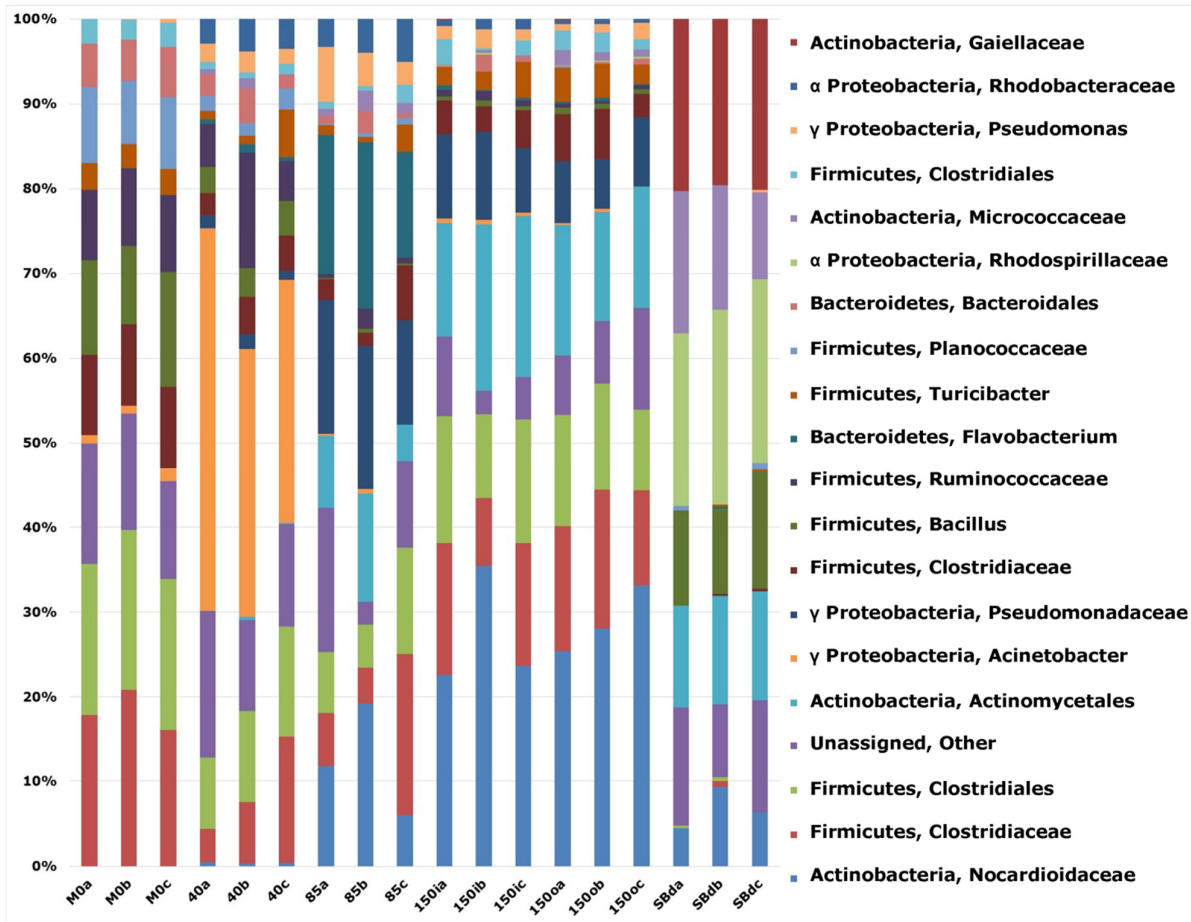
**Fig. S4. Cluster analysis ordination of the fungal data.** The Neighbor Joining dendrogram based on the Bray Curtis distances of the ITS amplicons data is shown.

Sample	Fungal genera	Bacterial genera
M0a	89	62
M0b	96	60
M0c	99	68
40a	44	127
40b	59	132
40c	45	127
85a	50	143
85b	64	155
85c	84	139
150ia	66	138
150ib	77	172
150ic	75	136
150oa	70	146
150ob	73	148
150oc	66	152
SBda	108	193
SBdb	98	201
SBdc	96	202

