Supplementary Material

Microbial Community Dynamics in Soil Depth Profiles over 120,000 Years of Ecosystem Development

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1 Supplementary Tables and Figures

1.1 Supplementary Tables

Table S1 Initial concentrations of organic carbon (OC) and total nitrogen (TN) per g soil fraction of samples used for the soil incubation experiment (Turner et al., 2017).

Site age (kyr)	Fraction	OC (mg g^{-1})	TN (mg g^{-1})
0.5	Bulk	144.2	7.9
5		137.3	7.7
12		77.7	3.6
120		65.5	3.6
0.5	HF	126.1	6.7
5		99.8	5.5
12		63.7	3.0
120		57.1	3.1
0.5	LF	403.5	14.0
5		409.5	14.1
12		387.9	11.9
120		381.8	10.5

Table S2 Physico-chemical and soil mineralogical properties of soil horizons along the Franz Josef chronosequence (Turner et al., 2014). Soil horizons used for the incubation experiment are highlighted in green. Hor. – horizon, OC – organic carbon, ON – organic nitrogen, TP – total phosphorus, Fe$_d$ – dithionite-extractable Fe phases representing poorly crystalline and crystalline Fe oxides as well as Fe-humus-complexes, (Fe+Al)$_o$ – oxalate-extractable Fe and Al phases representing poorly crystalline minerals and metal-humus-complexes, Fe$_{d\text{-}o}$ – difference between dithionite-extractable Fe and oxalate-extractable Fe representing crystalline Fe phases, (Fe+Al)$_p$ – Fe and Al from organic complexes.

Site age (kyr)	Hor.	pH	OC (g kg^{-1})	ON (g kg^{-1})	TP (mg kg^{-1})	(Fe+Al)$_o$ (g kg^{-1})	Fe$_d$ (g kg^{-1})	(Fe+Al)$_p$ (g kg^{-1})	Fe$_{d\text{-}o}$ (g kg^{-1})	Clay (%)	Silt (%)	Sand (%)
0.06	AO	5.8	201.5	13.2	1103	4.2	3.3	4.3	0.4	11	31	58
0.06	CA	6.0	7.3	0.5	686	2.3	2.9	1.1	1.1	3	17	80
0.06	C	6.4	7.2	0.4	804	2.7	3.6	1.7	1.5	6	31	63
0.5	OA	4.5	241.7	10.2	785	ND	ND	11.6	ND	ND	ND	ND
0.5	A	4.7	158.4	6.9	583	7	3.6	7.7	0.0	17	55	28
0.5	C	5.6	3.6	0.2	744	1.7	1.3	1.5	0.1	5	46	49
1	O	3.9	327.6	13.4	544	ND	ND	2.9	ND	ND	ND	ND
1	AE	4.1	66.6	4.0	220	2.4	1.3	3.6	0.1	12	58	30
1	E	4.7	17.2	0.7	103	3.2	2.0	3.3	0.6	12	55	33
1	B	5.3	9.5	0.5	272	8.5	6.8	5.5	1.1	5	43	52
5	O	3.9	367.9	14.4	681	ND	ND	2.3	ND	ND	ND	ND
5	AE	4.3	47.3	2.8	195	2.7	1.9	1.4	0.4	13	56	30
5	E	4.9	16.6	0.6	100	4.2	3.2	1.8	0.6	13	58	29
5	B	5.1	8.1	0.4	231	8.5	7.2	2.2	1.1	6	51	43
12	O	3.8	448.1	12.8	499	ND	ND	1.2	ND	ND	ND	ND
12	AE	4.1	143.6	6.8	244	1.6	0.6	1.2	0.0	7	56	36
12	EA	4.5	30.5	0.9	76	1.4	0.7	0.9	0.4	5	52	43
12	B	5.0	36.4	1.3	328	13.4	8.2	4.7	2.6	9	34	57
12	C	5.5	4.7	0.2	664	6.6	0.8	0.6	0.3	8	28	64
60	O	4.0	220.3	6.2	296	ND	ND	1.6	ND	ND	ND	ND
60	AE	4.1	60.4	2.9	161	1.7	0.7	1	0.2	11	68	20
60	EA	4.7	24.0	1.0	92	5.9	3.8	3.1	0.5	14	67	19
60	B	5.1	13.3	0.7	137	13.9	9.4	3.9	1.8	16	59	25
60	C	5.5	2.2	0.1	591	2.6	1.2	0.9	0.5	7	43	50
120	O	4.4	190.4	5.4	136	ND	ND	0.6	ND	ND	ND	ND
120	A	4.2	72.1	4.0	78	0.5	0.2	0.5	0.0	10	77	13
120	E	4.9	7.7	0.4	16	2.9	2.3	1.7	1.4	12	74	13
120	B	5.2	12.5	0.4	77	7.8	24.7	4	22.0	20	51	29

