Dispersal-competition tradeoff in microbiomes in the quest for land colonization

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Supplementary Information

Supplementary Tables

Supplementary Table S1. Location and physicochemical parameters measured for the samples collected along the salt marsh chronosequence at the island of Schiermonnikoog, The Netherlands. Sampling was performed in the referred months in the year of 2012.

		Soil stage (in years)																		
		Sta	ge 0			Sta	ge 5			Stage 35		Stage 65				Stage 105				
Soil physical structure																				
Silt (%)		2.7	±0.5			2.9	±0.1			22.3	±0.4			44.5	±2.2			49.0	±0.7	
Clay (%)		5.0	±0.0			5.0±0.0				23.4±1.5		36.2±1.3				37.7±0				
Sand (%)		92.3	±0.5			92.1±0.1 54.3±1.5			19.3±1.0			13.3±0.7								
Soil chemical parameters	Sampling time (in the year of 2012)																			
	May ^a	July ^{a,b}	Sept ^a	Nov ^a	May ^a	July ^{a,b}	Sept ^a	Nov ^a	May ^a	July ^{a,b}	Sept ^a	Nov ^a	May ^a	July ^{a,b}	Sept ^a	Nov ^a	May ^a	July ^{a,b}	Sept ^a	Nov ^a
Soil water content (%)	11±1	11±1	12±1	13±1	7±1	7±1	10±1	13±0	36±2	38±2	36±3	38±1	48±1	46±2	45±3	56±4	38±2	35±1	35±1	44±3
pH	8.7±0.1	8.7±0.1	8.6±0.1	8.6±0.1	8.3±0.1	8.3±0.1	8.3±0.1	8.3±0.1	7.7±0.1	7.8 ± 0.1	7.8±0.1	7.8±0.0	7.4±0.0	7.4 ± 0.1	7.4±0.1	7.4±0.1	7.6±0.0	7.4±0.1	7.4±0.1	7.5±0.1
Soil organic matter (g dm-3)	1±0	1±0	1±0	1±0	2±1	2±1	2±0	3±1	17±1	15±1	14±1	15±1	47±1	38±2	33±1	33±2	32±2	31±1	34±2	28±1
Sulphate (mg dm ⁻³)	16±3	13±1	95±4	75±4	32±4	43±5	180±4	203±9	670±13	130±12	113±10	53±4	704±5	550±26	452±23	67±11	539±13	423±12	498±5	416±26
Sodium (mg dm ⁻³)	155±13	178±19	752±88	647±182	170±15	187±9	1248±38	1587±89	6836±240	3782±340	4799±457	3755±94	4533±384	6447±204	5888±346	5200±438	5188±624	3541±170	4654±731	5039±687
Total nitrogen (mg kg ⁻¹)	462±50	329±44	371±116	378±105	490±76	530±74	467±172	478±78	3232±150	3253±389	2606±539	2679±209	9604±1187	8132±1955	8580±1785	8276±1212	6300±1646	5833±3003	5693±2660	5087±2151
Ammonium (mg kg ⁻¹)	5±1	4±1	4±1	4±1	6±1	6±1	5±2	6±1	38±3	38±4	30±6	33±4	115±16	94±18	102±21	99±15	75±20	70±37	67±32	60±30
Nitrate (mg kg ⁻¹)	3±0	2±0	2±0	2±0	3±1	3±1	3±1	3±1	21±2	21±3	17±4	18±2	63±7	51±8	57±12	55±8	42±11	39±20	38±18	34±17

^aQuantitative PCR (bacteria and fungi)

^aParallel amplicon bacterial 16S rRNA sequencing ^aParallel amplicon fungal ITS sequencing

^bShotgun metagenome sequencing

Supplementary Table S2. Characteristics of the metagenomes generated by Illumina HiSeq2000 for soil samples collected along the salt marsh chronosequence at the island of Schiermonnikoog, The Netherlands.

