SUPPLEMENTARY MATERIALS

Table S1. Chemical characteristics of the two digestate forms (SD and WD). Values quoted are expressed as % of air-dry digestate (means followed by standard error in brackets). References for the employed methods used for determination of each chemical characteristic is also reported.

	SD		WD		Reference
Org C %	44.4	(0.33)	1.1	(0.01)	[81]
Tot N %	1.4	(0.01)	0.4	(0.01)	[82]
C/N	31.4	(0.17)	3.1	(0.04)	
NH_4-N %	n.d		0.2	(0.00)	[83]
K %	1.7	(0.00)	n.d.		[84]
P %	0.9	(0.01)	n.d.		[84]
S %	0.23	(0.02)	n.d.		[85]

SD = solid digestate; WD = whole digestate.

Table S2. Soil physical and chemical characteristics at the beginning of trial (t_0) (means from 9 observations followed by standard errors in brackets).

Clay (%)	41.9	(1.22)
Silt (%)	47.8	(2.13)
Moisture (%)	24.46	(1.24)
Bulk density (g cm^{-3})	1.39	(0.04)
pH	8.3	(0)
$CaCO_3$ (%)	11.4	(0.7)
TOC (g kg^{-1})	12.8	(0.3)
TN (g kg^{-1})	1.4	(0)
C/N	9.4	(0.3)
CEC (cmol(+) kg^{-1})	21.0	(0.7)
Exchangeable Bases (mg kg^{-1})		
K	278.7	(8.0)
Na	22.0	(3.0)
Mg	201.7	(25.6)
Ca	3718.6	(176.3)
Available Microelements (mg kg^{-1})		
Cu	28.0	(5.0)
Zn	1.7	(0.2)
Fe	15.4	(0.5)
Mn	16.3	(0.5)

TOC = total organic C; TN = total N; CEC = cation exchange capacity

Table S3. PCR primers and amplification annealing temperatures used in this study.

Target	PCR conditions		Reference
	primers	annealing	
Bacteria	[a]986f, UNI1401r	56 °C	[86]
Fungi	EF390, [a]FR1	48 °C	[87]
Archaea	[a]Arc1106F, 1378R	55 °C	[88]
Clostridiaceae Cluster I & II	[a]CHIS150F, CLOST1R	55 °C	[89,90]
Clostridiaceae Cluster III	[a]CT110F, CT325R	55 °C	[91]
Clostridiaceae Cluster IV	[a]CLEPF, CLEPR	55 °C	[92]
Lachnospiraceae Cluster XIV	[a]EREC482F, CCOC1112R	52 °C	[93]

[a]A GC-clamp was added for DGGE analysis

Table S4. Species richness, Shannon-Weiner index of diversity (*H′*) and Simpson index (*D*) calculated on 16S rDNA and 18S rDNA DGGE profiles according to sampling time and fertilization treatment (means followed by standard error in brackets). Different letters in a column indicate statistically significant differences between soil samples at $p \leq 0.05$ (One-way ANOVA followed by Fisher LSD test). In the part below of the table are reported the statistical significance of multifactor ANOVA considering as factors: sampling time (ST) and fertilization treatment (FT).

		bacteria (16S-rDNA DGGE)			**fungi (18S-rDNA DGGE)**		
Plots	**Time**	**Richness**	***H′***	***D***	**Richness**	***H′***	***D***
D0	t_0	34.3 (0.3) ab	3.51 (0.01) ab	0.030 (0.000) abcd	21.7 (2.7) abc	3.05 (0.12) abc	0.049 (0.005) abc
	t_1	34.3 (2.3) ab	3.51 (0.07) ab	0.031 (0.002) abcd	20.3 (1.2) abcd	2.98 (0.06) abcd	0.053 (0.003) abcd
	t_2	37.0 (1.5) ab	3.58 (0.04) ab	0.029 (0.001) abcd	19.3 (1.2) bcd	2.93 (0.06) bcd	0.055 (0.003) bcd
	t_3	38.7 (0.3) a	3.63 (0.01) a	0.027 (0.001) cd	17.0 (1.0) cd	2.81 (0.06) cd	0.062 (0.003) cd
D50	t_0	35.3 (0.7) ab	3.54 (0.02) ab	0.029 (0.001) abcd	25.0 (0.6) a	3.19 (0.02) a	0.042 (0.001) a
	t_1	33.7 (1.7) b	3.49 (0.05) b	0.031 (0.002) ab	20.0 (0.6) abcd	2.97 (0.03) abcd	0.052 (0.002) abcd
	t_2	34.7 (0.3) ab	3.53 (0.01) ab	0.030 (0.001) abcd	21.3 (2.2) abc	3.02 (0.11) abc	0.051 (0.006) abc
	t_3	36.0 (1.0) ab	3.55 (0.03) ab	0.029 (0.001) abcd	16.0 (2.5) d	2.74 (0.15) d	0.067 (0.009) d
D100	t_0	38.7 (1.3) a	3.63 (0.04) a	0.027 (0.001) bcd	23.7 (1.5) ab	3.14 (0.06) ab	0.044 (0.003) ab
	t_1	38.7 (1.7) a	3.63 (0.04) a	0.027 (0.001) d	17.7 (0.9) cd	2.84 (0.05) cd	0.060 (0.003) cd
	t_2	34.0 (1.5) b	3.50 (0.04) b	0.031 (0.001) abc	25.0 (2.0) a	3.20 (0.09) a	0.042 (0.004) a
	t_3	34.0 (3.1) b	3.49 (0.09) b	0.032 (0.003) a	20.0 (2.6) abcd	2.96 (0.13) abcd	0.053 (0.007) abcd
Main effects							
ST		n.s.	n.s.	n.s.	***	***	***
FT		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction							
STxFT		*	*	*	n.s.	n.s.	n.s.