ND = not determined

Table S3 Results of the Tukey's post hoc test following the two-way ANOVA (soil age, horizon, their interaction and depth as a covariate) on microbial abundances along soil profiles of the Franz Josef chronosequence determined by qPCR (Figure 1). Letters indicate differences in abundances between different soil ages within each horizon (horizontal direction). Equal letters denote that the abundances not significantly differed from each other (Tukey test, $P < 0.05$).

	Soil age (kyr)						
	0.06	0.5	1	5	12	60	120
Archaea							
O	-	ab	a	a	a	ab	b
A	a	ab	ab	ab	b	a	a
E	-	-	a	a	a	a	a
B	-	-	a	a	a	a	a
C	a	a	-	-	b	a	-
Bacteria							
O	-	ab	a	a	a	ab	b
A	abc	abc	abc	ab	c	ac	b
E	-	-	a	ab	a	a	b
B	-	-	ab	ab	a	ab	b
C	a	ab	-	-	-	b	-
Fungi							
O	-	ab	a	a	a	ab	b
A	ab	ab	ab	a	ab	b	a
E	-	-	a	ab	a	a	b
B	-	-	a	a	a	a	a
C	a	ab	-	-	-	b	-
Eukarya							
O	-	a	a	a	a	a	a
A	ab	ab	ab	a	ab	b	ab
E	-	-	ab	ab	a	a	b
B	-	-	ab	ab	ab	a	b
C	a	ab	-	-	-	b	-
Archaea:Bacteria							
O	-	a	a	a	a	a	a
A	a	bc	bc	bc	b	ac	bc
E	-	-	ab	a	ab	a	b
B	-	-	a	a	a	a	b
C	a	b	-	-	-	b	-
Fungi:Bacteria							
O	-	a	a	a	a	a	a
A	a	a	a	a	a	a	a
E	-	-	a	a	a	a	a
B	-	-	ab	ab	ab	a	b
C	a	a	-	-	-	a	-

Table S4 Spearman rank order correlation coefficients for SSU rRNA gene copy numbers g^{-1} dry weight soil and soil chemical and mineralogical properties of the soil chronosequence. Significant results at $P < 0.01$ are typed in bold.

	NO_3^-	NH_4^+	TP	$(Fe+Al)_o$	Fe_d	$(Fe+Al)_p$	Fe_{d-o}	Clay	Silt	Sand
Archaea	0.19	**0.56**	-0.02	-0.20	-0.23	0.18	**-0.33**	0.14	0.23	-0.20
Bacteria	**0.36**	**0.69**	0.18	-0.17	-0.27	0.08	**-0.44**	0.13	0.14	-0.09
Fungi	**0.40**	**0.78**	0.08	-0.16	-0.28	0.00	**-0.38**	**0.31**	**0.33**	**-0.31**
Eukarya	**0.42**	**0.76**	0.06	-0.06	-0.14	0.04	-0.30	**0.30**	0.24	-0.22

Table S5 Number of archaeal and bacterial observed OTUs, richness estimator, and diversity index of the 16S rRNA gene sequences of samples along the Franz Josef chronosequence.

	Archaea			Bacteria		
Sample	No. OTUs[1]	Chao1[2]	H[3]	No. OTUs[1]	Chao1[2]	H[3]
0.5_CB	323	745 (603-959)	3.10 (3.04-3.17)	635	2134 (1748-2653)	5.54 (5.45-5.62)
5_O	239	565 (441-765)	2.60 (2.54-2.67)	922	4272 (3507-5264)	6.28 (6.21-6.35)
5_AE	288	685 (541-911)	3.12 (3.06-3.18)	500	1359 (1116-1697)	5.20 (5.12-5.29)
5_E	177	410 (308-590)	2.56 (2.51-2.62)	454	1276 (1030-1629)	5.08 (5.00-5.16)
5_B	199	442 (343-609)	2.62 (2.57-2.68)	456	1429 (1134-1853)	5.13 (5.06-5.10)
12_EA	343	1029 (799-1374)	2.77 (2.70-2.84)	498	1319 (1087-1642)	5.18 (5.10-5.27)
12_B	n.d.	n.d.	n.d.	501	1815 (1424-2374)	5.21 (5.13-5.29)
120_E1	225	608 (459-852)	2.26 (2.19-2.32)	361	812 (662-1036)	4.89 (4.81-4.96)
120_E2	163	358 (275-503)	1.69 (1.63-1.75)	219	552 (421-767)	3.68 (3.59-3.77)
120_B	176	419 (314-603)	1.82 (1.75-1.89)	267	850 (626-1214)	4.18 (4.09-4.26)

[1] Number of observed OTUs

[2] Chao1 richness estimator with higher and lower 95% confidence interval in parentheses.