		Soil stage (in years)													
		Stage 0			Stage 5			Stage 35			Stage 65			Stage 105	
	Plot A	Plot B	Plot C	Plot A	Plot B	Plot C	Plot A	Plot B	Plot C	Plot A	Plot B	Plot C	Plot A	Plot B	Plot C
Geographic coordinates															
Latitude	53°30'18''N	53°30'18''N	53°30'18''N	53°30'7''N	53°30'7''N	53°30'7''N	53°29'59''N	53°29'59''N	53°29'59''N	53°29'35''N	53°29'35''N	53°29'35''N	53°28'56''N	53°28'56''N	53°28'56''N
Longitude	6°19'51''E	6°19'51''E	6°19'51''E	6°19'54''E	6°19'54''E	6°19'54''E	6°18'57''E	6°18'57"'E	6°18'57''E	6°16'20''E	6°16'20''E	6°16'20''E	6°14'3''E	6°14'3''E	6°14'3''E
*Metagenomes															
MG-RAST ID	4558897.3	4558900.3	4558903.3	4558906.3	4558909.3	4558912.3	4558915.3	4558918.3	4558921.3	4558924.3	4558927.3	4558930.3	4558933.3	4558936.3	4558939.3
Metagenome size (bp)	1 946 098 904	3 039 009 260	1 729 121 135	1 985 828 415	2 470 040 059	2 541 714 674	2 876 930 138	1 658 957 366	2 169 497 340	1 883 521 083	1 814 228 985	1 103 890 408	384 386 953	503 545 455	2 659 532 084
Average sequence length (bp)	156 ± 27	157 ± 28	159 ± 29	164 ± 32	159 ± 29	150 ± 24	156 ± 26	157 ± 28	155 ± 27	155 ± 27	165 ± 33	167 ± 32	170 ± 34	167 ± 34	156 ± 28
Number of sequences	12 450 878	19 321 585	10 839 811	12 107 696	15 534 513	16 934 258	18 408 001	10 533 784	13 947 451	12 123 756	10 969 050	6 599 703	2 258 688	2 999 891	16 987 073
GC content (%)	56 ± 12	56 ± 12	54 ± 12	56 ± 12	56 ± 12	56 ± 12	55 ± 11	54 ± 11	55 ± 11	57 ± 10	56 ± 11	55 ± 11	55 ± 11	57 ± 10	57 ± 10
Number of predicted ORFs	11 596 311	17 647 296	10 070 537	11 124 734	14 473 485	15 374 314	17 155 698	9 823 968	13 024 803	11 275 236	10 201 327	6 140 974	2 057 545	2 794 317	15 889 995
ORFs with predicted function	3 934 757	5 853 920	3 272 425	4 105 274	5 645 080	5 925 433	5 285 542	3 104 792	3 894 995	3 794 182	3 610 530	2 181 005	755 878	1 025 318	5 627 796
Bacteria (%)	96.1	95.8	95.7	97	97.5	97.5	95.5	95.6	95.8	96.7	97.2	96.9	97.1	97.1	97.1
Eukarya (%)	2.1	2.5	2.4	1.8	1.6	1.8	2.1	2.1	1.9	1.5	1.4	1.5	1.8	1.3	1.3
Archaea (%)	1.5	1.4	1.6	1.1	0.8	0.6	2.1	2	2.1	1.6	1.2	1.4	0.9	1.4	1.4
KO matches	1 565 010	2 391 486	1 293 459	1 618 989	2 173 227	2 387 195	2 065 112	1 202 620	1 526 363	1 486 222	1 392 578	835 141	298 105	396 573	2 188 639
CAZy matches	102 392	157 078	87 041	126 660	161 093	161 471	174 120	99 232	130 412	116 255	118 351	73 099	25 784	33 566	175 447
ARG matches	84 995	131 065	73 283	100 002	120 458	113 877	123 456	72 389	93 161	88 463	99 059	60 103	21 635	28 837	13 7611

*Post QC values

Supplementary Table S3. Overall and pairwise comparisons of KO, ARGs and CAZy community profiles across five soil stages analysed using PERMANOVA (Bray-Curtis distance) with 10³ permutations. Abbreviations: MS, mean sum of squares; SS, sum of squares.