*$p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; n.s. = not significant

Table S5. Identification of 16S rDNA fragments selected from PCR-DGGE. Taxonomic identification was achieved by using different sequence similarity thresholds: a similarity ≥97%, ≥95%, ≥90%, ≥85%, ≥80% and ≥75% for assignment at the species-, genus-, family-, order-, class- and phylum-levels identification, respectively [51].

Band	Nearest match (GenBank accession no.; % similarity)	Taxonomic classification
A2.3	*Nitrososphaera viennensis* EN76 (NR_134097; 92%)	Unclassified *Nitrososphaeraceae*
A2.5	*Nitrososphaera viennensis* EN76 (NR_134097; 93%)	Unclassified *Nitrososphaeraceae*
A2.6	*Methanosarcina thermophila* TM-1 (NR_118372; 99%)	*Methanosarcina thermophila*
A2.8	*Nitrososphaera viennensis* EN76 (NR_134097; 91%)	Unclassified *Nitrososphaeraceae*
A3.1	*Nitrososphaera viennensis* EN76 (NR_134097; 91%)	Unclassified *Nitrososphaeraceae*
A5.2	*Nitrososphaera viennensis* EN76 (NR_134097; 87%)	Unclassified *Nitrososphaerales*
A5.5	*Nitrososphaera viennensis* EN76 (NR_134097; 88%)	Unclassified *Nitrososphaerales*
CI5.1	*Clostridium chromiireducens* GCAF-1 (NR_122090; 97%)	*Clostridium chromiireducens*
CI5.2	*Clostridium punicem* BL70/20 (NR_026105; 99%)	*Clostridium punicem*
CI6.1	*Clostridium bowmanii* A-1/C-an/C1 (NR_114765; 98%)	*Clostridium bowmanii*
CI6.2	*Clostridium bowmanii* A-1/C-an/C1 (NR_114765; 97%)	*Clostridium bowmanii*
CI7.1	*Clostridium bowmanii* A-1/C-an/C1 (NR_114765; 96%)	Unclassified *Clostridium*
CI8.1	*Nitrosospira multiformis* ATCC25196 (NR_074736; 99%)	*Nitrosospira multiformis*
CI9.1	*Clostridium tyrobutyricum* ATCC25755 (NR_044718; 99%)	*Clostridium tyrobutyricum*
CI9.2	*Clostridium kogasensis* YHK0403 (NR_136452; 96%)	Unclassified *Clostridium*
CI10.1	*Rheinheimera muenzenbergensis* E49 (NR_133839; 98%)	*Rheinheimera muenzenbergensis*
CI10.2	*Rheinheimera muenzenbergensis* E49 (NR_133839; 98%)	*Rheinheimera muenzenbergensis*
CI11.1	*Rheinheimera hassiensis* E48 (NR_133838; 96%)	Unclassified *Rheinheimera*
CI11.2	*Alishewanella aestuarii* B11 (NR_044344; 99%)	*Alishewanella aestuarii*
CI12.1	*Rheinheimera japonica* KP17 (NR_136858; 98%)	*Rheinheimera japonica*
CI13.2	*Oxalobacter formigenes* OXB (NR_029188; 91%)	Unclassified *Oxalobacteraceae*
CI13.3	*Clostridium saudiense* JCC (NR_144696; 100%)	*Clostridium saudiense*
CIII6.1	*Caloranaerobacter azorensis* MV1087 (NR_028919; 85%)	Unclassified *Clostridiales*
CIII6.2	*Clostridium stercorarium* DSM8532 (NR_102513; 90%)	Unclassified *Ruminococcaceae*
CIII6.3	*Clostridium stercorarium* DSM8532 (NR_102513; 89%)	Unclassified *Clostridiales*
CIII6.4	*Phocea massiliensis* P2769 (NR_144748; 90%)	Unclassified *Ruminococcaceae*
CIII10.1	*Geobacter bemidjiensis* Bem (NR_075007; 85%)	Unclassified *Desulfuromonadales*
CIII11.1	*Iamia majanohamensis* NBRC102561 (NR_041634; 87%)	Unclassified *Acidimicrobiales*
CIII11.2	*Iamia majanohamensis* NBRC102561 (NR_041634; 87%)	Unclassified *Acidimicrobiales*
CIII11.4	*Clostridium caenicola* EBR596 (NR_126170; 89%)	Unclassified *Clostridiales*
CIV10.4	*Caproiciproducens galactitolivorans* BS-1 (NR_145929; 98%)	*Caproiciproducens galactitolivorans*
CIV11.1	*Caproiciproducens galactitolivorans* BS-1 (NR_145929; 93%)	Unclassified *Ruminococcaceae*
CIV12.4	*Pyrinomonas methylaliphatogenes* K22 (NR_133835; 90%)	Unclassified Acidobacteria
CIV12.5	*Pyrinomonas methylaliphatogenes* K22 (NR_133835; 91%)	Unclassified Acidobacteria
CIV13.1	*Stenotrophobacter namibiensis* Ac17 (NR_146021; 95%)	Unclassified *Stenotrophobacter*
CIV14.2	*Pyrinomonas methylaliphatogenes* K22 (NR_133835; 93%)	Unclassified Acidobacteria
CIV15.3	*Clostridium cellulosi* AS1.1777 (NR_044624; 99%)	*Clostridium cellulosi*
CIV15.4	*Acutalibacter muris* KB18 (NR_144605; 86%)	Unclassified *Clostridiales*
CIV17.1	*Mageebacillus indolicus* 0009-5 (NR_146698; 91%)	Unclassified *Ruminococcaceae*
CIV19.1	*Caproiciproducens galactitolivorans* BS-1 (NR_145929; 94%)	Unclassified *Ruminococcaceae*
CIV19.2	*Caproiciproducens galactitolivorans* BS-1 (NR_145929; 93%)	Unclassified *Ruminococcaceae*
CIV19.6	*Caproiciproducens galactitolivorans* BS-1 (NR_145929; 93%)	Unclassified *Ruminococcaceae*
CIV19.8	*Caproiciproducens galactitolivorans* BS-1 (NR_145929; 94%)	Unclassified *Ruminococcaceae*
CXIV13.1	*Conexibacter arvalis* KV-962 (NR_113264; 90%)	Unclassified *Thermoleophilia*
CXIV14.1	*Herbinix hemicellulosilytica* T3/55 (NR_136763; 95%)	*Herbinix hemicellulosilytica*
CXIV14.1	*Herbinix hemicellulosilytica* T3/55 (NR_136763; 97%)	*Herbinix hemicellulosilytica*