[3] Shannon diversity index with higher and lower 95% confidence interval in parentheses.

n.d. no data

Table S6 Effects of the different factors on microbial abundances in the soil microcosm incubation experiment (bulk and HF, without the LF) as revealed by five-way ANOVA with significant interactions. Frac – fraction.

	O_2	Fraction	Site age	P	C	Significant interactions
Archaea	*	n.s.	***	n.s.	n.s.	O_2×Age*, Age×P*, O_2×Frac×Age***, O_2×Frac×P**, O_2×P×C***, Frac×Age×C*, Frac×Age×P**
Bacteria[1]	***	***	***	***	n.s.	O_2×Frac***, O_2×Age***, O_2×P***, Frac×Age***, Frac×P***, Age×C***, O_2×Frac×Age***, O_2×Age×P*, O_2×Age×C***, O_2×P×C***, Frac×Age×C*, Frac×P×C*
Fungi[1]	***	n.s.	**	***	***	O_2×Age***, O_2×C*, Frac×Age**, Frac×P**, Frac×C**, Age×C**, O_2×Frac×Age**, O_2×Frac×P*, O_2×Frac×C***, O_2×Age×P*
Archaea:*Bacteria*[1]	***	**	**	n.s.	n.s.	O_2×Frac***, O_2×Age***, O_2×P*, Age×P**, O_2×Frac×Age**, O_2×Frac×P**, Frac×Age×P*, Frac×Age×C*, Frac×P×C*
Fungi:Bacteria[1]	***	n.s.	***	***	***	O_2×Frac***, O_2×P***, Frac×C**, Age×C***, P×C**, O_2×Frac×Age**, O_2×Frac×C***, O_2×Age×C**

*** $P < 0.001$, ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$; n.s. – not significant ($P > 0.05$).

[1] data not normal-distributed, see Table S7 for analysis of normal-distributed oxic and anoxic data subset.

Table S7 Results of four-way ANOVA with significant interactions for the oxic and anoxic subsets of the soil incubation experiment (HF vs. bulk samples, without the LF). Frac – fraction.

	Fraction	Site age	P	C	Significant interactions
Bacteria oxic	***	***	***	**	Frac×Age***, Age×C***, P×C***, Frac×Age×P*
Bacteria anoxic	***	***	n.s.	n.s.	Frac×P**, Age×P**, P×C**, Frac×P×C*
Fungi oxic	n.s.	***	***	***	Frac×Age***, Frac×P*, Frac×C***, Age×P***, Age×C***, Frac×Age×C*, Frac×P×C***, Age×P×C*
Fungi anoxic	n.s.	***	***	***	Frac×P**, Frac×C***, Age×C*; P×C*
Archaea:*Bacteria* oxic	n.s.	***	*	n.s.	Frac×P*, Frac×Age×C*
Archaea:*Bacteria* anoxic	***	***	n.s.	n.s.	Frac×Age**, Frac×P*, Age×P*, Frac×Age×C*
Fungi:Bacteria oxic	***	***	**	***	Frac×Age***, Frac×C***, Age×P***, Age×C***, P×C***, Frac×Age×P**, Frac×Age×C***, Frac×P×C**, Age×P×C***
Fungi:Bacteria anoxic	***	*	***	***	Frac×Age*, Frac×C***

*** $P < 0.001$, ** $0.001 < P < 0.01$; * $0.01 < P < 0.05$; n.s. – not significant ($P > 0.05$).

Table S8 Results of variance component estimation of factors (fraction, soil age, P and C addition and their four-way interactions) controlling microbial abundances and abundance ratios in the oxic and anoxic subsets (without the LF, controls and inoculum) for the soil microcosm incubation experiment.