	KL.	EG Orthology	(KOs)		
Source	d.f.	SS	MS	Pseudo-F	<i>P</i> -value
Soil stages	4	282.24	70.559	6.7683	0.001
Residuals	10	104.25	10.425		
Total	14	386.48			
Average similarity between stages					
	Stage 0	Stage 5	Stage 35	Stage 65	Stage 105
Stage 0	96.267	Singer	Singette	Singe or	Singe 100
Stage 5	92 361	95 262			
Stage 35	92.801	91 858	96.612		
Stage 65	91.003	92 525	93 597	95 363	
Stage 105	90.31	92.323	92.086	94 408	94 222
Stage 105	A	Cs (Soil resis	()2.000	74.400	J 4 .222
Source	df	SS	MS	Pseudo-F	P-value
Soil stages	4	330 33	82 583	7 1732	0.001
Desiduals	4 10	115 13	11 513	1.1752	0.001
Total	10	115.15	11.515		
A vorage similarity between stages	14	445.40			
Average similarity between stages	Store ()	Store 5	Store 35	Store 65	Stago 105
Stage 0	07 527	Stage 5	Stage 55	Stage 05	Stage 105
Stage 5	97.337	04 617			
Stage 3	95.373	94.017	07 625		
Stage 55	93.919	95.420	97.055	04.456	
Stage 05	90.300	92.045	91.591	94.430	04.007
Stage 105	88.0//	91.458	89.337	94.091	94.097
S	3.6	CALY	MC	Decede F	D li a
Source	a.i.	33	MIS	12 200	<i>P</i> -value
Soll stages	4	439.44	109.80	12.209	0.001
Residuals	10	89.979	8.9979		
Total	14	529.42			
Average similarity between stages	<i>a. a</i>	a	a	G. (.	C
	Stage 0	Stage 5	Stage 35	Stage 65	Stage 105
Stage 0	96.917				
Stage 5	89.661	95.625			
Stage 35	90.774	92.961	97.228		
~			02 207	05 157	
Stage 65	87.841	92.526	93.280	95.157	
Stage 65 Stage 105	87.841 86.475	92.526 91.703	93.286	94.829	94.813
Stage 65 Stage 105	87.841 86.475 B	92.526 91.703 acterial 16S r	93.286 91.377 RNA	94.829	94.813
Stage 65 Stage 105 Source	87.841 86.475 B d.f.	92.526 91.703 acterial 16S rl SS	93.286 91.377 RNA MS	94.829 Pseudo-F	94.813 <i>P</i> -value
Stage 65 Stage 105 Source Soil stages	87.841 86.475 B d.f. 4	92.526 91.703 acterial 16S r SS 20051	93.286 91.377 RNA MS 5012.7	94.829 Pseudo-F 3.9968	94.813 P-value 0.001
Stage 65 Stage 105 Source Soil stages Residuals	87.841 86.475 B d.f. 4 10	92.526 91.703 acterial 16S rl SS 20051 12542	93.286 91.377 RNA 5012.7 1254.2	94.829 94.829 Pseudo-F 3.9968	94.813 P-value 0.001
Stage 65 Stage 105 Source Soil stages Residuals Total	87.841 86.475 B d.f. 4 10 14	92.526 91.703 acterial 16S rl 8S 20051 12542 32592	93.286 91.377 RNA MS 5012.7 1254.2	93.137 94.829 Pseudo-F 3.9968	94.813 P-value 0.001
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages	87.841 86.475 B d.f. 4 10 14	92.526 91.703 acterial 16S r SS 20051 12542 32592	93.286 91.377 RNA MS 5012.7 1254.2	93.137 94.829 Pseudo-F 3.9968	94.813 P-value 0.001
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages	87.841 86.475 B d.f. 4 10 14 Stage 0	92.526 91.703 acterial 16S rJ SS 20051 12542 32592 Stage 5	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35	93.137 94.829 Pseudo-F 3.9968 Stage 65	94.813 <i>P</i> -value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955	92.526 91.703 acterial 16S r SS 20051 12542 32592 Stage 5	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35	93.137 94.829 Pseudo-F 3.9968 Stage 65	94.813 <i>P</i> -value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826	92.526 91.703 acterial 16S rJ SS 20051 12542 32592 Stage 5 46.589	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35	93.137 94.829 Pseudo-F 3.9968 Stage 65	94.813 P-value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308	92.526 91.703 acterial 16S rJ SS 20051 12542 32592 Stage 5 46.589 24.118	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268	93.137 94.829 Pseudo-F 3.9968 Stage 65	94.813 P-value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179	92.526 91.703 acterial 16S rJ SS 20051 12542 32592 Stage 5 46.589 24.118 27.235	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475	94.813 P-value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663	92.526 91.703 acterial 16S r SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805	94.813 P-value 0.001 Stage 105 53.684
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663	92.526 91.703 acterial 16S rl SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805	94.813 <i>P</i> -value 0.001 Stage 105 53.684
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105 Source	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f.	92.526 91.703 acterial 16S r SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 MS	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F	94.813 P-value 0.001 Stage 105 53.684 P-value