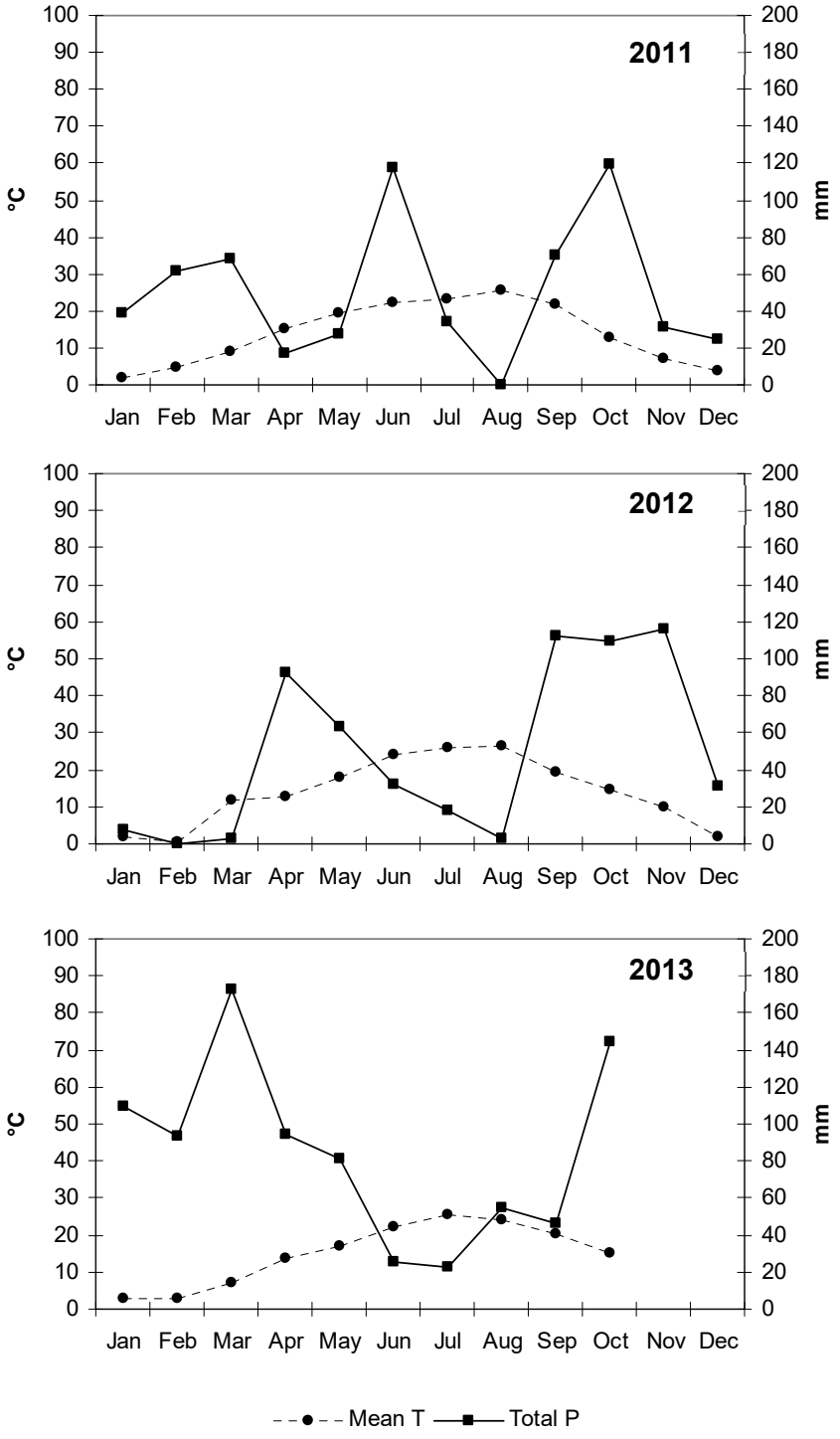

Figure S1. Ombrothermic diagrams [94] of the study site showing rainfall (P, mm) and temperatures (T, °C) during the three experimental years (by Agenzia Regionale per l'Ambiente of Emilia-Romagna, ARPAE; https://www.arpae.it).

Figure S2. Spreader machinery developed in this study to apply liquid digestate on soil surface to fertilizer plots of experimental trial (CAT and Cavazzuti Franco).