	% of total variation				
Oxic					
	Archaea	*Bacteria*	*Fungi*	*Archaea:Bacteria*	*Fungi:Bacteria*
Fraction	0	33	0	0	17
Age	6	5	9	31	1
P	0	14	12	2	0
C	0	0	50	0	32
Fraction × Age	10	13	10	0	0
Fraction × P	1	1	0	3	0
Fraction × C	0	0	2	0	8
Age × P	0	0	5	3	5
Age × C	0	15	2	0	19
P × C	0	9	0	0	5
Fraction × Age × P	14	0	0	5	0
Fraction × Age × C	3	0	2	5	1
Fraction × P × C	5	0	2	1	0
Age × P × C	0	0	2	0	2
Fraction × Age × P × C	0	3	0	0	5
Residual	62	8	4	49	3
Anoxic					
	Archaea	*Bacteria*	*Fungi*	*Archaea:Bacteria*	*Fungi:Bacteria*
Fraction	0	53	0	29	0
Age	0	35	5	3	0
P	0	0	16	0	18
C	0	0	3	0	1
Fraction × Age	10	0	2	10	6
Fraction × P	0	1	8	1	3
Fraction × C	0	0	21	0	35
Age × P	0	2	0	0	0
Age × C	0	0	6	0	2
P × C	7	0	5	0	0
Fraction × Age × P	5	0	0	4	0
Fraction × Age × C	0	1	1	0	0
Fraction × P × C	0	1	0	0	0
Age × P × C	0	0	0	0	0
Fraction × Age × P × C	20	0	0	17	1
Residual	58	7	34	36	34

Table S9 Total number of T-RFs and number of T-RFs that were unique either in bulk, HF, or LF samples of the soil incubation experiment (without inoculum and control).

	Total	Bulk	HF	LF
Archaea (HaeIII)	31	3	3	1
Archaea (RsaI)	23	8	1	0
Bacteria (HaeIII)	52	5	6	0
Bacteria (HhaI)	44	7	4	1

1.2 Supplementary Figures

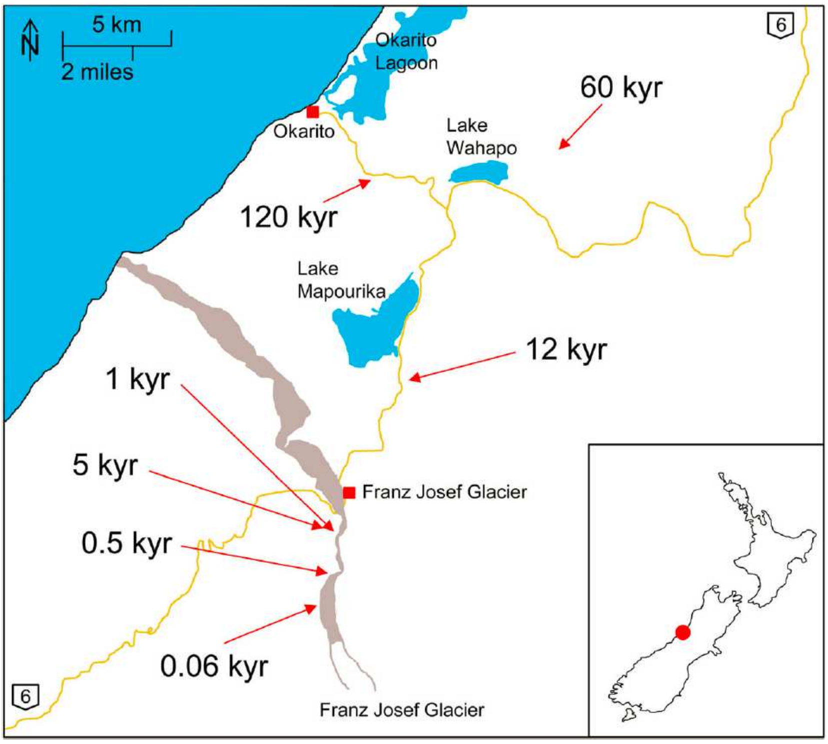

Figure S1 Map of sampling sites along the 120 kyr old Franz Josef chronosequence modified after Dietel et al. (2017).