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105 Source Soil stages	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4	92.526 91.703 acterial 16S rl SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 MS 7751.8	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323	94.813 <i>P</i> -value 0.001 Stage 105 53.684 <i>P</i> -value 0.001
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105 Source Soil stages Residuals	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4 10	92.526 91.703 acterial 16S rl SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007 11346	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 MS 7751.8 1134.6	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323	94.813 <i>P</i> -value 0.001 Stage 105 53.684 <i>P</i> -value 0.001
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105 Source Soil stages Residuals Total	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4 10 14	92.526 91.703 acterial 16S rl SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007 11346 42353	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 MS 7751.8 1134.6	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323	94.813 <i>P</i> -value 0.001 Stage 105 53.684 <i>P</i> -value 0.001
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4 10 14	92.526 91.703 acterial 16S rl SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007 11346 42353	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 5 MS 7751.8 1134.6	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323	94.813 <i>P</i> -value 0.001 Stage 105 53.684 <i>P</i> -value 0.001
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4 10 14 Stage 0 14 Stage 0 14 14 14 14 14 10 14 14 14 14 10 14 14 14 14 14 14 14 14 14 14	92.526 91.703 acterial 16S rl SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007 11346 42353 Stage 5	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 5 MS 7751.8 1134.6 Stage 35	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323 Stage 65	94.813 P-value 0.001 Stage 105 53.684 P-value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4 10 14 Stage 0 43.268	92.526 91.703 acterial 16S rJ SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007 11346 42353 Stage 5	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 5 MS 7751.8 1134.6 Stage 35	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323 Stage 65	94.813 P-value 0.001 Stage 105 53.684 P-value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4 10 14 Stage 0 43.268 17.903	92.526 91.703 acterial 16S rJ SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007 11346 42353 Stage 5 49.15	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 5 MS 7751.8 1134.6 Stage 35	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323 Stage 65	94.813 <i>P</i> -value 0.001 Stage 105 53.684 <i>P</i> -value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 35	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4 10 14 Stage 0 43.268 17.903 15.766	92.526 91.703 acterial 16S rJ SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007 11346 42353 Stage 5 49.15 18.295	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 5 MS 7751.8 1134.6 Stage 35 57.567	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323 Stage 65	94.813 <i>P</i> -value 0.001 Stage 105 53.684 <i>P</i> -value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 35 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 65 Stage 35 Stage 35 Stage 35 Stage 65	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4 10 14 Stage 0 43.268 17.903 15.766 15.624	92.526 91.703 acterial 16S rJ SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007 11346 42353 Stage 5 49.15 18.295 22.596	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 5 MS 7751.8 1134.6 Stage 35 57.567 12.554	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323 Stage 65 53.396	94.813 <i>P</i> -value 0.001 Stage 105 53.684 <i>P</i> -value 0.001 Stage 105
Stage 65 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 35 Stage 105 Source Soil stages Residuals Total Average similarity between stages Stage 0 Stage 5 Stage 35 Stage 65 Stage 35 Stage 65 Stage 105	87.841 86.475 B d.f. 4 10 14 Stage 0 49.955 30.826 20.308 21.179 22.663 d.f. 4 10 14 Stage 0 43.268 17.903 15.766 15.624 15.112	92.526 91.703 acterial 16S rJ SS 20051 12542 32592 Stage 5 46.589 24.118 27.235 30.452 Fungal ITS SS 31007 11346 42353 Stage 5 49.15 18.295 22.596 22.053	93.286 91.377 RNA MS 5012.7 1254.2 Stage 35 51.268 37.319 36.088 5 MS 7751.8 1134.6 Stage 35 57.567 12.554 11.167	93.137 94.829 Pseudo-F 3.9968 Stage 65 48.475 46.805 Pseudo-F 6.8323 Stage 65 53.396 34.472	94.813 P-value 0.001 Stage 105 53.684 P-value 0.001 Stage 105 60.832