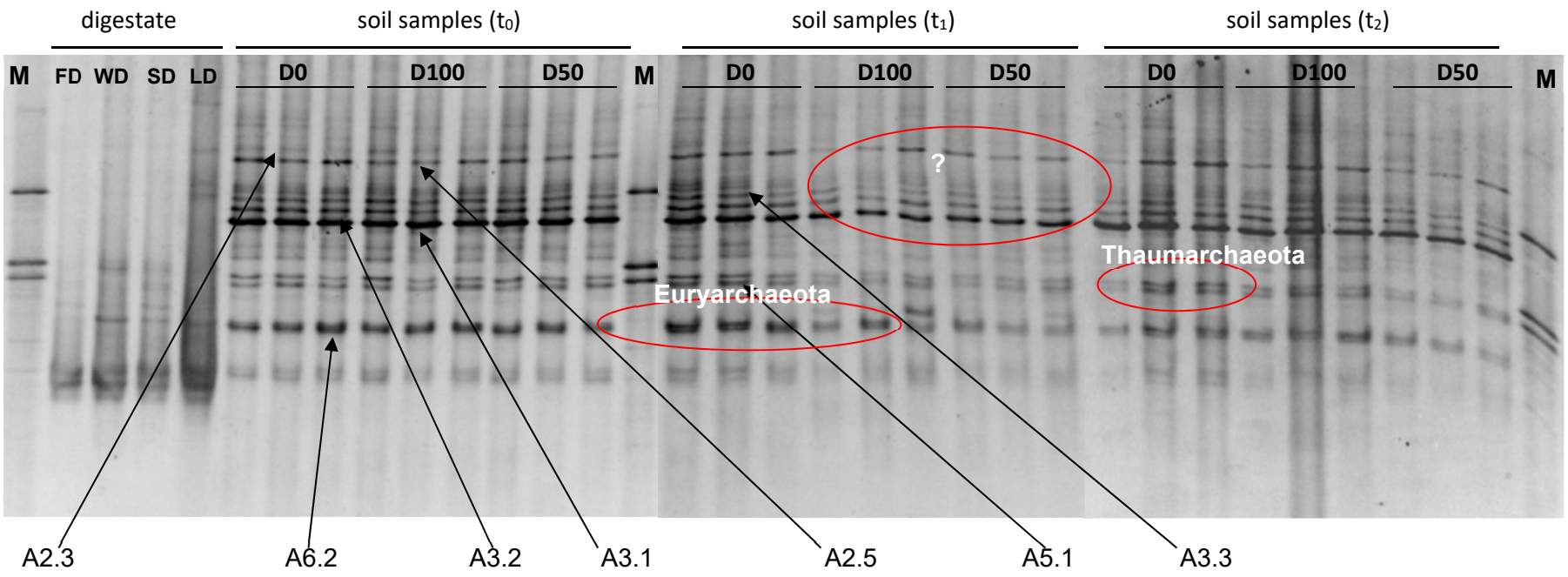

Figure S3a. DGGE profiles of 16S rDNA PCR products obtained from DNA extracted from digestate fractions and soil samples using primer specific for Archaea (1106F-1378R). DGGE conditions: 8% acrylamide; 32–60% denaturing gradient. The excised bands used for sequencing and phylogenetic analyses are pointed out. The letter M on the gel images indicates the marker used for normalization of bands. FD = liquid fraction collected from the anaerobic fermentation silos of the biogas plant; WD = whole digestate; SD = digestate solid fraction; LD = digestate liquid fraction; D0 = mineral fertilizer only (control); D50 = WD + mineral fertilizer; D100 = WD only.