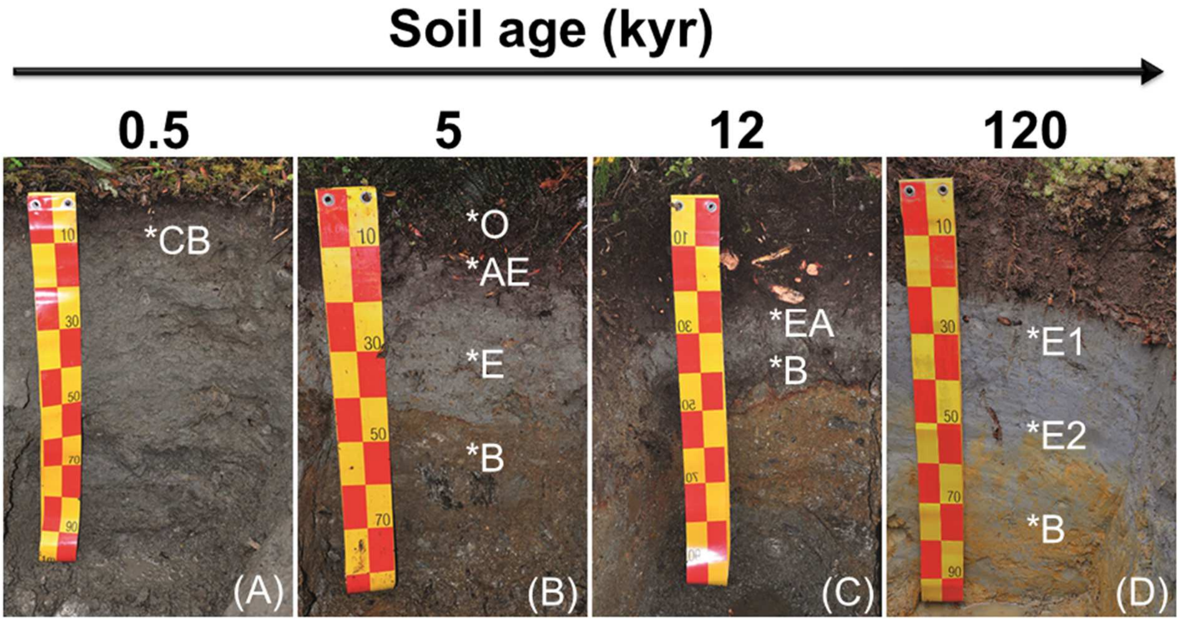

Figure S2 Soil profiles at four sampling sites along the Franz Josef chronosequence of **(A)** 0.5, **(B)** 5, **(C)** 12, and **(D)** 120 kyr soil age. Asterisks mark horizons that were analyzed by pyrosequencing.