Supplementary Table S4. Spearman's correlation (ρ values), determined via RELATE tests, relating pairwise community composition (bacterial 16S rRNA, fungal ITS) and metagenomic distances (KO, ARGs, CAZy) based on Bray-Curtis distances.

	ρ	P-value
Bacterial 16S rRNA, KO	0.782	0.001
Bacterial 16S rRNA, ARG	0.263	0.021
Bacterial 16S rRNA, CAZy	0.729	0.001
Fungal ITS, CAZy	0.556	0.001

Supplementary Table S5. Layer decision of KOs, ARG mechanisms and CAZy families identified using random forest analysis with 1,000 trees followed by the Boruta algorithm for feature selection (average *z*-scores of 1,000 runs > 4).

	KEE	G Orthologs (KOs)								
KO level 3	KO ID	Layer decision	Layer importance							
Bacterial chemotaxis	K00575	Rejected	Inf							
Bacterial chemotaxis	K03406	Confirmed	10.1505							
Bacterial chemotaxis	K03407	Confirmed	12.9054							
Bacterial chemotaxis	K03408	Confirmed	8.8730							
Bacterial chemotaxis	K03412	Confirmed	8.7897							
Bacterial chemotaxis	K03413	Confirmed	10.5524							
Bacterial chemotaxis	K05874	Confirmed	11.9026							
Bacterial chemotaxis	K05875	Confirmed	8.3797							
	KEE	G Orthologs (KOs)								
KO level 3 KO ID Layer decision Layer importance										
Flagellar assembly	K02387	Rejected	Inf							
Flagellar assembly	K02388	Tentative	3.7512							
Flagellar assembly	K02389	Rejected	Inf							
Flagellar assembly	K02390	Confirmed	4.8603							
Flagellar assembly	K02391	Confirmed	6.2569							
Flagellar assembly	K02392	Confirmed	4.2732							
Flagellar assembly	K02393	Rejected	Inf							
Flagellar assembly	K02394	Confirmed	5.9072							
Flagellar assembly	K02396	Confirmed	4.5166							
Flagellar assembly	K02397	Rejected	Inf							
Flagellar assembly	K02400	Rejected	Inf							
Flagellar assembly	K02401	Tentative	3.9386							
Flagellar assembly	K02406	Confirmed	6.7124							
Flagellar assembly	K02407	Confirmed	5.6035							
Flagellar assembly	K02408	Confirmed	7.8791							
Flagellar assembly	K02409	Confirmed	4.5325							
Flagellar assembly	K02410	Confirmed	5.6311							
Flagellar assembly	K02411	Confirmed	4.7666							
Flagellar assembly	K02412	Confirmed	5.6361							
Flagellar assembly	K02413	Rejected	Inf							
Flagellar assembly	K02414	Rejected	Inf							
Flagellar assembly	K02416	Tentative	4.0559							
Flagellar assembly	K02417	Confirmed	5.8813							
Flagellar assembly	K02418	Rejected	Inf							
Flagellar assembly	K02419	Rejected	Inf							
Flagellar assembly	K02420	Rejected	Inf							
Flagellar assembly	K02421	Tentative	3.8170							
Flagellar assembly	K02556	Rejected	Inf							
Flagellar assembly	K02557	Confirmed	5.4375							

AR	Gs (Soil resistomes)	
Mechanism	Layer decision	Layer importance
ABC transporter	Confirmed	7.1482
Acetyltransferase	Confirmed	6.9241
Aminotransferase	Rejected	Inf
Beta-lactamase	Confirmed	6.8547
D-ala D-ala ligase	Rejected	Inf
Gene modulating resistance	Confirmed	5.6460
Gylcopeptide resistance	Rejected	Inf
Methyltransferase	Rejected	Inf
MFS transporter	Confirmed	8.7414
Other efflux	Confirmed	4.9721
Phosphotransferase	Confirmed	7.8383
Quinolone resistance	Rejected	Inf
RND antibiotic efflux	Rejected	Inf
rRNA methyltransferase	Rejected	Inf
Stress response	Rejected	Inf
Target protection	Rejected	Inf
Target redundancy/overexpression	Rejected	Inf