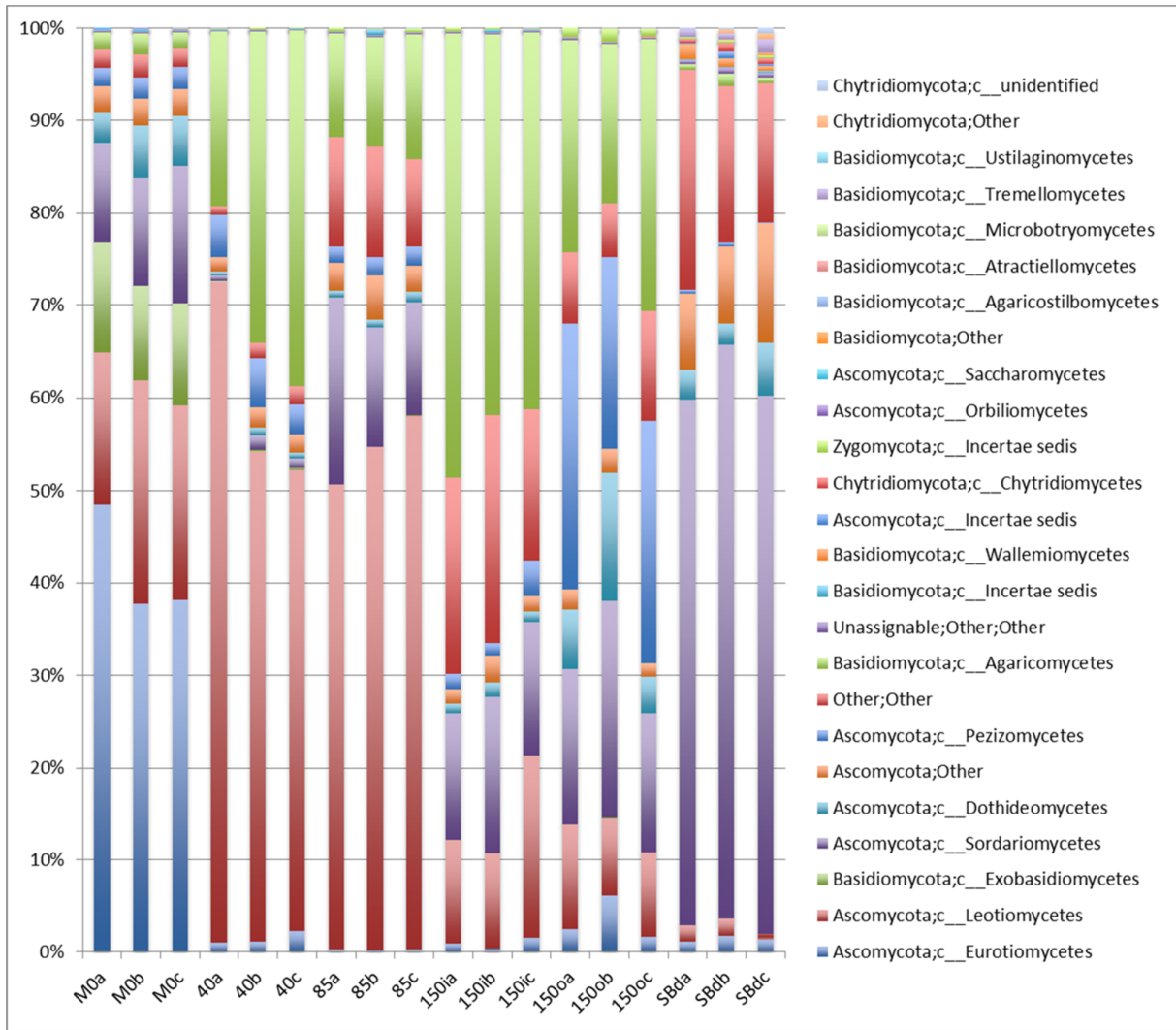
**Table S1. Bacterial and fungal genera numbers recorded at different time points.** M0: manure at time zero (faecal material); 40, 85 150: material rescued after 40, 85, 150 days from burying respectively. For the endpoint analysis of the mature material (150 days) the sampling from the inner core (i) was distinguished from that of the outer portion of the cone (o), closer to the horn shell. SBd: Soil of the biodynamic agricultural farm from which the manure originates and in which the final product is used. Letters a,b,c indicate the three biological replicates.

	M0	40	85	150i	150o	SBd
<b>Bacteria Simpson 1-D</b>	0.9152	0.9175	0.9629	0.9361	0.9357	0.9802
<b>Bacteria Shannon H</b>	2.918	3.543	3.988	3.689	3.658	4.548
<b>Bacteria Evenness</b>	0.1908	0.1869	0.2593	0.1795	0.1709	0.3222
<b>Fungi Simpson 1-D</b>	0.7798	0.5846	0.6776	0.7981	0.8903	0.896
<b>Fungi Shannon H</b>	2.191	1.311	1.783	2.106	2.558	2.841
<b>Fungi Evenness</b>	0.06527	0.04946	0.05667	0.07604	0.1207	0.1181

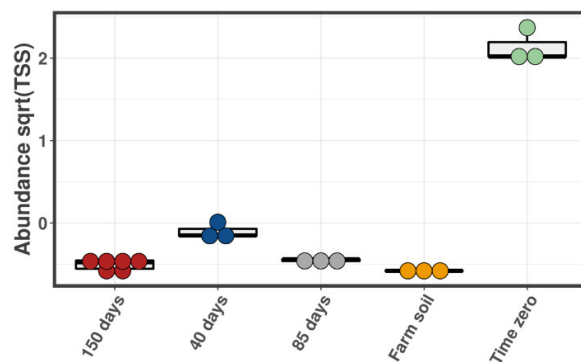
**Table S2. Ecological indexes of diversity and evenness.** Input data: abundance matrix featuring the means of the three replicates for each OTU detected. M0: manure time zero; 40: sampling at time 40 days, 85: sampling at time 85 days; 150i: sampling at 150 days, inner core; 150o: sampling at 150 days, outer layer; SBd: soil of the Biodynamic farm.



**Fig. S5. Bacterial first 20 most abundant taxa.** The nomenclature reported shows the Phylum (or the Class for Proteobacteria) followed by the deepest rank (L6) achievable by the annotation procedure.



**Fig. S6. Fungal classes observed.** their relative percent contribution to each time point community and to the soil of the project's farm (SBd) is shown.



**Fig. S7. Onygenales fungi abundance comparison at different timepoints.** The corresponding anova p value for the order resulted =  $7.8 \times 10^{-14}$ . The units on the Y axis are abundance values after square root transformation of their data (TSS= total sum of squares).