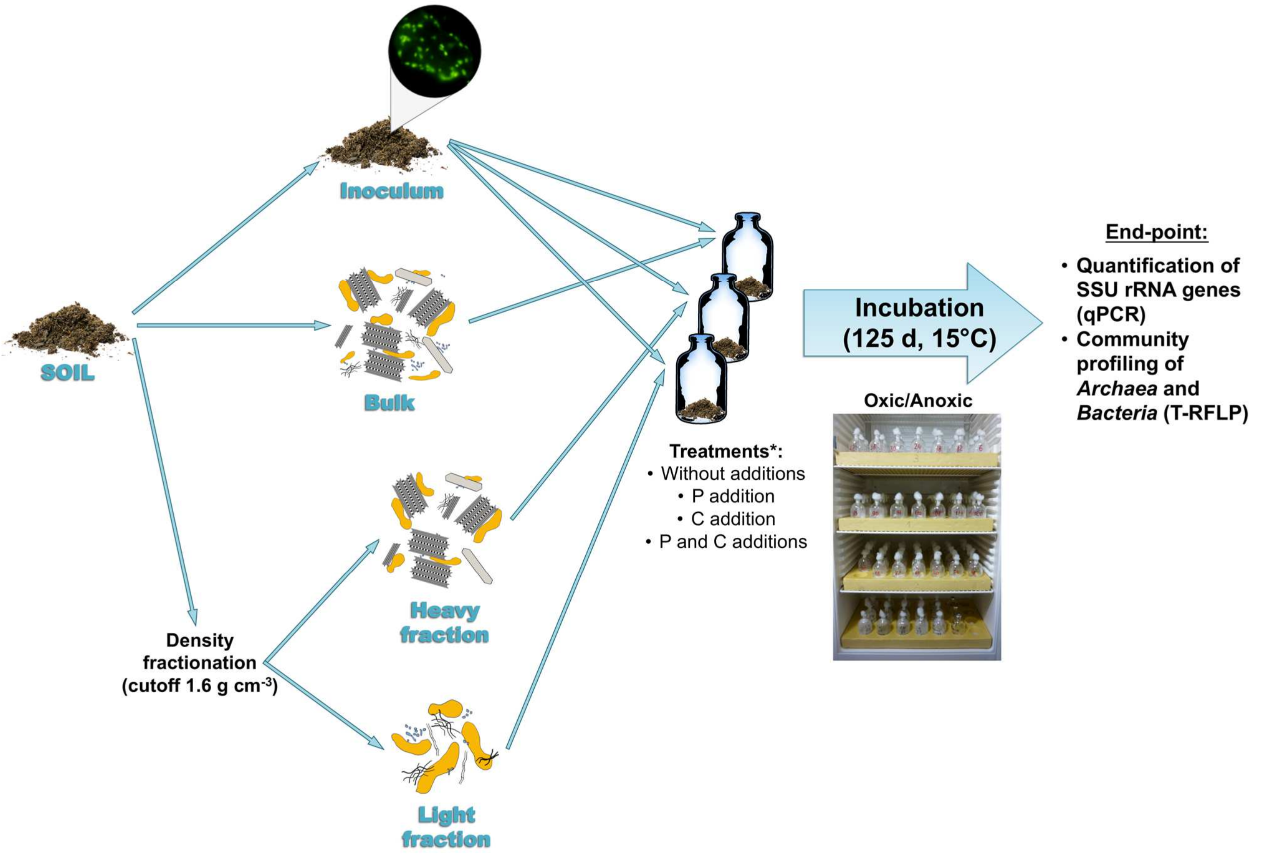

Figure S3 Schematic overview of the microcosm incubation experiment setup modified after Turner et al. (2017). *The C and P treatments were not tested for the light fraction samples. Photographs courtesy of Christian Siebenbürgen and Norman Gentsch.