Class Gene family Layer decision Layer importance AA AA10 Confirmed 3.5571 AA AA2 Rejected Inf AA AA3 Confirmed 3.7742 AA AA4 Confirmed 3.7742 AA AA5 Rejected Inf AA AA5 Rejected Inf AA AA6 Rejected Inf CBM CBM10 Rejected Inf CBM CBM11 Rejected Inf CBM CBM12 Rejected Inf CBM CBM12 Rejected Inf CBM CBM12 Rejected Inf CBM CBM			CAZymes	
AA AA10 Tentarive 2.8021 AA AA10 Confirmed 3.5571 AA AA2 Rejected Inf AA AA3 Confirmed 3.7742 AA AA4 Tentarive 2.9352 AA AA5 Rejected Inf AA AA6 Rejected Inf AA AA6 Rejected Inf CBM CBM1 Rejected Inf CBM CBM13 Rejected Inf CBM CBM14 Rejected Inf CBM CBM15 Rejected Inf CBM CBM18 Rejected Inf CBM CBM18 Rejected Inf CBM CBM2 Rejected Inf <th>Class</th> <th>Gene family</th> <th>Laver decision</th> <th>Laver importance</th>	Class	Gene family	Laver decision	Laver importance
AA AA2 Rejected Inf AA AA3 Confirmed 3.7742 AA AA4 Tentative 2.9352 AA AA5 Rejected Inf AA AA6 Rejected Inf AA AA6 Rejected Inf CBM CBM10 Rejected Inf CBM CBM11 Rejected Inf CBM CBM12 Rejected Inf CBM CBM12 Rejected Inf CBM CBM23 Rejected I	AA	AA1	Tentative	2.8021
AA AA3 Confirmed 3.7742 AA AA4 Tentative 2.9352 AA AA5 Rejected Inf AA AA6 Rejected Inf AA AA70 Confirmed 3.4470 AA AA70 Rejected Inf CBM CBM1 Rejected Inf CBM CBM1 Rejected Inf CBM CBM1 Rejected Inf CBM CBM11 Rejected Inf CBM CBM12 Rejected Inf CBM CBM22 Rejected Inf CBM CBM22 Rejected Inf CBM CBM22 Rejected Inf CBM CBM22 Rejected Inf CBM CBM23 Rejected Inf CBM CBM23 Rejected Inf CBM CBM23 Rejected <t< td=""><td>AA</td><td>AA10</td><td>Confirmed</td><td>3.5571</td></t<>	AA	AA10	Confirmed	3.5571
AA AA4 AA43 Confirmed 3.7742 AA AA5 Rejected Inf AA AA5 Rejected Inf AA AA7 Conjected Inf CBM CBM10 Rejected Inf CBM CBM11 Rejected Inf CBM CBM12 Rejected Inf CBM CBM2 Rejected<	AA	AA2	Rejected	Inf
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AA AA Rejected Inf AA AA AA Statut AA AA Rejected Inf CBM CBM10 Rejected Inf CBM CBM11 Rejected Inf CBM CBM11 Rejected Inf CBM CBM12 Rejected Inf CBM CBM13 Confirmed 4.1255 CBM CBM14 Rejected Inf CBM CBM15 Rejected Inf CBM CBM17 Rejected Inf CBM CBM18 Rejected Inf CBM CBM19 Rejected Inf CBM CBM19 Rejected Inf CBM CBM19 Rejected Inf CBM CBM20 Rejected Inf CBM CBM22 Rejected Inf CBM CBM23 Rejected Inf CBM CBM23 Rejected Inf CBM CBM23 Rejected Inf CBM CBM23 Rejected Inf CBM CBM33 Rejected Inf CBM CBM33 Rejected In	AA	AA4 A A 5	Paiastad	2.9352 Inf
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CBM CBM10 Repeted Inf CBM CBM110 Repeted Inf CBM CBM12 Repeted Inf CBM CBM13 Confirmed 4.1255 CBM CBM13 Repeted Inf CBM CBM13 Repeted Inf CBM CBM14 Repeted Inf CBM CBM17 Repeted Inf CBM CBM19 Repeted Inf CBM CBM20 Rejected Inf CBM CBM20 Rejected Inf CBM CBM22 Rejected Inf CBM CBM23 Rejected Inf CBM CBM32 Rejected Inf CBM CBM32 Rejected Inf CBM CBM33 Rejected Inf CBM CBM33 Rejected Inf CBM CBM33 Rejected Inf CBM CBM33 Rejected	AA	AA9	Rejected	Inf
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CBMCBM12RejectedInfCBMCBM52RejectedInfCBMCBM54RejectedInfCBMCBM55RejectedInfCBMCBM57RejectedInfCBMCBM57RejectedInfCBMCBM59RejectedInfCBMCBM59RejectedInfCBMCBM60RejectedInfCBMCBM61RejectedInfCBMCBM62Tentative 3.0064 CBMCBM63RejectedInfCBMCBM64RejectedInfCBMCBM65RejectedInfCBMCBM66RejectedInfCBMCBM66RejectedInfCBMCBM67Tentative 2.9440 CBMCBM67Tentative 2.9440 CBMCBM68RejectedInfCBMCBM9RejectedInfCECE11RejectedInfCECE11RejectedInfCECE13RejectedInfCECE14Confirmed 3.2091 CECE15Tentative 2.9137 CECE16RejectedInfCECE5Confirmed 3.2828 CECE5Confirmed 3.2828 CECE8RejectedInfCECE9RejectedInfCECE5Confirmed 3.8010 CECE6Re	CBM	CBM51 CPM52	Rejected	Inf
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GH GH101 Rejected Inf GH GH102 Rejected Inf	GH	GH10 GH100	Confirmed	3.0285
GH GH102 Rejected Inf	GH	GH100 GH101	Rejected	5.8900 Inf
	GH	GH102	Rejected	Inf