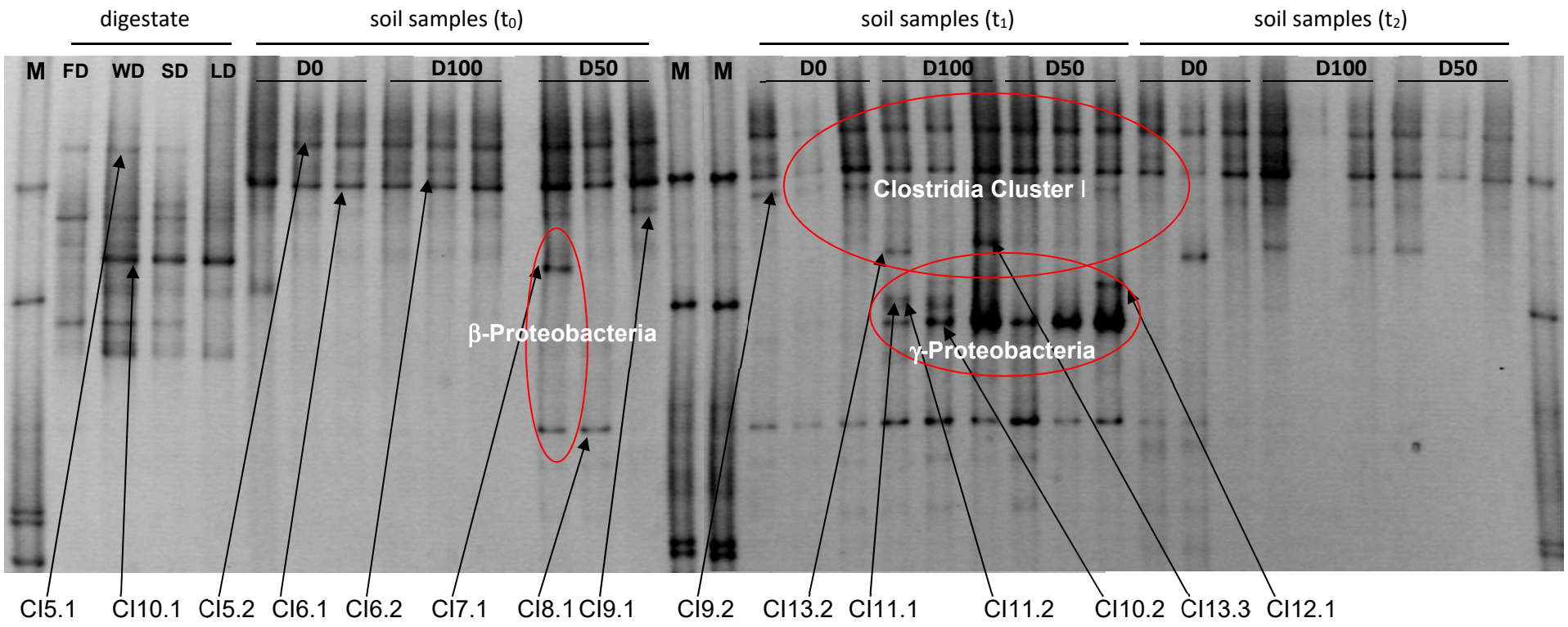

Figure S3b. DGGE profiles of 16S rDNA PCR products obtained from DNA extracted from digestate fractions and soil samples using primers specific for *Clostridiaceae* Cluster I & II (CHIS150F-CLOST1R). DGGE conditions: 6% acrylamide; 50–60% denaturing gradient. The excised bands used for sequencing and phylogenetic analysis are pointed out. The letter M on the gel images indicates the marker used for normalization of bands. FD = liquid fraction collected from the anaerobic fermentation silos of the biogas plant; WD = whole digestate; SD = digestate solid fraction; LD = digestate liquid fraction; D0 = 100% N as urea; D50 = 50% N as urea + 50% N as WD; D100 = 100% N as WD.