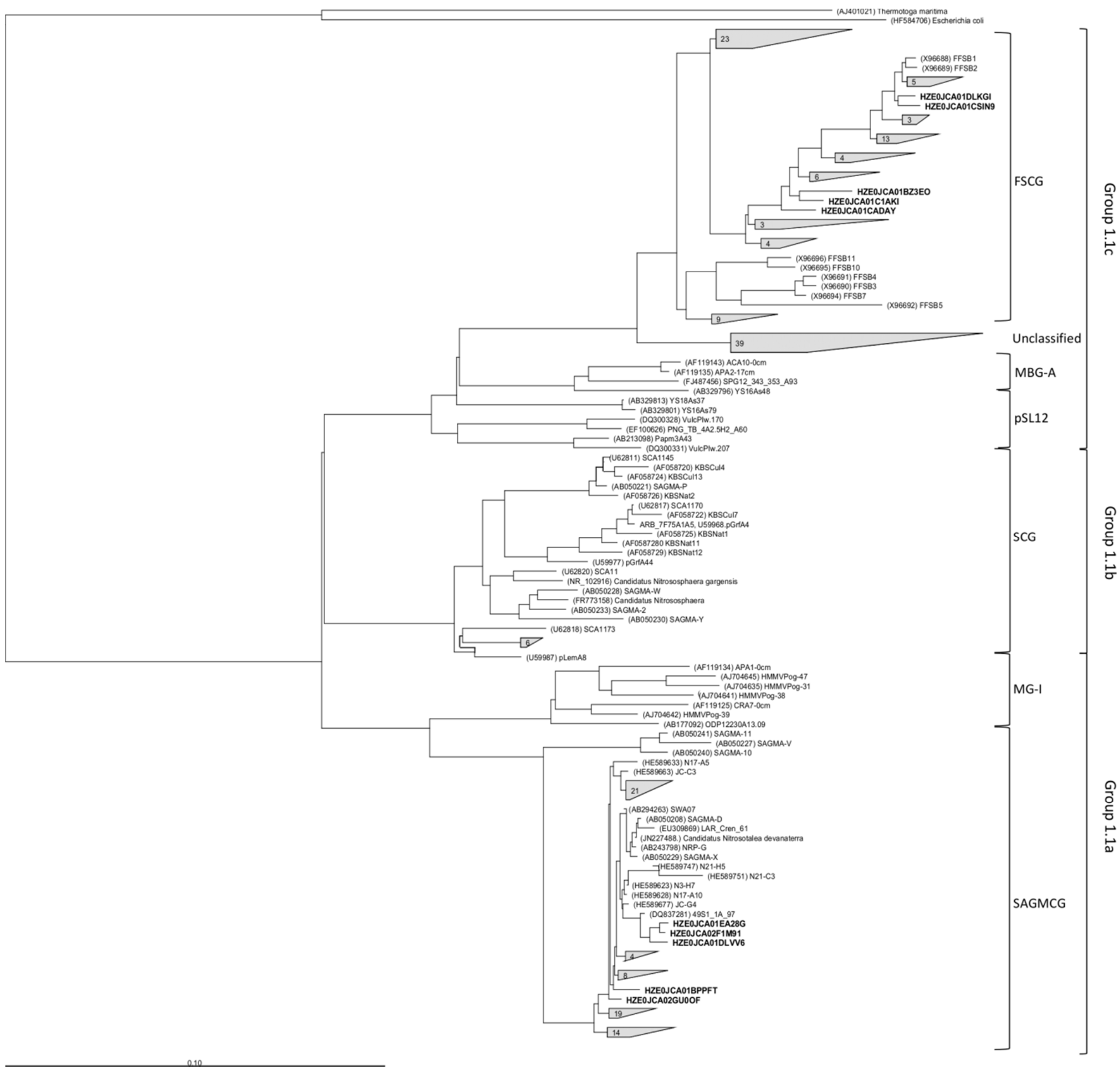

Figure S4 Phylogenetic affiliation of *Thaumarchaeota* 16S rRNA gene sequences from soils along the Franz Josef chronosequence (in bold or grouped). The scale bar indicates 10% estimated phylogenetic divergence.

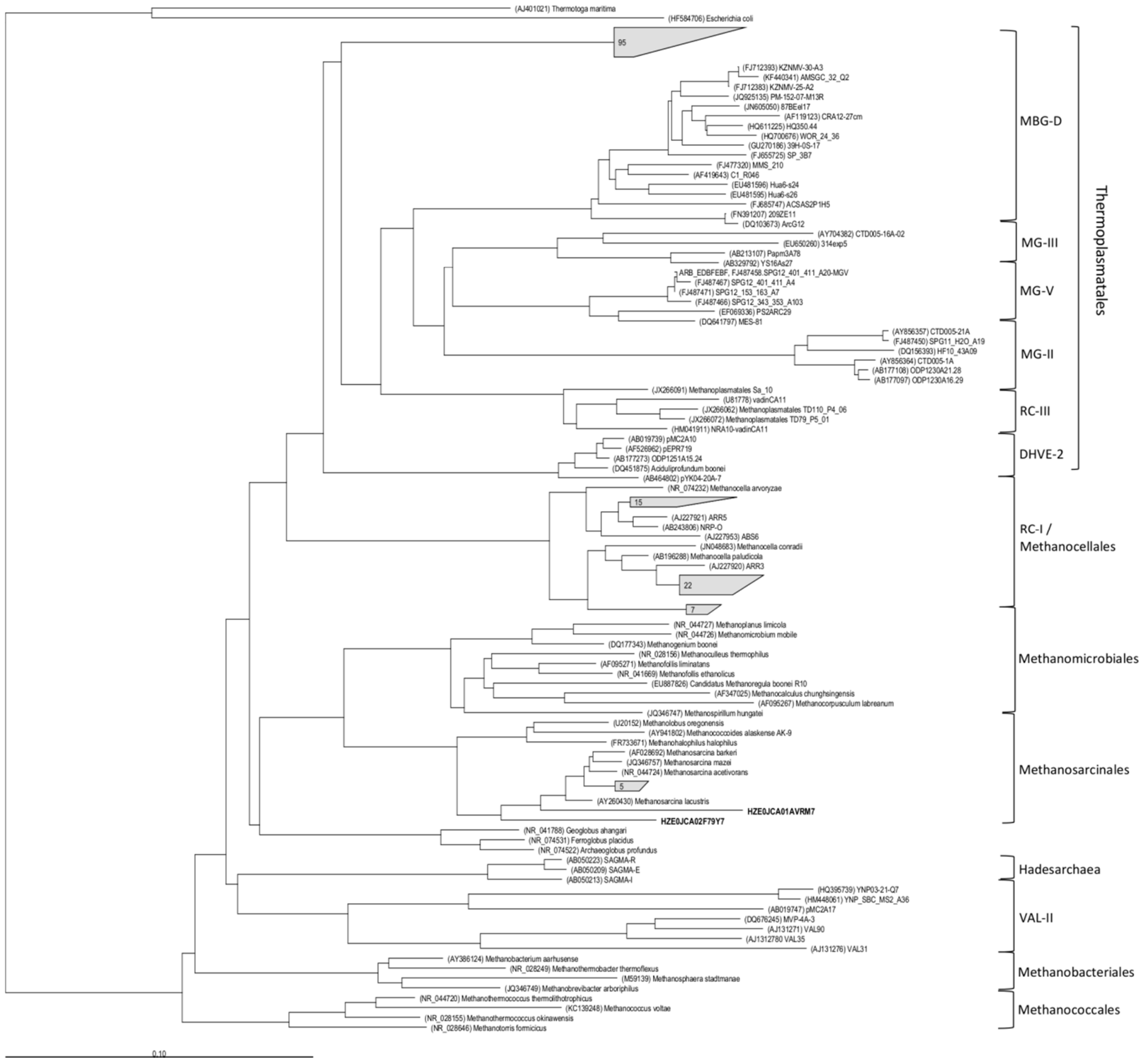

Figure S5 Phylogenetic affiliation of *Euryarchaeota* 16S rRNA gene sequences from soils along the Franz Josef chronosequence (in bold or grouped). The scale bar indicates 10% estimated phylogenetic divergence.