GH	GH103	Rejected	Inf
GH	GH104 GH105	Rejected	Inf
GH	GH106 GH107	Rejected	Inf
GH	GH107 GH108	Rejected	Inf
GH GH	GH109 GH11	Confirmed Rejected	3,3174 Inf
GH	GH110	Rejected	Inf
GH	GH111 GH112	Rejected	Inf
GH	GH112 GH113	Rejected	Inf
GH GH	GH114 GH115	Rejected Confirmed	Inf 3 1135
GH	GH116	Rejected	Inf
GH GH	GH117 GH118	Rejected Rejected	Inf Inf
GH	GH119	Rejected	Inf
GH GH	GH12 GH120	Rejected Rejected	Inf Inf
GH	GH121	Rejected	Inf
GH GH	GH122 GH123	Rejected	Inf
GH	GH124	Rejected	Inf
GH	GH125 GH126	Rejected	Inf
GH	GH127	Confirmed	3.4860
GH	GH128 GH129	Rejected	Inf
GH	GH13 GH120	Rejected	Inf 2 7615
GH	GH131	Rejected	Inf
GH GH	GH132 GH14	Rejected Rejected	Inf Inf
GH	GH15	Rejected	Inf
GH GH	GH16 GH17	Rejected Rejected	Inf Inf
GH	GH18	Rejected	Inf
GH GH	GH19 GH2	Rejected Tentative	Inf 2 8451
GH	GH20	Rejected	Inf
GH GH	GH22 GH23	Rejected Rejected	Inf Inf
GH	GH24	Rejected	Inf
GH GH	GH25 GH26	Rejected Rejected	Inf Inf
GH	GH27	Rejected	Inf
GH GH	GH28 GH29	Rejected Tentative	Inf 3.0446
GH	GH3	Rejected	Inf
GH GH	GH30 GH31	Rejected	3.0678 Inf
GH	GH32	Rejected	Inf
GH	GH35 GH35	Confirmed	4.0238
GH	GH36 CH27	Confirmed Paineted	3.8866
GH	GH38	Rejected	Inf
GH	GH39 GH4	Confirmed	3.8047
GH	GH42	Confirmed	3.4475
GH GH	GH43 GH44	Rejected Confirmed	Inf 3 1113
GH	GH45	Rejected	Inf
GH GH	GH46 GH47	Rejected Rejected	Inf Inf
GH	GH48	Rejected	Inf
GH GH	GH49 GH5	Rejected Rejected	Inf Inf
GH	GH50	Confirmed	3.4836
GH GH	GH51 GH52	Rejected	Inf
GH	GH53 GH54	Rejected	Inf 3 0008
GH	GH54 GH55	Confirmed	3.5308
GH	GH56 GH57	Rejected	Inf
GH	GH58	Rejected	Inf
GH	GH59 GH6	Rejected Confirmed	Inf 3 2089
GH	GH62	Rejected	Inf
GH GH	GH63 GH64	Rejected Rejected	Inf Inf
GH	GH65	Rejected	Inf
GH GH	GH66 GH67	Rejected Rejected	Inf Inf
GH	GH68	Rejected	Inf
GH	GH70	Rejected	ini Inf
GH	GH71	Rejected	Inf
GH	GH73	Rejected	Inf
GH	GH74 GH75	Rejected	Inf
GH	GH76	Rejected	Inf
GH GH	GH77 GH78	Rejected Confirmed	Inf 3 7183
GH	GH79	Rejected	Inf
GH	GH8	Rejected	Inf

GH GH81 Kejected Inf GH GH823 Kejected Inf GH GH843 Rejected Inf GH GH853 Rejected Inf GH GH854 Rejected Inf GH GH854 Rejected Inf GH GH89 Rejected Inf GH GH89 Rejected Inf GH GH99 Rejected Inf	GH	GH80	Rejected	Inf
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GH GH89 Rejected Inf GH GH89 Rejected Inf GH GH90 Rejected Inf GH GH91 Rejected Inf GH GH92 Rejected Inf GH GH93 Rejected Inf GH GH94 Confirmed 3.3032 GH GH95 Rejected Inf GH GH96 Rejected Inf GH GH99 Rejected Inf GH GH99 Rejected Inf GH GH99 Rejected Inf GH GH99 Rejected Inf GT GT11 Confirmed 3.1354 GT GT12 Rejected Inf GT GT13 Rejected Inf GT GT14 Rejected Inf GT GT17 Rejected Inf GT GT18 Rejected Inf GT GT19 Rejected Inf GT GT21 Rejected Inf GT GT21 Rejected Inf GT GT22 Rejected Inf <	GH	GH87	Rejected	Inf
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Gri GriPs Confirmed 3.032 GH GH95 Rejected Inf GH GH96 Rejected Inf GH GH98 Confirmed 3.1538 GH GF98 Confirmed 3.1548 GT GT1 Confirmed 3.1544 GT GT1 Confirmed 3.1544 GT GT12 Rejected Inf GT GT13 Rejected Inf GT GT14 Rejected Inf GT GT17 Rejected Inf GT GT18 Rejected Inf GT GT19 Rejected Inf GT GT22 Rejected Inf GT GT22 Rejected Inf GT GT22 Rejected Inf GT GT24 Tentative 2.7951 GT GT25 Confirmed 3.0824 GT GT33 Rejected<	GH	GH93	Rejected	Inf 2 2022
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GT	GT82	Rejected	Inf	
GT	GT83	Tentative	2.9641	
GT	GT84	Rejected	Inf	
GT	GT85	Rejected	Inf	
GT	GT87	Rejected	Inf	
GT	GT88	Rejected	Inf	
GT	GT89	Rejected	Inf	
GT	GT9	Rejected	Inf	
GT	GT90	Rejected	Inf	
GT	GT91	Rejected	Inf	
GT	GT92	Rejected	Inf	
GT	GT93	Tentative	2.8188	
GT	GT94	Rejected	Inf	
PL	PL1	Confirmed	3.8243	
PL	PL10	Rejected	Inf	
PL	PL11	Confirmed	4.6552	
PL	PL12	Confirmed	3.6877	
PL	PL13	Rejected	Inf	
PL	PL14	Rejected	Inf	
PL	PL15	Rejected	Inf	
PL	PL16	Rejected	Inf	
PL	PL17	Rejected	Inf	
PL	PL18	Rejected	Inf	
PL	PL2	Rejected	Inf	
PL	PL20	Rejected	Inf	
PL	PL21	Rejected	Inf	
PL	PL22	Confirmed	3.2002	
PL	PL3	Rejected	Inf	
PL	PL4	Rejected	Inf	
PL	PL5	Rejected	Inf	
PL	PL6	Rejected	Inf	
PL	PL7	Rejected	Inf	
PL	PL8	Rejected	Inf	
PL.	PL9	Confirmed	3.3642	