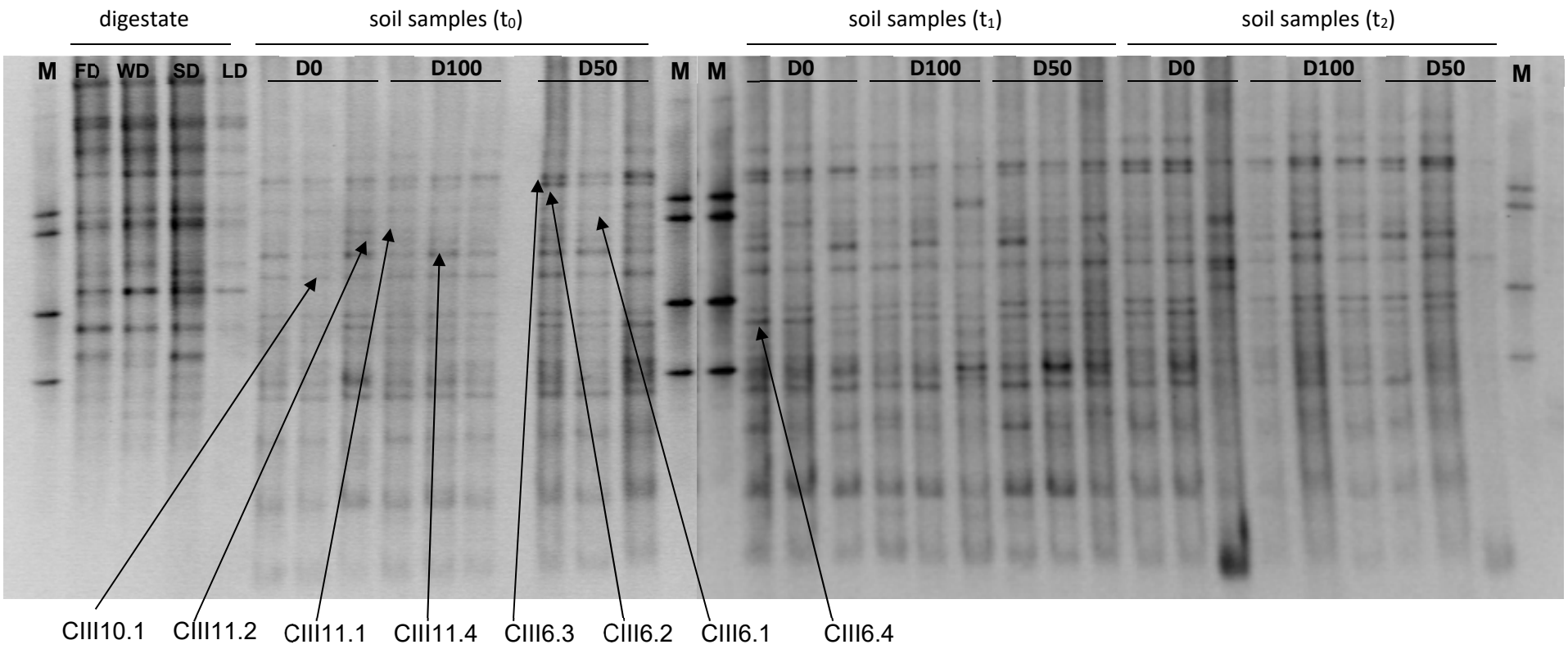

Figure S3c. DGGE profiles of 16S rDNA PCR products obtained from DNA extracted from digestate fractions and soil samples using primers specific for *Clostridiaceae* Cluster III (CT110F-CT325R). DGGE conditions: 6% acrylamide; 50–60% denaturing gradient. The excised bands used for sequencing and phylogenetic analysis are pointed out. The letter M on the gel images indicates the marker used for normalization of bands. FD = liquid fraction collected from the anaerobic fermentation silos of the biogas plant; WD = whole digestate obtained from the biogas plant; SD = digestate solid fraction; LD = digestate liquid fraction; D0 = 100% N as urea; D50 = 50% N as urea + 50% N as WD; D100 = 100% N as WD.