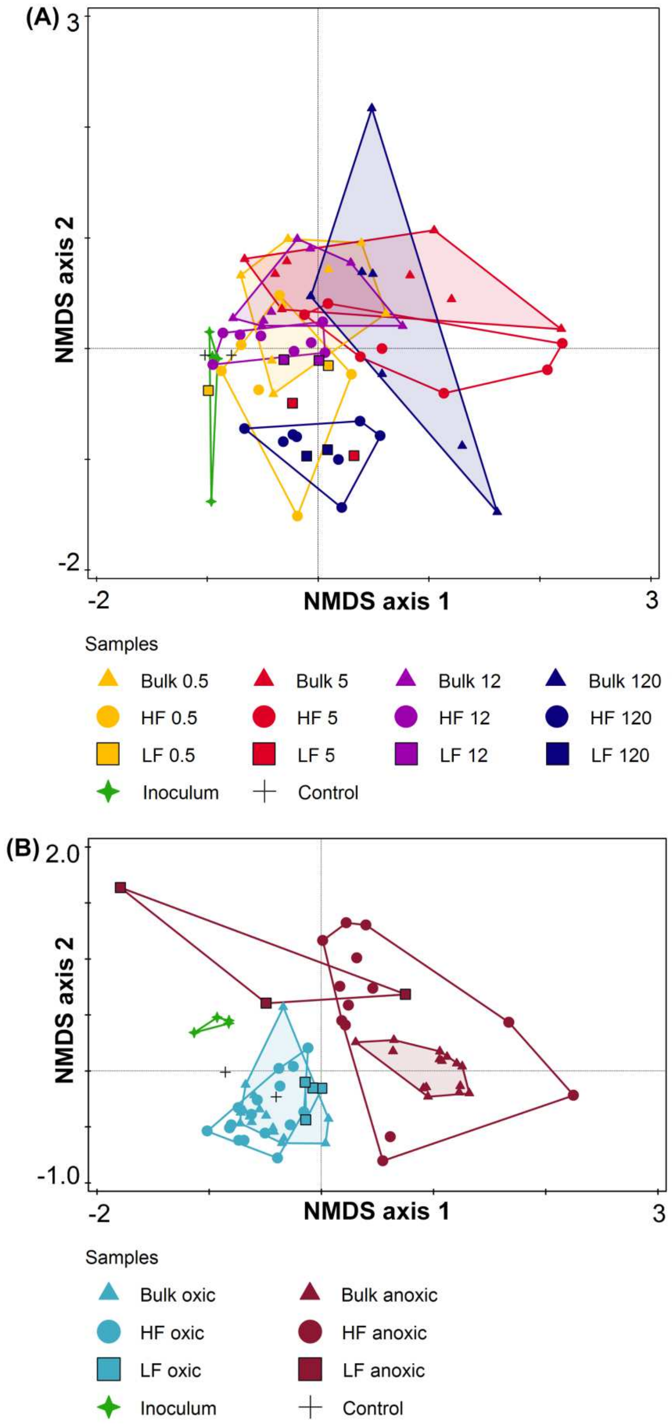

Figure S6 Non-metric multidimensional scaling (NMDS) of **(A)** archaeal (stress value = 0.120) and **(B)** bacterial community composition (stress value = 0.100) for the soil microcosm incubation experiment based on relative abundance of T-RFs (after digestion of PCR products with RsaI or HhaI, respectively) using Bray-Curtis distance measure. Different symbols encode the different soil fractions (triangle – bulk soil; circle - heavy fraction, HF; and square – light fraction, LF). Different colors encode the different soil ages (0.5, 5, 12, and 120 kyr) for archaeal community analysis **(A)** and O_2 status (oxic and anoxic) for bacterial community analysis **(B)**.

References

Dietel, J., Dohrmann, R., Guggenberger, G., Meyer-Stüve, S., Turner, S., Schippers, A., et al. (2017). Complexity of clay mineral formation during 120,000 years of soil development along the Franz Josef chronosequence, New Zealand. *New Zeal. J. Geol. Geophys.* 60, 23–35. doi:10.1080/00288306.2016.1245668.

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