Supplementary Figures



Supplementary Fig. S1. Map of the island of Schiermonnikoog, the Netherlands (N53°30' E6°10') and characteristics of the soil stages across the chronosequence. (a) The scaled map depicts the chronology of the geogenesis along the gradual eastward extension of the island, dating from 1809 to 2014. Throughout the development of this ecosystem, different stages of soil formation were precisely located (see Materials and Methods), named as stages 0, 5, 35, 65 and 105 (in years of soil development). (b) Photographs of each plot taken in July 2012. (c) Bar charts depict the flooding frequency (i.e., number of times each plot was flooded) per month along the year of 2012 (scaled 0 to 50). (d) Physicochemical data display soil sand:silt:clay content (%), sulphate (S-SO₄²⁻), sodium (Na), soil organic matter (SOM), total nitrogen (N), ammonium (N-NH₄⁺), nitrate (N-NO₃⁻), and pH — at the five stages of soil formation. The map was created using the software ArcGIS Pro v. 2.0 (http://pro.arcgis.com/en/pro-app/).



Supplementary Fig. S2. Differences in bacterial and fungal β -diversities across the stages of soil formation. (a) Bacterial and (b) fungal principal coordinate analyses (PCoA) based on Bray-Curtis distances calculated using the bacterial 16S rRNA and the fungal ITS amplicon sequencing data, respectively. PERMANOVA was performed using the homonymous routines in PRIMER6+ with 10³ permutations (Supplementary Table S3).



Supplementary Fig. S3. Distribution of KOs involved in bacterial chemotaxis and flagellar assembly across the stages of soil formation – the 'dispersal' *modus*. Heatmap displays the relative abundance (row *z*-scores) of the KOs involved in bacterial chemotaxis and flagellar assembly. Circles are proportional to the relative abundance of each KO in all samples. Asterisks denote KOs that differentially segregated across the soil stages, identified by random forest analysis with Boruta feature selection (average *z*-scores of 1000 runs > 4) (Supplementary Table S5).



Supplementary Fig. S4. Patterns of soil resistomes across the stages of soil formation. (a) Principal coordinate analysis (PCoA) based on Bray-Curtis distances calculated from normalized ARG annotations. PERMANOVA was performed using the homonymous routines in PRIMER6+ with 10³ permutations (Supplementary Table S3). (b) Procrustes analysis depicts the significant correlation between soil resistomes (Bray-Curtis) and bacterial community composition (Bray-Curtis) across the soil stages.



Supplementary Fig. S5. CAZy profiles across the stages of soil formation. (a) Principal coordinate analysis (PCoA) based on Bray-Curtis distances calculated from normalized CAZy family annotations. PERMANOVA was performed using the homonymous routines in PRIMER6+ with 10^3 permutations (Supplementary Table S3). Procrustes analyses depict significant correlations between CAZy profiles (Bray-Curtis) and bacterial (b) and fungal (c) community compositions (Bray-Curtis) across the soil stages. (d) Extended version of Fig. 3a that includes glycosyltransferase (GT) families. Heatmap displays the relative abundance (row *z*-scores) of CAZy gene families that differentially segregated across the soil stages, identified by random forest analysis with Boruta feature selection (average *z*-scores of 1000 runs > 4) (Supplementary Table S5). Circles are proportional to the relative abundance of each gene family in all samples.