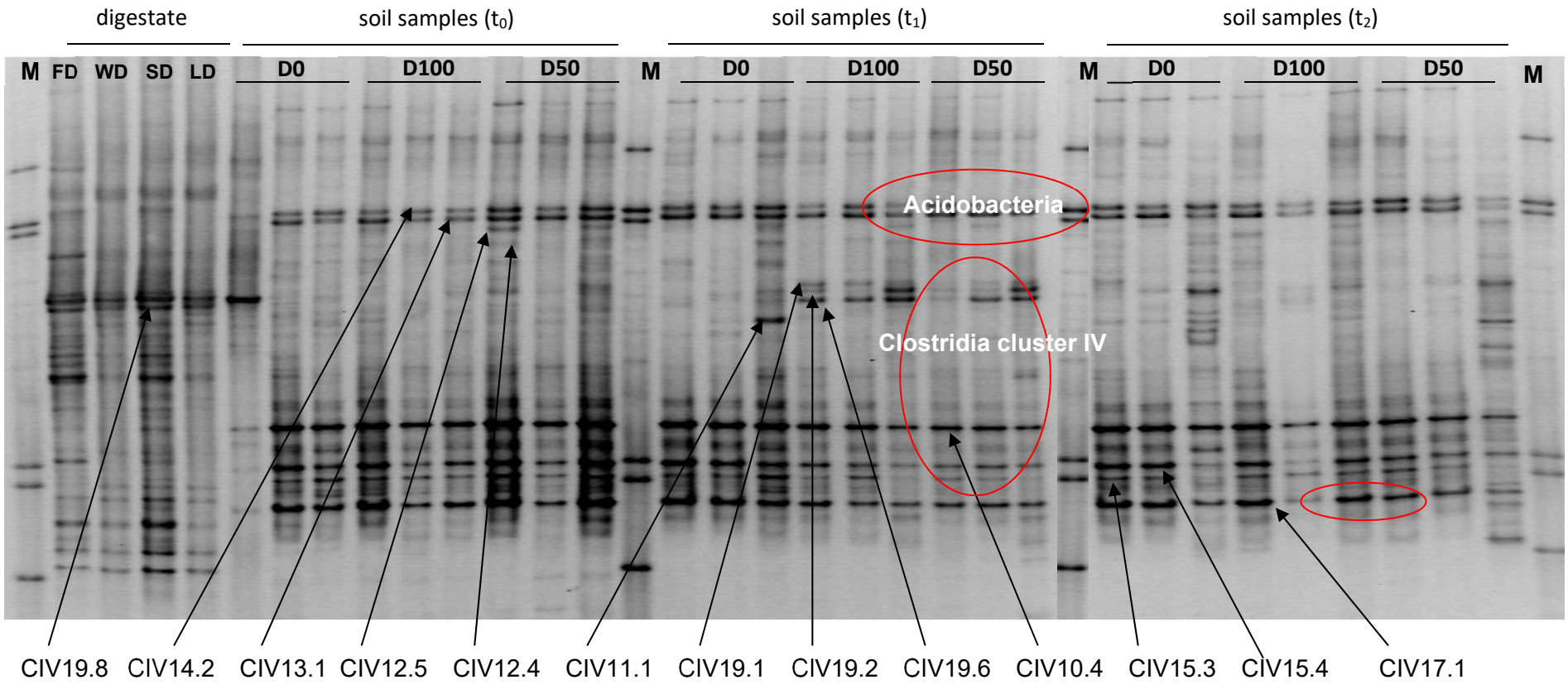

Figure S3d. DGGE profiles of 16S rDNA PCR products obtained from DNA extracted from digestate fractions and soil samples using primers specific for *Clostridiaceae* Cluster IV (CLEPF-CLEPR). DGGE conditions: 6% acrylamide; 50–60% denaturing gradient. The excised bands used for sequencing and phylogenetic analysis are pointed out. The letter M on the gel images indicates the marker used for normalization of bands. FD = liquid fraction collected from the anaerobically fermentation silos of the biogas plant; WD = whole digestate obtained from the biogas plant; SD = digestate solid fraction; LD = digestate liquid fraction; D0 = 100% N as urea; D50 = 50% N as urea + 50% N as WD; D100 = 100% N as WD.

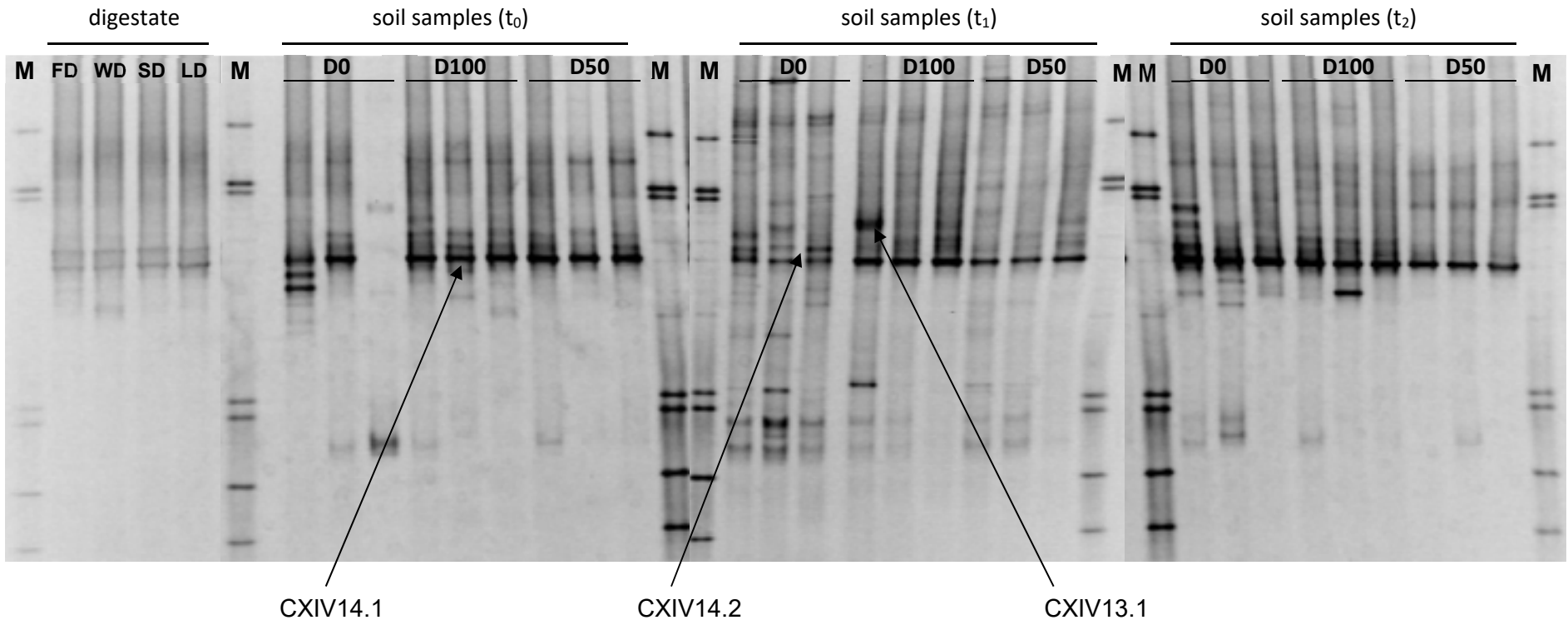

Figure S3e. DGGE profiles of 16S rDNA PCR products obtained from DNA extracted from digestate fractions and soil samples using primers specific for *Lachnospiraceae*-Cluster XIV (EREC482F-CCOC1112R). DGGE conditions: 8% acrylamide; 45–65% denaturing gradient. The excised bands used for sequencing and phylogenetic analysis are pointed out. The letter M on the gel images indicates the marker used for normalization of bands. FD = liquid fraction collected from the anaerobic fermentation silos of the biogas plant; WD = whole digestate obtained from the biogas plant; SD = digestate solid fraction; LD = digestate liquid fraction; D0 = 100% N as urea; D50 = 50% N as urea + 50% N as WD; D100 = 100% N as